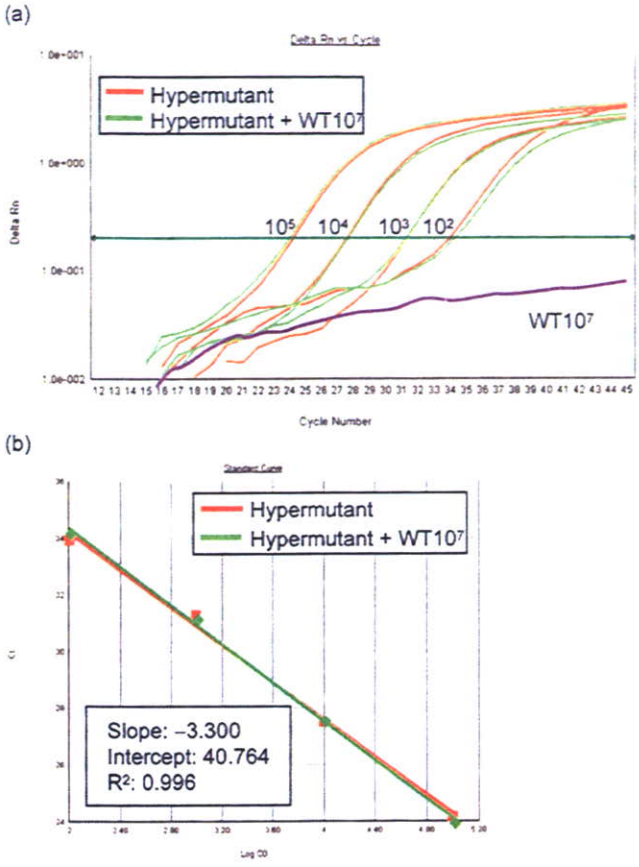

Fig. 2. Quantitative measurement of hypermutated HBV DNA using 3D-PCR combined with real-time PCR. The indicated numbers (10^2–10^5) of hypermutated genomes alone (orange lines) and a mixture of wild-type plus hypermutated genomes (green lines) were amplified by 3D-PCR. 3D-PCR did not result in amplification of wild-type sequence (purple line). Denaturation temperature was 88 °C.

showed detection of more heavily hypermutated genomes at lower denaturation temperatures (Fig. 1b). To develop quantitative measurement, we selected sequences with many G residues, designed primers that contained only a small number of G residues and used degenerate primers. A probe sequence was designed without a G residue. Using this primer and probe set, we could amplify only hypermutated genomes (Fig. 2). When hypermutated and non-mutated genomes were co-amplified, only hypermutated genomes were successfully amplified using the above primer and probe set (Fig. 2b). Non-hypermutated genomes (10^7 copies) were not amplified, although conventional PCR amplified both mutated and non-mutated genomes equally (data not shown). We also tried to detect only slightly (four of the 58 G residues) mutated genomes by 3D-PCR, but could not detect such genomes. It should thus be noted that the quantitative measurement we developed in this study detects only hypermutated genomes.

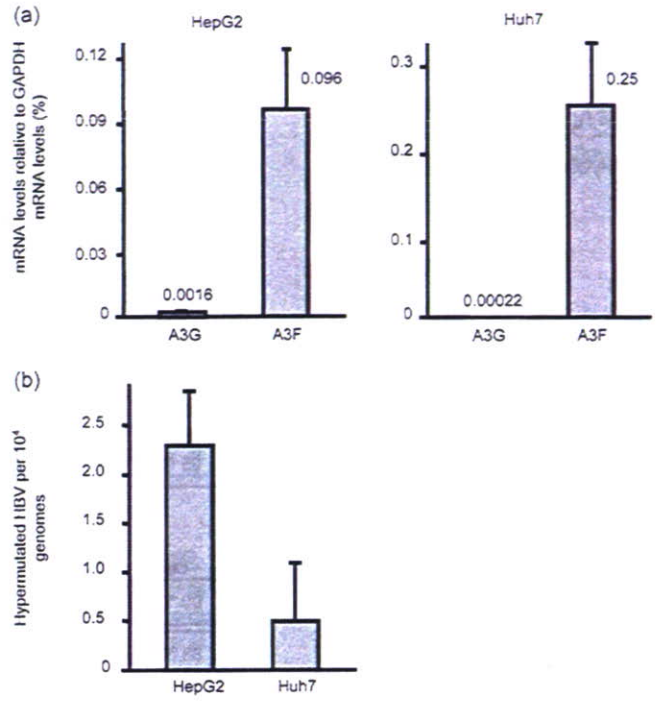

Fig. 3. Expression levels of APOBEC3G and -3F protein mRNAs in HepG2 and Huh7 cell lines. (a) mRNAs were extracted from cultured cell lines and the number of mRNA was quantified by real-time PCR with a probe for APOBEC3G and -3F. The expression levels were expressed as a percentage of GAPDH mRNA. (b) Number of hypermutated HBV genomes measured by real-time 3D-PCR in HepG2 and Huh7 cell lines transiently transfected with pTRE-HBV-wt. Results are means ± SD values of three independent experiments.

Detection of APOBEC3G mRNA and hypermutated genomes in semi-permissive and permissive cell lines

In retrovirus studies, it is known that some cell lines allow production of infectious retrovirus virions with Vif deficiency (permissive cells) while others do not. The difference between semi-permissive and permissive cell lines is the expression of APOBEC3G (Mangeat *et al.*, 2003; Zhang *et al.*, 2003; Lecossier *et al.*, 2003; Harris *et al.*, 2003; Shirakawa *et al.*, 2006). Thus, we examined the expression of APOBEC3G in both HepG2 and Huh7 cell lines. The APOBEC3G mRNA level detected by real-time PCR was very low (approx. 0.002 % relative to GAPDH mRNA) and about ten times greater in HepG2 cells than in Huh7 cells (Fig. 3a).

The number of hypermutated genomes in HepG2 cells transiently transfected with pTRE-HB-wt was about five times that in Huh7 cells (Fig. 3b). Vif-deficient HIV-1 virions produced from HepG2 cell exhibited very low infectivity compared with wild-type (Fig. 4a). In contrast, the infectivity of HIV-1 virions produced by Huh7 was

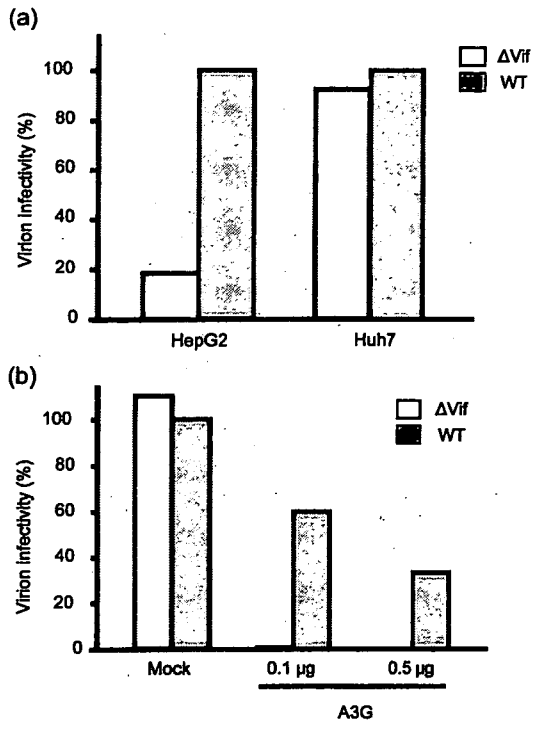

Fig. 4. Infectivity of HIV-1 virions produced from HepG2 and Huh7 cell lines. (a) Wild-type and mutant viruses lacking Vif protein produced from the two cell lines were examined for infectivity as described in Methods. The relative infectivity of the wild-type is shown. (b) Effect of APOBEC3G (A3G) expression on infectivity. HIV-1 virions produced by Huh7 cells co-transfected with the indicated number of APOBEC3G expression plasmid were used for measurement of infectivity.

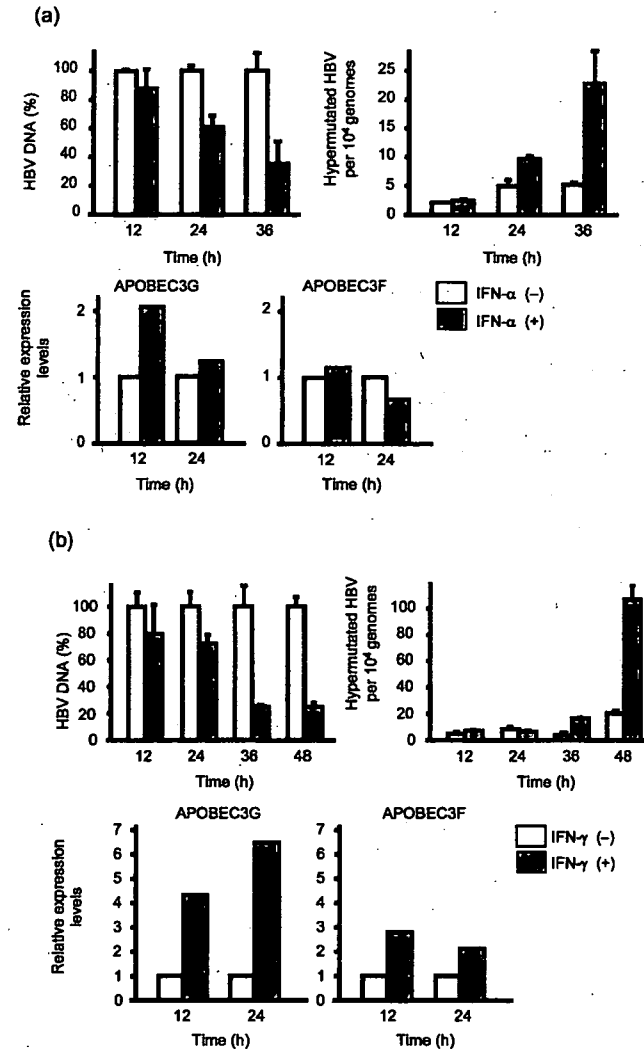

Fig. 5. Effects of IFN-α and -γ on HBV-producing cells. (a) The IFN-α-treated and -untreated HBV-producing T23 cell line was harvested at the indicated time after IFN treatment and examined for the number of core-associated HBV DNA, the number of hypermutated genome and mRNAs of APOBEC3G and APOBEC3F. (b) IFN-γ-treated and -untreated HBV-producing T23 cell line was examined as described in (a). Results are means ± SD values of three independent experiments.

similar to that of the wild-type virus (Fig. 4a). Transient expression experiments showed that the expression of APOBEC3G in Huh7 cell lines reduced infectivity of wild-type HIV-1 produced in these cell lines in a dose-dependent manner (Fig. 4b). Infectivity of Vif-deficient HIV-1 was reduced to almost undetectable levels (Fig. 4b). Thus, APOBEC3G effectively suppressed the production of infectious HIV in these cell lines.

Both IFN-α and -γ induce APOBEC3G mRNA expression and hypermutation of HBV genomes and reduce replication of HBV

We treated HepG2 cell lines stably transfected with 1.4 genome length construct HBV (Tsuge *et al.*, 2005) with either IFN-α or -γ to examine their influence on the expression of APOBEC3G mRNA and G to A hypermutation of HBV genomes. Chronological studies showed that the core-associated HBV DNA in the stably HBV-producing cell line gradually decreased until 36 h after IFN-α treatment (Fig. 5a). Expression levels of APOBEC3G mRNA, but not those of APOBEC3F, increased in this cell line at 12 h after the IFN treatment (Fig. 5a). Hypermutated genomes in this cell line increased with time until 36 h after IFN-α treatment. Similarly, the core-associated HBV DNA decreased gradually to about 20 % of the levels in untreated cells after IFN-γ treatment (Fig. 5b). The increase in APOBEC3G mRNA expression was more prominent after IFN-γ than after IFN-α treatment. The level of APOBEC3F mRNA was also about double that of untreated cells. G to A hypermutation of HBV genomes increased markedly with time after IFN-γ treatment (Fig. 5b).

We further examined the effect of IFN on reduction of HBV replication and induction of hypermutation by comparing the effects of different doses of IFN-α and -γ. Both IFN-α

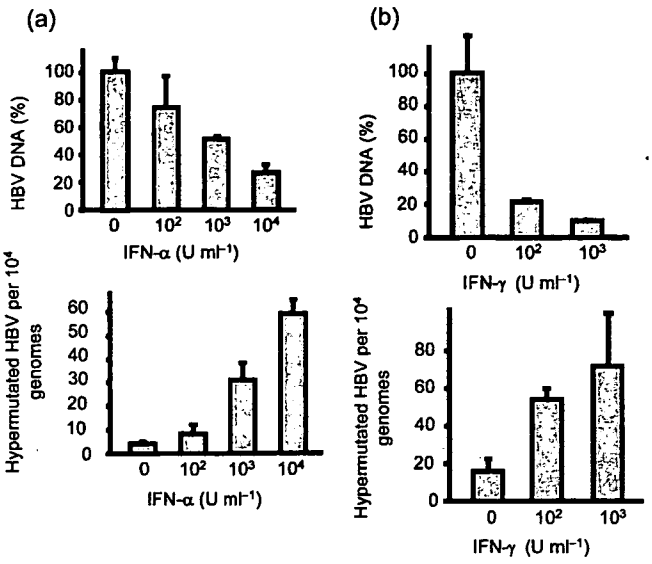

Fig. 6. Dose-dependent reduction of HBV replication and hypermutation of genomic sequences. HBV-producing cell line T23 was harvested after (a) IFN-α and (b) IFN-γ treatment for 72 h. The number of core-associated HBV DNA and the number of hypermutated genomes were measured. Results are means ± SD values of three independent experiments.

and -γ treatment decreased core-associated HBV DNA in a dose-dependent manner (Fig. 6). Hypermutation of HBV genomes also increased with higher doses of IFN (Fig. 6).

Expression of APOBEC3G increases hypermutation of the HBV genome

To confirm that the increase in hypermutation of the HBV genome is induced by the effect of APOBEC3G, we performed expression experiments of APOBEC3G and its deaminase function-deficient mutants into HepG2 cell lines and measured the number of hypermutated HBV genomes. Transient expression experiments showed that the number of HBV DNA was decreased by co-transfection of APOBEC3G in HepG2 cells (Fig. 7a). 3D-PCR and detection with HA-yellow agarose gel electrophoresis showed the presence of heavily hypermutated genomes (Fig. 7b). No amplification was observed at the 81 °C denaturation temperature (data not shown). Quantitative analysis showed an about 334-fold increase in hypermutated genomes compared with mock-transfected control cells (Fig. 7c). However, the proportion of hypermutated genomes was 9.68 % (968 in 10^4 genomes).

To confirm the effect of APOBEC3G on HBV hypermutation, we transfected wild-type and inactive mutants of APOBEC3G (Fig. 8a, b) into Huh7 cells. Wild-type APOBEC3G effectively induced hypermutation of HBV genomes and reduced the replication of HBV. In contrast, insufficient deaminase activity in the E67Q mutant induced less hypermutation of HBV genomes than in the wild-type. No increase in hypermutation was observed in cell lines transfected with deamination-defective E259Q and E67Q/E259Q mutants, although the number of HBV replication was reduced in these cells (Fig. 8a). We observed similar reduction in HBV replication by transient transfection of APOBEC3F. Induction of hypermutation by APOBEC3F was less efficient than by wild-type and the E67Q mutant of

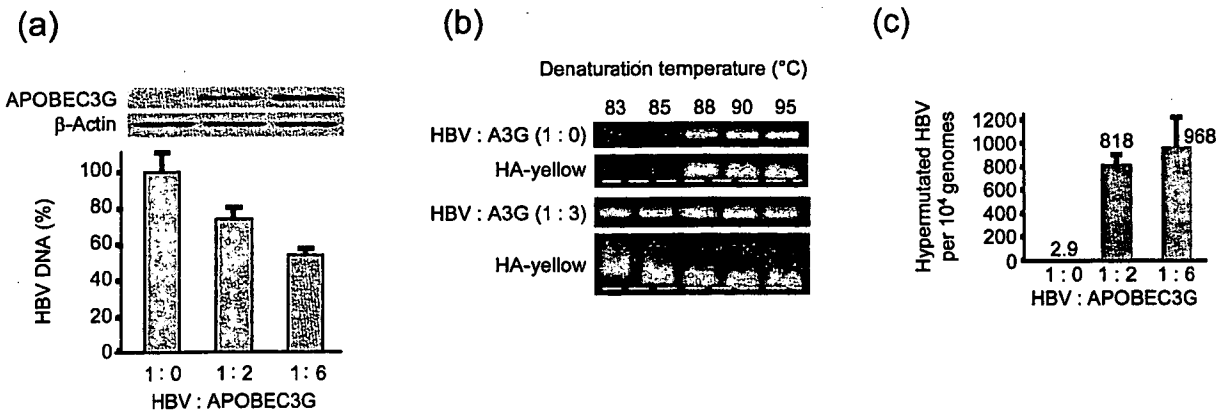

Fig. 7. Effects of APOBEC3G expression on HBV hypermutation. A plasmid containing 1.4 genome length HBV DNA was co-transfected with pcDNA3/HA-A3G into HepG2 cells. At 72 h after transfection, the cells were harvested. (a) Quantification of core-associated HBV DNA and Western blot analysis of cytoplasmic extracts with anti-HA or anti-β-actin antibody. (b) Detection of hypermutated genomes by HA-yellow agarose gel electrophoresis. Hypermutated genomes in the presence or absence of APOBEC3G-HA were amplified by 3D-PCR. The white dotted line was added to help visualize the retardation of AT-rich DNA in HA-yellow agarose gel. (c) Quantification analysis of hypermutated genomes by real-time 3D-PCR. Results are means ± SD values of three independent experiments.

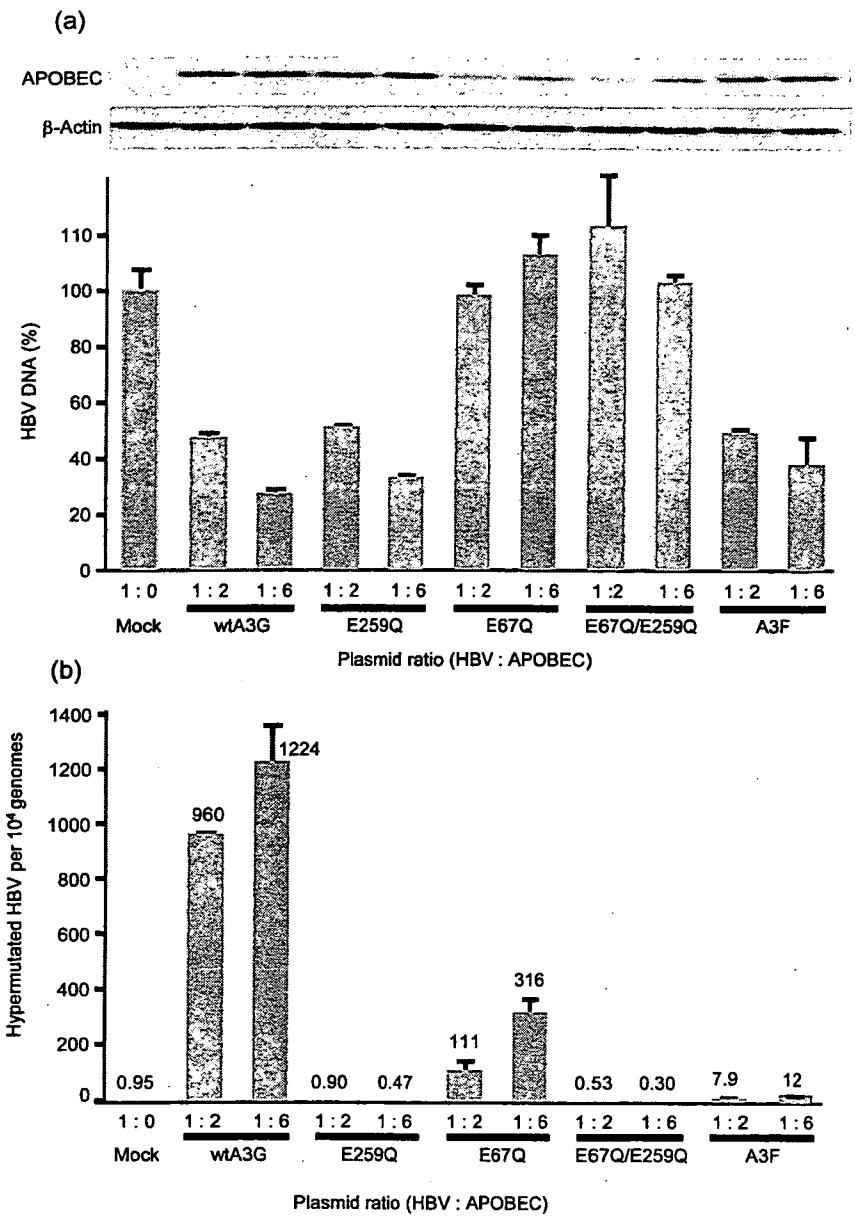

Fig. 8. Effect of APOBEC proteins on HBV hypermutation. A plasmid containing 1.4 genome length HBV DNA was co-transfected with wild-type, enzymically impaired APOBEC3G mutants (E67Q, E259Q, E67Q/E259Q) and APOBEC3F into Huh7 cells (plasmid ratio HBV : APOBEC = 1 : 2 or 1 : 6). The cells were harvested at 96 h after transfection. (a) Quantification of core-associated HBV DNA and Western blot analysis of cytoplasmic extracts with anti-HA or anti-β-actin antibody. (b) Quantification of hypermutated genomes by real-time 3D-PCR. Results are means ± SD values of three independent experiments.

APOBEC3G. These results suggest that hypermutation of HBV contributes very little to reduce the number of replicative intermediate.

DISCUSSION

Induction of G to A hypermutation in HIV has been reported as part of host innate immunity against virus infection (Mangeat *et al.*, 2003; Zhang *et al.*, 2003; Lecossier *et al.*, 2003; Harris *et al.*, 2003; Sheehy *et al.*, 2002). We and others have reported the presence of hypermutated genomes of HBV in serum samples of chronically infected patients and in HepG2 cell lines (Gunther *et al.*, 1997; Suspene *et al.*, 2005a; Noguchi *et al.*, 2005; Rosler *et al.*, 2004). Hypermutation of HBV was induced in hepatocytes (Noguchi *et al.*, 2005), and expression of APOBEC proteins in liver cell-derived cell lines increased hypermutation (Suspene *et al.*, 2005b; Rosler *et al.*, 2004). However, the estimated number of hypermutated genomes in chronically infected patients is very low (Noguchi *et al.*, 2005; Suspene *et al.*, 2005b). The reason for the partial hypermutation of HBV remains an enigma. It might be due to the low expression levels of APOBEC proteins in liver cells (Jarmuz *et al.*, 2002). Alternatively, rapid packaging of pregenome RNA into capsid might prevent access of APOBEC3G to the first strand DNA. Furthermore, rapid degradation of edited HBV genomes by uracil DNA glycosylase in liver cells might also explain the low number of hypermutated genomes.

The mechanism that controls the activities of APOBEC proteins to cause hypermutation has not been analysed until

recently. Tanaka *et al.* (2006) reported that IFN-α increases the expression levels of APOBEC3G mRNA. They reported the presence of ISRE elements in the promoter region of APOBEC3G and that the promoter was activated by IFN-α. However, they did not examine the occurrence of G to A hypermutation in their experiments. Moreover, Peng *et al.* (2006) showed that IFN-α and -γ cooperatively induce APOBEC3G expression and that the inhibition of HIV production by a small number of IFN is cancelled by a small interfering RNA (siRNA) against APOBEC3G. More recently, Bonvin *et al.* (2006) demonstrated that IFN-α induces transcription of APOBEC proteins. They showed that IFN treatment increased APOBEC3B, -3C, -3F and -3G mRNAs, particularly when they used primary cultured hepatocytes. They also reported that they were able to detect hypermutated genomes after transfection of APOBEC3 plasmids, but did not measure the direct effect of IFN on G to A hypermutation.

These studies did not analyse quantitatively the increase in hypermutation of viral genomes. The studies that analysed the expression of APOBEC protein and reduction of HBV DNA also did not analyse quantitatively the number of hypermutated genome (Suspene *et al.*, 2005a; Noguchi *et al.*, 2005; Turelli *et al.*, 2004a, b; Rosler *et al.*, 2005). In the present study, we developed a method that accurately measures the level of hypermutation using real-time PCR. It is often difficult to design a primer set and a probe to detect G to A hypermutation because they are located in a region with many G residues, but the primer and probe sequences should not contain any. It is thus possible that we did not see any C to T substitution because we did not design a primer–probe set to detect this substitution. We also tried to select such a primer–probe set applicable for all genotypes of HBV, but were able to select only one suitable for genotype C.

Using this method, we demonstrated that both IFN-α and -γ increased G to A hypermutation of the HBV genome. Although the expression levels of APOBEC3G increased after IFN treatment, we did not observe an apparent shift of preferred dinucleotide sequence of APOBEC proteins from 3F to 3G. This is probably because the increase in APOBEC3G is only slight (Fig. 5).

The exact mechanism by which IFNs activate the transcription of APOBEC3G is unknown. Furthermore, what kind of sensor(s) detects HBV infection and how the signal is communicated for the production of IFNs and subsequent induction of effector molecules have not been analysed yet. Although the importance of the IFN system in eliminating HBV and its possible mechanism have been reported (Wieland *et al.*, 2004a, b, 2005), further studies are needed to fully describe the mechanism of action of IFNs including the activation of APOBEC3G.

We also demonstrated that the number of hypermutated genomes increased with the expression of APOBEC3G and APOBEC3F (Fig. 8), but not in deaminase-inactive mutants, as demonstrated previously in HIV studies (Shindo *et al.*, 2003; Newman *et al.*, 2005). However, these mutants also reduced the replication of HBV almost to the wild-type level. This suggests that the contribution of hypermutation of HBV to the reduction of virus replication is only minimal and supports the previous report that showed that APOBEC3G reduced the replication of HBV through inhibition of packaging of the pregenome (Turelli *et al.*, 2004a). However, the effect of hypermutation on infectivity of the virus should be investigated further. The effects of APOBEC proteins, including other family members, especially under physiological conditions, should also be examined further. Whether any HBV protein inhibits deamination of the genomic DNA awaits further investigation. Furthermore, the mechanism that enables HBV to cause chronic infection, especially escape from innate antiviral immunity, should also be clarified in order to control chronic HBV infection and reduce HBV-related morbidity.

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REFERENCES

Abu-Daya, A., Brown, P. M. & Fox, K. R. (1995). DNA sequence preferences of several AT-selective minor groove binding ligands. *Nucleic Acids Res* **23**, 3385–3392.

Bonvin, M., Achermann, F., Greeve, I., Stroka, D., Keogh, A., Inderbitzin, D., Candinas, D., Sommer, P., Wain-Hobson, S. & other authors (2006). Interferon-inducible expression of APOBEC3 editing enzymes in human hepatocytes and inhibition of hepatitis B virus replication. *Hepatology* **43**, 1364–1374.

Bruix, J. & Llovet, J. M. (2003). Hepatitis B virus and hepatocellular carcinoma. *J Hepatol* **39** (Suppl. 1), S59–S63.

Colonna, M., Krug, A. & Cella, M. (2002). Interferon-producing cells: on the front line in immune responses against pathogens. *Curr Opin Immunol* **14**, 373–379.

Ganem, D. & Schneider, R. (2001). *Hepadnaviridae*: the viruses and their replication. In *Fields Virology*, 4th edn, pp. 2923–2969. Edited by D. M. Knipe & P. M. Howley. Baltimore: Lippincott Williams & Wilkins.

Ganem, D. & Prince, A. M. (2004). Hepatitis B virus infection - natural history and clinical consequences. *N Engl J Med* **350**, 1118–1129.

Grandvaux, N., tenOever, B. R., Servant, M. J. & Hiscott, J. (2002). The interferon antiviral response: from viral invasion to evasion. *Curr Opin Infect Dis* **15**, 259–267.

Gunther, S., Sommer, G., Plikat, U., Iwanska, A., Wain-Hobson, S., Will, H. & Meyerhans, A. (1997). Naturally occurring hepatitis B virus genomes bearing the hallmarks of retroviral G→A hypermutation. *Virology* **235**, 104–108.

Harris, R. S., Bishop, K. N., Sheehy, A. M., Craig, H. M., Petersen-Mahrt, S. K., Watt, I. N., Neuberger, M. S. & Malim, M. H. (2003). DNA deamination mediates innate immunity to retroviral infection. *Cell* **113**, 803–809.

Janini, M., Rogers, M., Birx, D. R. & McCutchan, F. E. (2001). Human immunodeficiency virus type 1 DNA sequences genetically damaged by hypermutation are often abundant in patient peripheral blood mononuclear cells and may be generated during near-simultaneous infection and activation of CD4(+) T cells. *J Virol* **75**, 7973–7986.

Jarmuz, A., Chester, A., Bayliss, J., Gisbourne, J., Dunham, I., Scott, J. & Navaratnam, N. (2002). An anthropoid-specific locus of orphan C to U RNA-editing enzymes on chromosome 22. *Genomics* **79**, 285–296.

Kobayashi, M., Takaori-Kondo, A., Shindo, K., Abudu, A., Fukunaga, K. & Uchiyama, T. (2004). APOBEC3G targets specific virus species. *J Virol* **78**, 8238–8244.

Lecossier, D., Bouchonnet, F., Clavel, F. & Hance, A. J. (2003). Hypermutation of HIV-1 DNA in the absence of the Vif protein. *Science* **300**, 1112.

Mangeat, B., Turelli, P., Caron, G., Friedli, M., Perrin, L. & Trono, D. (2003). Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. *Nature* **424**, 99–103.

Newman, E. N., Holmes, R. K., Craig, H. M., Klein, K. C., Lingappa, J. R., Malim, M. H. & Sheehy, A. M. (2005). Antiviral function of APOBEC3G can be dissociated from cytidine deaminase activity. *Curr Biol* **15**, 166–170.

Noguchi, C., Ishino, H., Tsuge, M., Fujimoto, Y., Imamura, M., Takahashi, S. & Chayama, K. (2005). G to A hypermutation of hepatitis B virus. *Hepatology* **41**, 626–633.

Norder, H., Courouce, A. M. & Magnius, L. O. (1994). Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology* **198**, 489–503.

Peng, G., Lei, K. J., Jin, W., Greenwell-Wild, T. & Wahl, S. M. (2006). Induction of APOBEC3 family proteins, a defensive maneuver underlying interferon-induced anti-HIV-1 activity. *J Exp Med* **203**, 41–46.

Rosler, C., Kock, J., Malim, M. H., Blum, H. E. & von Weizsacker, F. (2004). Comment on 'Inhibition of hepatitis B virus replication by APOBEC3G'. *Science* **305**, 1403 (author reply 1403).

Rosler, C., Kock, J., Kann, M., Malim, M. H., Blum, H. E., Baumert, T. F. & von Weizsacker, F. (2005). APOBEC-mediated interference with hepadnavirus production. *Hepatology* **42**, 301–309.

Samuel, C. E. (2001). Antiviral actions of interferons. *Clin Microbiol Rev* **14**, 778–809.

Seeger, C. & Mason, W. S. (2000). Hepatitis B virus biology. *Microbiol Mol Biol Rev* **64**, 51–68.

Sheehy, A. M., Gaddis, N. C., Choi, J. D. & Malim, M. H. (2002). Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature* **418**, 646–650.

Shindo, K., Takaori-Kondo, A., Kobayashi, M., Abudu, A., Fukunaga, K. & Uchiyama, T. (2003). The enzymatic activity of CEM15/Apobec-3G is essential for the regulation of the infectivity of HIV-1 virion but not a sole determinant of its antiviral activity. *J Biol Chem* **278**, 44412–44416.

Shirakawa, K., Takaori-Kondo, A., Kobayashi, M., Tomonaga, M., Izumi, T., Fukunaga, K., Sasada, A., Abudu, A., Miyauchi, Y. & other authors (2006). Ubiquitination of APOBEC3 proteins by the Vif-Cullin5-ElonginB-ElonginC complex. *Virology* **344**, 263–266.

Skalka, A. M. & Goff, S. P. (1993). *Reverse Transcriptase.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Summers, J. & Mason, W. S. (1982). Replication of the genome of a hepatitis B-like virus by reverse transcription of an RNA intermediate. *Cell* **29**, 403–415.

Suspene, R., Guetard, D., Henry, M., Sommer, P., Wain-Hobson, S. & Vartanian, J. P. (2005a). Extensive editing of both hepatitis B virus DNA strands by APOBEC3 cytidine deaminases in vitro and in vivo. *Proc Natl Acad Sci U S A* **102**, 8321–8326.

Suspene, R., Henry, M., Guillot, S., Wain-Hobson, S. & Vartanian, J. P. (2005b). Recovery of APOBEC3-edited human immunodeficiency virus G→A hypermutants by differential DNA denaturation PCR. *J Gen Virol* **86**, 125–129.

Tanaka, Y., Marusawa, H., Seno, H., Matsumoto, Y., Ueda, Y., Kodama, Y., Endo, Y., Yamauchi, J., Matsumoto, T. & other authors (2006). Anti-viral protein APOBEC3G is induced by interferon-α stimulation in human hepatocytes. *Biochem Biophys Res Commun* **341**, 314–319.

Tsuge, M., Hiraga, N., Takaishi, H., Noguchi, C., Oga, H., Imamura, M., Takahashi, S., Iwao, E., Fujimoto, Y. & other authors (2005). Infection of human hepatocyte chimeric mouse with genetically engineered hepatitis B virus. *Hepatology* **42**, 1046–1054.

Turelli, P., Jost, S., Mangeat, B. & Trono, D. (2004a). Response to comment of 'Inhibition of hepatitis B virus replication by APOBEC3G'. *Science* **305**, 1403b.

Turelli, P., Mangeat, B., Jost, S., Vianin, S. & Trono, D. (2004b). Inhibition of hepatitis B virus replication by APOBEC3G. *Science* **303**, 1829.

Wieland, S., Thimme, R., Purcell, R. H. & Chisari, F. V. (2004a). Genomic analysis of the host response to hepatitis B virus infection. *Proc Natl Acad Sci U S A* **101**, 6669–6674.

Wieland, S. F., Spangenberg, H. C., Thimme, R., Purcell, R. H. & Chisari, F. V. (2004b). Expansion and contraction of the hepatitis B virus transcriptional template in infected chimpanzees. *Proc Natl Acad Sci U S A* **101**, 2129–2134.

Wieland, S. F., Eustaquio, A., Whitten-Bauer, C., Boyd, B. & Chisari, F. V. (2005). Interferon prevents formation of replication-competent hepatitis B virus RNA-containing nucleocapsids. *Proc Natl Acad Sci U S A* **102**, 9913–9917.

Wright, T. L. & Lau, J. Y. (1993). Clinical aspects of hepatitis B virus infection. *Lancet* **342**, 1340–1344.

Zhang, H., Yang, B., Pomerantz, R. J., Zhang, C., Arunachalam, S. C. & Gao, L. (2003). The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. *Nature* **424**, 94–98.

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Journal of Gastroenterology

Pretreatment predictor of response, time to progression, and survival to intraarterial 5-fluorouracil/interferon combination therapy in patients with advanced hepatocellular carcinoma

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Background. Several studies have reported survival benefits of combination therapy with intraarterial 5-fluorouracil (5-FU) and subcutaneous interferon (IFN) α for advanced hepatocellular carcinoma (HCC) with portal vein tumor thrombosis (PVTT). We investigated the pretreatment predictive factors of early response, time to progression (TTP), and survival in response to intraarterial 5-FU/IFN combination therapy. ***Methods.*** Patients with nonresectable HCC and variable PVTT grades (without PVTT to PVTT in the trunk) received intraarterial 5-FU/IFN combination therapy (n = 55). ***Results.*** After two courses of the combination therapy, 1 (2%), 15 (27%), 16 (29%), 12 (22%), and 11 (20%) of 55 patients showed complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD), or had dropped out (DO), respectively, when their early response to treatment was assessed. Univariate analysis identified only hepatitis C virus (HCV) antibody positivity as having significantly influenced the early response (P = 0.028) and TTP (P = 0.021). Multivariate analysis identified performance status (P = 0.003) and HCV antibody positivity (P = 0.007) as significant and independent determinants of survival. PVTT grade did not influence early response, TTP, or survival. The survival rate was significantly higher in patients who achieved CR or PR than in those that assessed as SD or PD, or DO (P < 0.0001, each). ***Conclusions.*** HCV antibody positivity may be a significant pretreatment predictor of early response, TTP, and survival of patients with advanced HCC treated with 5-FU/IFN. CR or PR as the early response to the combination therapy might indicate a more favorable prognosis in patients with advanced HCC. PVTT grade did not seem to influence the efficacy of combination therapy.

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Key words: advanced hepatocellular carcinoma, 5-fluorouracil and interferon, early response, survival, HCV

Introduction

Hepatocellular carcinoma (HCC) is a life-threatening neoplasm and one of the most common neoplasms in Africa and Asia, including Japan. Deaths due to HCC are increasing worldwide.[1–3] Advances in biotechnology have resulted in new diagnostic techniques, such as ultrasonography, computed tomography (CT), magnetic resonance imaging, and angiography. Similarly, new treatment options have become available, such as surgical resection, radiofrequency ablation (RFA), percutaneous ethanol injection (PEI), and transcatheter arterial chemoembolization (TACE). As a result, the prognosis of HCC patients has gradually improved. Nevertheless, the survival rates of patients with advanced HCC and complications such as portal vein tumor thrombosis (PVTT) or distant metastasis remains extremely poor.[4–8]

Advances in implantable drug delivery systems have allowed repeated arterial infusions of anticancer agents. First, monotherapy with intraarterial 5-fluorouracil (5-FU) for unresectable HCC was reported.[9,10] However, such treatment resulted in a low response rate (13.0% and 22.0%). Next, several authors reported favorable results with low-dose cisplatin and 5-FU for advanced HCC with PVTT, with a response rate ranging from 33.0% to 48.0%.[11–13] Recently, several studies have reported survival benefits of combination therapy with intraarterial 5-FU and subcutaneous interferon (IFN) α for advanced HCC with PVTT, with a response rate ranging from 43.6% to 72.7%.[14–17] In these studies, only HCC patients with PVTT (in the main trunk or first branch) without distant metastases were treated. The

pretreatment predictive factors of response, time to progression (TTP), and survival of HCC patients treated with the combination therapy remain unclear. At present, some patients with nonresectable HCC are treated with TACE. However, some patients are not suitable candidates for TACE because of PVTT or poor response to TACE. Because of the poor prognosis of patients with nonresectable HCC who are not treatable by TACE, effective treatment is needed. There is little information about assessment of patients with advanced HCC (e.g., nonresectable HCC with PVTT in the second branch or nonresectable HCC without PVTT but with poor response to TACE) treated with combination therapy of intraarterial 5-FU and IFN. In the present retrospective cohort study, we assessed the efficacy of intraarterial 5-FU with IFN for various types of nonresectable advanced HCC and investigated the pretreatment predictive factors of early response, TTP, and survival in response to the combination therapy.

Materials and methods

Patients

From June 2003 to December 2006, 265 consecutive patients with unresectable HCC were admitted to our hospital. Of the 265 patients with advanced HCC, 94 were treated with TACE, 34 patients received systemic chemotherapy, and 13 patients received best supportive care. The remaining 124 patients were selected as suitable candidates for intraarterial 5-FU and IFN combination therapy. Forty-one patients refused the therapy. Thus, 83 patients with advanced HCC were treated with intraarterial 5-FU and IFN. Of these 83 patients, 24 with distant metastases and four with hepatic venous invasion were excluded from this study, so we assessed 55 patients without distant metastases or hepatic venous invasion in this retrospective cohort study. Of the 55 patients, 30 had been treated with TACE before enrollment. Table 1 lists the baseline characteristics of the 55 patients. PVTT grade, based on the location of the tumor thrombus, was determined according to the criteria of the Liver Cancer Study Group of Japan (LCSGJ).[18] PVTT grading was as follows: Vp 0, no PVTT; Vp 1, tumor thrombus in a third or more of the peripheral branches of the portal vein; Vp 2, tumor thrombus in a second branch of the portal vein; Vp 3, tumor thrombus in the first branch of the portal vein; and Vp 4, tumor thrombus in the trunk of the portal vein. Tumor staging was defined based on the TNM staging system of the LCSGJ:[18] stage I (fulfilling three intrahepatic conditions: solitary, <2 cm, no vessel invasion), stage II (fulfilling two of the three intrahepatic conditions), stage III (fulfilling one of the three intrahepatic conditions), stage IVA (fulfilling none of the three intrahepatic conditions with no distant metastases or any intrahepatic conditions with lymph node metastases), and stage IVB (any intrahepatic conditions with distant metastases).

Eligibility

This was a retrospective cohort study to investigate pretreatment predictive factors of TTP, survival, and

Table 1. Clinical profile of the 55 HCC patients

Age (years)[a]	67 (38–79)
Sex (M/F)	44/11
Etiology: HBV/HCV/other	15/36/4
Total bilirubin (mg/dl)	1.1 (0.4–6.4)
Platelet count ($\times 10^4$ mg/dl)	13.0 (5.1–54.5)
Albumin (mg/dl)	3.5 (2.4–4.8)
Child Pugh stage (A/B/C)	43/10/2
PS (0/1)	45/10
Intrahepatic tumor volume (≤50%/>50%)	38/17
Tumor stage (III/IVA)	20/35
Vp[a] (0/2/3/4)	20/6/15/14
AFP (ng/ml)	934 (14.3–525 900)
AFP-L3 (%)	47.3 (<0.5–87.6)
DCP (mAU/ml)	3729 (10–722 140)
Previous treatment (performed/not performed)	30/25

Data are expressed as median with range values in parentheses, or number of patients HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HBV, hepatitis B virus; PS, Eastern Cooperative Oncology Group performance status; AFP, α-fetoprotein; AFP-L3, lens culinaris agglutinin-reactive fraction of α-fetoprotein; DCP, des-γ-carboxy prothrombin; PVTT, portal vein tumor thrombosis

[a] PVTT grade: Vp 0, no PVTT; Vp 1, tumor thrombus in a third or more of the peripheral branches of the portal vein; Vp 2, tumor thrombus in a second branch of the portal vein; Vp 3, tumor thrombus in the first branch of the portal vein; Vp 4, tumor thrombus in the trunk of the portal vein

response to intraarterial 5-FU/IFN combination therapy. Eligibility criteria were as follows: age, 18–80 years; leukocyte count, >2000/μl; neutrophil count, >1200/μl; hemoglobin, >8 g/dl; platelet count, >50 000/μl; unresectable or not suitable for local ablation therapy, including RFA or PEI; with PVTT or TACE was ineffective; without hepatic venous invasion; without distant metastases; and Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–1.[19] There was no eligibility criterion regarding hepatic reserve function, including serum total bilirubin levels. All patients gave written informed consent to this study, which was approved by the Institutional Review Board of Hiroshima University.

Treatment protocol

Patients received repeated arterial infusions of anticancer agents via the injection port. One course of chemotherapy lasted 4 weeks. 5-FU (500 mg body weight/day, Kyowa Hakko, Tokyo, Japan) was administered over 5 h with a mechanical infusion pump on days 1 to 5 of the first and second weeks (5 g in one course). Recombinant IFN α-2b (Intron A, Schering-Plough Pharmaceuticals, Osaka, Japan); 3×10^6 U (3 MU), or natural IFN α (OIF, Otsuka Pharmaceuticals, Tokyo, Japan); 5×10^6 U (5 MU) was administered intramuscularly on days 1, 3, and 5 of each week (total dose, 36 and 60 MU, respectively). In principle, treatment was repeated several times unless PS changed to 3 or 4 during the treatment. A 2- to 4-week rest period of no treatment was allowed after each treatment course. As for the two types of IFN, we previously reported similar effects of recombinant IFN α-2b and natural IFN α when combined with intraarterial 5-FU for the treatment of advanced HCC.[20]

Implantation of the arterial catheter

A catheter was inserted through the right femoral artery by the Seldinger method. After localization of the HCC, a 3-French heparin-coated catheter was inserted and its tip advanced to the common hepatic artery or proper hepatic artery. The other end of the catheter was connected to the injection port, which was implanted in a subcutaneous pocket created in the right lower abdominal quadrant. The gastroduodenal artery and right gastric artery were occluded with steel coils to prevent gastroduodenal injury by the chemotherapeutic agents.

Evaluation

The early response to the combination therapy was assessed with contrast-enhanced CT after two courses of the combination therapy. The response was defined according to the criteria of the Response Evaluation Criteria in Solid Tumors (RECIST).[21] A complete response (CR) was defined as the complete disappearance of all target lesions. A partial response (PR) was defined as a decrease of at least 30% in the sum of the longest diameter of the target lesions with the baseline sum of the longest diameter of the target lesions as the reference. Progressive disease (PD) was defined as an increase of at least 20% in the sum of the longest diameter of target lesions. Stable disease (SD) was defined as meeting neither the PR nor the PD criteria. The duration of the response was measured from the date of the start of treatment to the date of documented progression. Adverse reactions were assessed with the National Cancer Institute Common Toxicity Criteria (NCI-CTC; version 3.0)[22] every week during the treatment.

Additional therapy

After two courses of the combination therapy, we assessed the response to therapy in all patients. According to the response, we provided various additional therapies such as RFA, TACE, or radiotherapy (RT) to patients treated with the combination therapy. These additional therapies were considered for patients with PS of 0–1 and a Child-Pugh stage of A or B. Patients assessed with PR continued to receive the combination therapy repeatedly. Then, when downstaging of advanced HCC was achieved (single tumor ≤50 mm in diameter or 1–3 tumors ≤30 mm in diameter) by the repeated combination therapy, RFA was considered. For patients assessed with SD or PD, in addition to the combination therapy, TACE with cisplatin–lipiodol suspension was performed. The catheter tip was advanced superselectively into the feeding artery so that sufficient anticancer agent was delivered. Among the patients assessed with SD or PD, RT was performed for PVTT if present. For patients assessed with CR, the clinical course was observed without adjuvant chemotherapy or additional therapy.

Statistical analysis

Statistical analysis was performed on 1 April 2007. Differences between groups were examined for statistical significance using the Mann-Whitney *U* test, logistic regression test, or χ-squared test as appropriate. Cumulative survival rate and TTP were calculated from the initial date of the combination therapy and assessed by the Kaplan-Meier life-table method, and differences were evaluated by the log rank test. Univariate and multivariate analyses of predictors for early response to the combination therapy were assessed by logistic

regression test. Univariate analysis of predictors of TTP and survival of patients with HCC who received the combination therapy was assessed by the Kaplan-Meier life-table method, and differences were evaluated by the log rank test. Multivariate analysis of predictors of TTP and survival was assessed by Cox proportional hazard model. Statistical significance was defined as a P value of less than 0.05. All analyses described above were performed with SPSS software (version 11, SPSS, Chicago, IL, USA). In this study, we investigated pretreatment predictive factors of early response, TTP, and survival in response to the combination therapy.

Results

Response to the combination therapy

The early response of the 55 patients was assessed after two courses of 5-FU/IFN combination therapy. As a result, 1 (2%), 15 (27%), 16 (29%), 12 (22%), and 11 (20%) patients showed CR, PR, SD, PD, or dropped out (DO), respectively. The reasons for DO were confusion (one patient), refusal after initiation of therapy (one patient), exanthema (one patient), infection around the catheter (four patients), and stenosis of the hepatic artery (four patients). We investigated the pretreatment determinants of the early response to the combination therapy. Univariate analysis identified positivity to HCV antibody as the only factor with significant influence on the early response (P = 0.028, Table 2, Fig. 1). Of the HCV antibody-positive patients, 38.9% (14/36) showed an early response of CR or PR, but only 10.5% (2/19) of other patients. When we compared the early response between patients with Vp 0–2 and those with Vp 3/4, 30.8% (8/26) of patients with Vp 0–2 and 27.6% (8/29) of those with Vp 3/4 achieved CR or PR, but the difference was not significant.

Time to progression

The median TTP in all 55 patients was 7.5 months [95% confidence interval (CI), 5.1–9.9 months], and the cumulative TTP rates at 6, 12, 18, and 24 months were 60%, 41%, 30%, and 24%, respectively. We investigated the pretreatment determinants of TTP after initiation of the combination therapy. Univariate analysis identified positivity for HCV antibody as the only factor with significant influence on TTP (P = 0.021, Table 3, Fig. 2). The median TTP in patients with Vp 0–2 and those with Vp 3/4 was 5.2 and 7.5 months, respectively. There was no significant difference in TTP between these two groups.

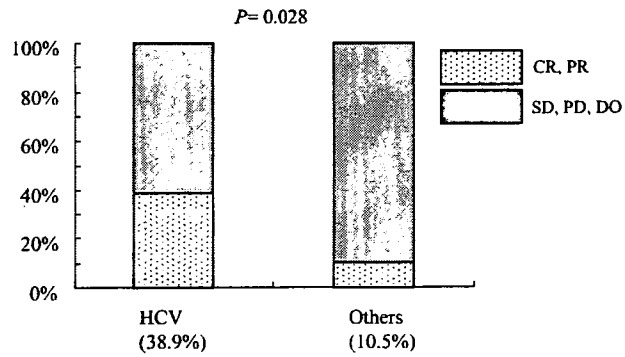

Fig. 1. Comparison of the early response rate between the hepatitis C virus (HCV)-positive group and others. The rate was significantly higher in the HCV-positive group (logistic regression test: $P = 0.028$). *CR*, complete response; *PR*, partial response; *SD*, stable disease; *PD*, progressive disease; *DO*, dropped out

Table 2. Univariate analysis of predictors for early response to 5-FU/IFN combination therapy

Variable	Odds Ratio	95% CI	P value
Age (≤65 vs. >65 years)	0.463	0.136–1.572	0.217
Sex (M vs. F)	2.327	0.445–12.168	0.317
HCV antibody (positive vs. negative)	6.071	1.216–30.314	0.028
Total bilirubin (≤1.5 vs. >1.5 mg/dl)	0.931	0.240–3.614	0.918
Platelet count (≤150000 vs. >150000 mg/dl)	0.978	0.278–3.437	0.972
Albumin (≤3.5 vs. >3.5 mg/dl)	1.390	0.441–4.376	0.574
Child Pugh stage (A vs. B, C)	2.172	0.413–11.420	0.360
PS (0 vs. 1)	4.965	0.576–42.810	0.145
Intrahepatic tumor volume (≤50% vs. >50%)	1.690	0.458–6.237	0.431
Tumor stage (III vs. IVA)	1.709	0.533–5.478	0.367
Vp (0–2 vs. 3, 4)	0.988	0.314–3.106	0.983
AFP (≤10000 vs. >10000 ng/ml)	0.978	0.278–3.437	0.972
AFP-L3 (≤50 vs. >50%)	0.776	0.229–2.625	0.683
DCP (≤10000 vs. >10000 mAU/ml)	0.606	0.186–1.974	0.406
Treatment (performed vs. not performed)	1.833	0.563–5.970	0.314

5-FU, 5-fluorouracil; IFN, interferon; CI, confidence interval

Table 3. Univariate analysis of predictors of time to progression

Variable	Hazard Ratio	95% CI	*P* value
Age (>65 vs. ≤65 years)	1.348	0.177–10.263	0.773
Sex (M vs. F)	1.788	0.403–7.935	0.445
HCV antibody (positive vs. negative)	2.775	1.169–6.590	0.021
Total bilirubin (≤1.5 vs. >1.5 mg/dl)	0.618	0.216–1.768	0.370
Platelet count (≤150000 vs. >150000 mg/dl)	0.739	0.307–1.777	0.500
Albumin (≤3.5 vs. >3.5 mg/dl)	0.705	0.300–1.655	0.421
Child Pugh stage (A vs. B, C)	2.381	0.314–18.045	0.401
PS (0 vs. 1)	1.348	0.177–10.263	0.773
Intrahepatic tumor volume (≤50% vs. >50%)	0.710	0.298–1.691	0.440
Tumor stage (III vs. IVA)	1.107	0.469–2.616	0.816
Vp (0–2 vs. 3, 4)	1.195	0.512–2.790	0.680
AFP (≤10000 vs. >10000 ng/ml)	1.325	0.484–3.626	0.584
AFP-L3 (≤50% vs. >50%)	2.371	0.696–8.076	0.167
DCP (≤10000 vs. >10000 mAU/ml)	1.145	0.486–2.701	0.756
Treatment (performed vs. not performed)	0.671	0.282–1.595	0.367

Table 4. Univariate analysis of predictors of survival of patients with HCC who received 5-FU/IFN combination therapy

Variable	Hazard Ratio	95% CI	*P* value
Age (≤65 vs. >65 years)	0.763	0.402–1.449	0.408
Sex (M vs. F)	1.208	0.527–2.769	0.655
HCV antibody (positive vs. negative)	2.283	1.165–4.474	0.016
Total bilirubin (≤1.5 vs. >1.5 mg/dl)	0.628	0.308–1.278	0.199
Platelet count (≤150000 vs. >150000 mg/dl)	0.690	0.355–1.340	0.273
Albumin (≤3.5 vs. >3.5 mg/dl)	0.760	0.398–1.451	0.406
Child Pugh stage (A vs. B, C)	0.527	0.228–1.216	0.133
PS (0 vs. 1)	3.413	1.391–8.375	0.007
Intrahepatic tumor volume (≤50% vs. >50%)	0.753	0.383–1.481	0.411
Tumor stage (III vs. IVA)	0.670	0.342–1.313	0.243
Vp (0–2 vs. 3, 4)	0.745	0.389–1.427	0.374
AFP (≤10000 vs. >10000 ng/ml)	0.947	0.445–2.017	0.888
AFP-L3 (≤50% vs. >50%)	0.898	0.430–1.871	0.773
DCP (≤10000 vs. >10000 mAU/ml)	0.753	0.394–1.438	0.390
Treatment (performed vs. not performed)	0.627	0.319–1.230	0.175
Additional therapy (performed vs. not performed)	1.129	0.583–2.188	0.719

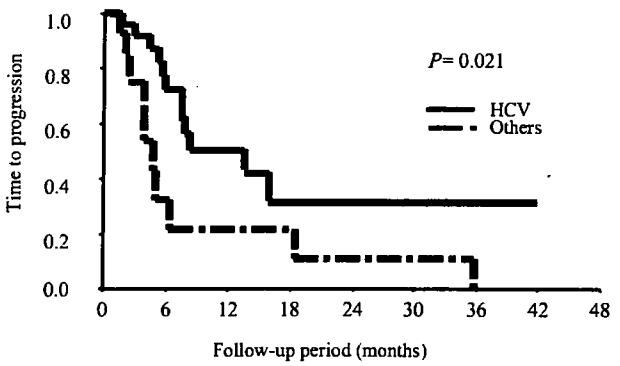

Fig. 2. Comparison of the time to progression between the HCV antibody-positive group and others. The rate was significantly higher in the HCV-positive group (log-rank test: $P = 0.021$)

Survival

The median survival in the whole group was 9.0 months (95% CI, 7.0–11.0 months), and the cumulative survival rates at 6, 12, 18, and 24 months were 67%, 39%, 22%, and 17%, respectively. We investigated the pretreatment determinants of survival after initiation of the 5-FU/IFN combination therapy. Univariate analysis identified PS = 0 ($P = 0.007$) and positivity for HCV antibody ($P = 0.016$) (Table 4, Fig. 3) as factors that significantly influenced survival. Since it was possible that the variables were mutually correlated, we performed a multivariate analysis and identified PS = 0 ($P = 0.003$) and positivity for HCV antibody ($P = 0.007$) as significant and independent determinants of survival (Table 5). The median survival time of patients with Vp 0–2 and of those with Vp 3/4 was 13.0 and 8.0 months, respectively. There was no significant difference in survival between these two groups.

Table 5. Multivariate analysis of predictors of survival of patients with HCC who received 5-FU/IFN combination therapy

Variable	Hazard Ratio	95% CI	*P* value
PS (0 vs. 1)	4.056	1.601–10.276	0.003
HCV antibody (positive vs. negative)	2.555	1.286–5.079	0.007

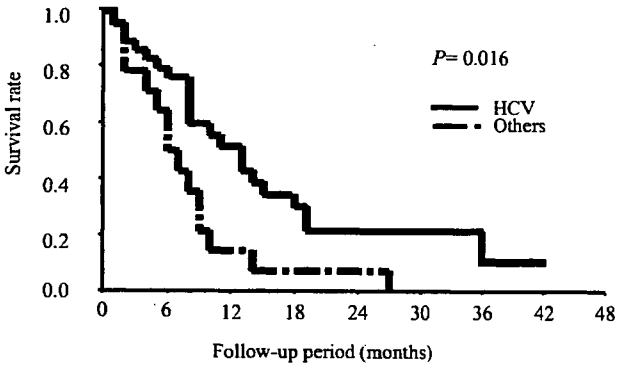

Fig. 3. Comparison of the cumulative survival rates between the HCV antibody-positive group and others. The rate was significantly higher in the HCV-positive group (log-rank test: $P = 0.016$)

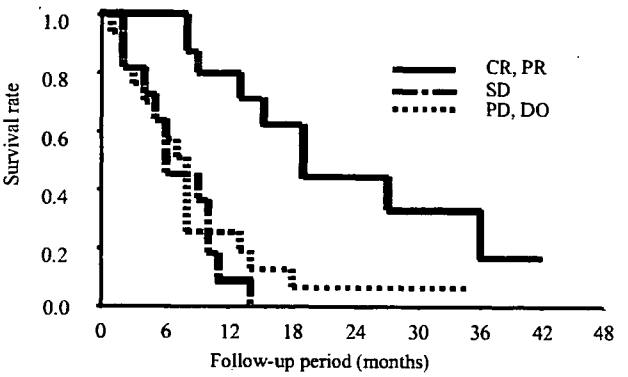

Fig. 4. Comparison of the cumulative survival rates among patients with CR/PR, SD, or PD/DO. The rate was significantly higher in patients who achieved CR/PR than those who showed SD (log-rank test: $P < 0.0001$) or PD/DO (log-rank test: $P < 0.0001$)

The cumulative survival rates of patients who achieved CR/PR at 6 and 12 months were 100% and 80%, respectively. On the other hand, the cumulative survival rates of patients who showed SD or PD/DO at 6 and 12 months were 64% and 9%, and 57% and 25%, respectively. The survival rate was significantly higher in patients who achieved CR/PR than in the other patients ($P < 0.0001$, Fig. 4).

Adverse reactions and complications

The most common adverse reactions were fever, nausea, and loss of appetite, but these were mostly NCI-CTC grade 1 or 2. Among patients with various NCI-CTC grade 3 adverse reactions, leukopenia was observed in seven (12.7%) patients, and thrombocytopenia in five (9.1%). None required administration of granulocyte colony-stimulating factor or blood transfusion. Five (9.1%) patients showed infection associated with the indwelling catheter. In this study, the number of patients with serum total bilirubin levels >3 mg/dl was three (3.7 mg/dl, 4.7 mg/dl, and 6.4 mg/dl). Other hepatic reserve functions and PS of the three patients was good (albumin, 4.1, 3.3, and 3.9 g/dl; prothrombin time, 60, 91, and 83%; PS, 0 in all cases). These three patients did not show any severe adverse reaction.

Additional therapy

Among the 55 patients, one (2%), ten (20%), and four (8%) patients were treated with RFA, TACE, and RT, respectively, as additional therapies for PVTT. The median survival time in patients receiving and in those not receiving additional therapies was the same at 9.0 months. There was no significant difference in survival between the two groups (Table 4).

Causes of death

Seventeen patients were still alive at the end of the observation period, and 38 patients had died. All 38 patients died of intrahepatic HCC-related disease.

Discussion

The median survival time of HCC patients with PVTT in the portal trunk is reported to be about 90 days with supportive care.[23] Recent studies have reported the efficacy and survival benefits of combination therapy with intraarterial 5-FU and IFN in a large number of patients with advanced HCC.[16,17] In particular, Ota et al.[16] assessed 55 patients with advanced HCC, multiple lesions, and Vp 3 or 4, and Obi et al.[17] assessed 116 patients with advanced HCC with Vp 3 or 4. These two studies assessed only patients with advanced HCC/

Vp 3 or 4. Thus, the favorable survival results they reported suggest that combination therapy with intra-arterial 5-FU and IFN is potentially useful also for HCC with Vp 0–2. Although TACE is the standard treatment option for nonresectable HCC, many patients with nonresectable HCC either show a poor response to TACE or are not suitable candidates for TACE. The prognosis of patients with nonresectable HCC who are not treated with TACE is poor, so an effective treatment for such patients is needed. In this study, we treated a heterogeneous group of patients with advanced HCC (i.e., patients with nonresectable HCC and Vp 3 or 4, those with nonresectable HCC and Vp 2 who were not suitable candidates for TACE, and those with nonresectable HCC without PVTT who showed a poor response to TACE). There was no significant difference in early response, TTP, or survival between HCC patients with Vp 0–2 and those with Vp 3/4. Hence, with regard to the response to 5-FU/IFN combination therapy, PVTT grade does not seem to be an important factor.

The objective response rates (CR and PR patients/all patients) reported in the above two studies[16,17] were 43.6% (24/55 patients) and 52.6% (61/116 patients). In our study, the objective response rate, based on the early response, was 29% (16/55 patients). One reason for the discrepancy may be that the response was evaluated differently in the three studies. Ota et al.[16] and Obi et al.[17] used ECOG criteria, but we used RECIST criteria. Second, the inclusion criteria were different. Ota et al.[16] included patients with aspartate aminotransferase (AST) or alanine aminotransferase (ALT) of less than 100 IU/l and patients with total bilirubin of less than 1.4 mg/dl, whereas Obi et al.[17] mentioned no inclusion criteria related to AST or ALT, though they used a total bilirubin level of 3.0 mg/dl as a cutoff. In our study, we used no inclusion criteria related to AST or ALT. Third, the assessment day in our protocol may be earlier than that in the other two studies. In our study, the early response cumulative survival rate was significantly higher in patients who achieved CR/PR than in those with SD or PD/DO ($P < 0.0001$, each). The early response is an important posttreatment predictor of survival of patients with advanced HCC on 5-FU/IFN combination therapy. An early response of CR or PR promises a good prognosis.

The cumulative survival rates reported by Ota et al.[16] and Obi et al.[17] at 12 and 24 months were 48.9% and 28.8%, and 34% and 18%, respectively. In our study, the cumulative survival rates at 12 and 24 months were 39% and 17%, respectively. We obtained survival rates almost identical to those reported by Obi et al.,[17] but Ota et al.[16] obtained better survival rates. This discrepancy may be due to the differences in the inclusion criteria, as described above.

Our results indicated that HCV antibody positivity was a significant pretreatment predictor of early response, TTP, and survival of patients with advanced HCC treated with 5-FU/IFN. On the other hand, PVTT grade and total bilirubin levels were not significant predictors. Though we established no eligibility criterion regarding serum total bilirubin levels, the median total bilirubin level was 1.1 mg/dl (range, 0.4–6.4). Therefore, total bilirubin levels may not be statistically significant predictors in this study. In this study, three patients had serum total bilirubin levels >3 mg/dl. These patient achieved PR, SD, and PD. Though the three patients with high bilirubinemia (≥3 mg/dl) were safely treated in this study, we think that 5-FU/IFN combination therapy should be used with caution in patients with advanced HCC with high bilirubinemia. In general, the prognosis of HCC patients with Vp 3 or 4 is poorer than those with Vp 0–2. In this study, we treated a heterogeneous group of patients with advanced HCC as described above. Therefore, HCC with Vp 0–2 cases were advanced HCC cases in this study. All 55 patients were thought to have a poor prognosis at the time of enrollment in this study. Achievement of a good early response is important for good survival. A study with larger sample size may show the importance of PVTT grade and total bilirubin level.

Obi et al.[17] also reported that positivity to HCV antibody might be a predictor of CR in patients with advanced HCC treated with 5-FU/IFN. Why is HCV antibody positivity a predictor of the efficacy of combination therapy? One reason may be the underlying mechanisms associated with hepatocarcinogenesis. In our study, 36 patients were infected with HCV, 15 with hepatitis B virus (HBV), and four with non-B non-C hepatitis. Although the probability of hepatocarcinogenesis is high for both HBV and HCV infections, some differences have been noted with regard to their relationship with HCC.[24,25] HCV is an RNA virus, and viral genes are not integrated into the host genome. On the other hand, HBV is a DNA virus with reverse-transcriptase activity. HBV-mediated hepatocarcinogenesis is reported to be associated with the integration of viral DNA into the host genome.[26–28] The integration of the HBV genome into the host genome may diminish the effect of intraarterial 5-FU/IFN combination therapy. A second reason may be the differentiation of the cytokine pattern in HBV and HCV hepatitis.[29] Falasca et al.[29] reported the presence of high levels of Th1 cytokines, particularly during the course of chronic hepatitis B. They also reported that interleukin (IL)-18 and IL-6 levels might play important roles in both inflammation and hepatic injury, particularly during the course of hepatitis C infection. IFN may play a different role in patients with advanced HCC associated with HBV or HCV. In this study, the efficacy of the

combination therapy for advanced HCC patients with non-B non-C hepatitis was not clear because of the small number ($n = 4$) of those patients.

The DO proportion was high in this study (20%). Two major reasons for DO were infection around the catheter and stenosis of the hepatic artery. In this study, we established no eligibility criterion regarding the hepatic reserve function, including serum total bilirubin. Poor hepatic reserve function and high bilirubinemia might affect infection around the catheter. On the other hand, previous treatment with TACE might injury the hepatic artery and affect hepatic artery stenosis.

In conclusion, HCV antibody positivity might be a pretreatment predictive factor for early response, TTP, and survival of patients with advanced HCC treated with intraarterial 5-FU/IFN combination therapy. Early response to the combination therapy might be a significant posttreatment predictor of survival. Thus, patients who do not achieve CR or PR during the early phase of combination therapy should be switched to another treatment modality. Our results also showed that PVTT grade does not seem to be an important factor in the prognosis of patients with advanced HCC treated with 5-FU/IFN combination therapy. Further studies with long-term follow-up and a larger sample size are needed.

References

1. Kobayashi M, Ikeda K, Hosaka T, Sezaki H, Someya T, Akuta N, et al. Natural history of compensated cirrhosis in the Child-Pugh class A compared between 490 patients with hepatitis C and 167 with B virus infections. J Med Virol 2006;78:459–65.
2. Okuda K, Fujimoto I, Hanai A, Urano Y. Changing incidence of hepatocellular carcinoma in Japan. Cancer Res 1987;47:4967–72.
3. Health and Welfare Statistics Association. Health and welfare statistics. J Health Welfare Stat 2000;47:421.
4. Cancer of the Liver Italian Program (CLIP) investigators. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients. Hepatology 1998;28:751–5.
5. Llovet JM, Bustamante J, Castells A, Vilana R, Ayuso Mdel C, Sala M, et al. Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. Hepatology 1999;29:62–7.
6. Uka K, Aikata H, Takaki S, Shirakawa H, Jeong SC, Yamashina K, et al. Clinical features and prognosis of patients with extrahepatic metastases from hepatocellular carcinoma. World J Gastroenterol 2007;13:414–20.
7. Kamada K, Kitamoto M, Aikata H, Kawakami Y, Kono H, Imamura M, et al. Combination of transcatheter arterial chemoembolization using cisplatin-lipiodol suspension and percutaneous ethanol injection for treatment of advanced small hepatocellular carcinoma. Am J Surg 2002;184:284–90.
8. Friedman M. Primary hepatocellular cancer-present results and future prospects. Int J Radiat Oncol Biol Phys 1983;9:1841–50.
9. Stehlin JS Jr, de Ipolyi PD, Greeff PJ, McGaff CJ Jr, Davis BR, McNary L. Treatment of cancer of the liver. Twenty years' experience with infusion and resection in 414 patients. Ann Surg 1988;208:23–35.
10. Doci R, Bignami P, Bozzetti F, Bonfanti G, Audisio R, Colombo M, et al. Intrahepatic chemotherapy for unresectable hepatocellular carcinoma. Cancer 1988;61:1983–7.
11. Ando E, Yamashita F, Tanaka M, Tanikawa K. A novel chemotherapy for advanced hepatocellular carcinoma with tumor thrombosis of the main trunk of the portal vein. Cancer 1997;79:1890–6.
12. Ando E, Tanaka M, Yamashita F, Kuromatsu R, Yutani S, Fukumori K, et al. Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma with portal vein tumor thrombosis: analysis of 48 cases. Cancer 2002;95:588–95.
13. Lai YC, Shih CY, Jeng CM, Yang SS, Hu JT, Sung YC, et al. Hepatic arterial infusion chemotherapy for hepatocellular carcinoma with portal vein tumor thrombosis. World J Gastroenterol 2003;9:2666–70.
14. Urabe T, Kaneko S, Matsushita E, Unoura M, Kobayashi K. Clinical pilot study of intrahepatic arterial chemotherapy with methotrexate, 5-fluorouracil, cisplatin, and subcutaneous interferon-alpha-2b for patients with locally advanced hepatocellular carcinoma. Oncology 1998;55:39–47.
15. Sakon M, Nagano H, Dono K, Nakamori S, Umeshita K, Yamada A, et al. Combined intraarterial 5-fluorouracil and subcutaneous interferon-alpha therapy for advanced hepatocellular carcinoma with tumor thrombi in the major portal branches. Cancer 2002;94:435–42.
16. Ota H, Nagano H, Sakon M, Eguchi H, Kondo M, Yamamoto T, et al. Treatment of hepatocellular carcinoma with major portal vein thrombosis by combined therapy with subcutaneous interferon-α and intra-arterial 5-fluorouracil: role of type I interferon receptor expression. Br J Cancer 2005;93:557–64.
17. Obi S, Yoshida H, Toune R, Unuma T, Kanda M, Sato S, et al. Combination therapy of intraarterial 5-fluorouracil and systemic interferon-alpha for advanced hepatocellular carcinoma with portal venous invasion. Cancer 2006;106:1990–7.
18. Liver Cancer Study Group of Japan. The general rules for the clinical and pathological study of primary liver cancer (in Japanese). 4th ed. Tokyo: Kanehara; 2000. p. 19.
19. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649–55.
20. Uka K, Aikata H, Takaki S, Miki D, Jeong SC, Hiramatsu A, et al. Similar effects of recombinant IFN alpha-2b and natural IFN alpha when combined with intraarterial 5-fluorouracil for the treatment of advanced HCC. Liver Int (in press).
21. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors (RECIST) guidelines. J Natl Cancer Inst 2000;92:205–16.
22. NCI Common Toxicity Criteria. http://ctep.cancer.gov/reporting/ctc.html
23. Lee HS, Kim JS, Choi IJ, Chung JW, Park JH, Kim CY. The safety and efficacy of transcatheter arterial chemoembolization in the treatment of patients with hepatocellular carcinoma and main portal vein obstruction: a prospective controlled study. Cancer 1997;79:2087–94.
24. Ohishi W, Kitamoto M, Aikata H, Kamada K, Kawakami Y, Ishihara H, et al. Impact of aging on the development of hepatocellular carcinoma in patients with hepatitis C virus infection in Japan. Scand J Gastroenterol 2003;38:894–900.
25. Kiyosawa K, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Gad A, et al. Hepatocellular carcinoma: recent trends in Japan. Gastroenterology 2004;127(5 Suppl 1):S17–26.
26. Buendia MA. Hepatitis B viruses and cancerogenesis. Biomed Pharmacother 1998;52:34–43.
27. Robinson WS. Molecular events in the pathogenesis of hepadnavirus-associated hepatocellular carcinoma. Annu Rev Med 1994;45:297–323.

28. Someya T, Ikeda K, Saitoh S, Kobayashi M, Hosaka T, Sezaki H, et al. Interferon lowers tumor recurrence rate after surgical resection or ablation of hepatocellular carcinoma: a pilot study of patients with hepatitis B virus-related cirrhosis. J Gastroenterol 2006;41:1206–13.

29. Falasca K, Ucciferri C, Dalessandro M, Zingariello P, Mancino P, Petrarca C, et al. Cytokine patterns correlate with liver damage in patients with chronic hepatitis B and C. Ann Clin Lab Sci 2006;36:144–50.

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CLINICAL STUDIES

Similar effects of recombinant interferon-α-2b and natural interferon-α when combined with intra-arterial 5-fluorouracil for the treatment of advanced hepatocellular carcinoma

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Abstract

Aim: Intra-arterial 5-fluorouracil (5-FU) plus interferon (IFN) combination therapy is effective against advanced hepatocellular carcinoma (HCC) with portal vein tumour thrombosis. In this study, we compared the efficiency and safety of recombinant IFN-α-2b with natural IFN-α as components of the combination therapy. *Methods:* Consecutive HCC patients ($n = 31$) with portal vein tumour thrombosis were enrolled in this prospective study. They received combination therapy of 5-FU and either recombinant IFN-α-2b (R group, $n = 15$) or natural IFN-α (N group, $n = 16$). We compared the two groups for the early response rate, adverse reactions, time to progression (TTP) and survival rates. In addition, we assessed the cost-effectiveness of each protocol. *Results:* The early response rate (R: 26.7%, N: 31.2%), median TTP (R: 5.8 months, N: 5.6 months) and median survival time (R: 7.5 months, N: 6.5 months) were not significantly different between the R and N groups. There were no differences in adverse reactions between the two groups. The estimated cost-effectiveness ratio of recombinant IFN-α-2b was better than natural IFN-α. *Conclusions:* In our protocol of combination therapy, there were no significant differences between recombinant IFN-α-2b and natural IFN-α with regard to early response to therapy, adverse effects, TTP and survival rates. 5-FU could be combined with either recombinant IFN-α-2b or natural IFN-α, although the cost-effectiveness of the former warrants its use clinically.

Hepatocellular carcinoma (HCC) is one of the most common neoplasms in Africa and Asia including Japan, and HCC-related deaths are increasing worldwide including Japan (1–3). Despite the progress in diagnostic techniques and therapeutic procedures, such as ultrasonography, computed tomography, magnetic resonance imaging, angiography, surgical resection, radiofrequency ablation (RFA), percutaneous ethanol injection (PEI) and transcatheter arterial chemoembolization (TACE), the prognosis of patients with HCC remains unsatisfactory. Furthermore, the survival rates of patients with advanced HCC and complications such as portal vein tumour thrombosis (PVTT) or distant metastasis remain extremely poor (4–8). PVTT frequently develops in HCC patients. In HCC patients with PVTT, tumour cells may spread out through the portal tract, resulting in intra-/extrahepatic metastases. Furthermore, portal vein occlusion may cause liver failure, ascites or variceal rupture. Thus, the performance status (PS) of HCC patients with PVTT gradually worsens, rendering them unsuitable for any treatment of HCC.

Advances in implantable drug-delivery systems have made it possible to administer repeated arterial infusions of anticancer agents. Recent studies reported the effectiveness of combination therapy of intra-arterial 5-fluorouracil (5-FU) and subcutaneous interferon (IFN)-α therapy for advanced HCC (9–11), with a response rate ranging from 47 to 73%. The majority of these studies used natural IFN-α. To our knowledge, there are no reports that have compared the effects of combination therapy of intra-arterial 5-FU and IFN

Table 1. Clinical profiles of the 31 patients with hepatocellular carcinoma

	Recombinant IFN-α-2b	Natural IFN-α	*P* value
N	15	16	
Age (years)	59 (26–79)	65(52–76)	NS
Sex (male/female)	12/3	14/2	NS
Grade of portal vein invasion (Vp 2/3/4)	2/3/10	1/8/7	NS
Main tumour size (mm)	52 (15–160)	57(25–140)	NS
Tumour volume (< 50%/≥ 50%)	9/6	10/6	NS
Child–Pugh grade (A/B/C)	10/5/0	13/3/0	NS
AFP (ng/mL)	3922.7 (51.2–708 100)	1957 (14.3–377 700)	NS
DCP (mAU/mL)	17 874 (< 10 – 233 450)	11 476 (46–722 140)	NS
Aetiology (HBV/HCV/others)	5/7/3	4/11/1	NS
Leucocyte count (/μL)	5790 (2210–8940)	6255 (2890–8910)	NS
Neutrophil count (/μL)	3395 (1260–6884)	4536 (1531–7008)	NS
Haemoglobin (g/dL)	12.5 (8.4–16.4)	12.9 (8.6–16.2)	NS
Platelet count (/μL) × 10^4	14.2 (5.1–54.4)	12.1 (5.9–34.2)	NS
Total bilirubin (mg/dL)	1.3 (0.6–2.7)	1.3 (0.7–2.8)	NS

Data are expressed as median values with ranges in parentheses, or number of patients. Portal invasion (Vp1, tumour thrombus in a third or more of the peripheral branches; Vp2, in the second branch; Vp3, in the first branch; Vp4, in the trunk).
AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; HBV, hepatitis B virus; HCV, hepatitis C virus; IFN, interferon; NS, not significant.

with those of recombinant IFN-α-2b and natural IFN-α. Such studies are important because the activity of recombinant IFN-α-2b differs from that of natural IFN (12, 13), and the former is less expensive than natural IFN-α. Thus, if the safety and efficacy of recombinant IFN-α-2b is equal to or better than that of natural IFN-α, recombinant IFN-α-2b may be recommended with regard to cost-effectiveness. In the present prospective study, we investigated the safety of intra-arterial 5-FU and IFN and compared recombinant IFN-α-2b with natural IFN-α. In addition, we assessed the cost-effectiveness of each treatment regimen.

Materials and methods

Study design and eligibility

This was a prospective study conducted at our hospital to compare the outcome of recombinant IFN-α-2b and natural IFN-α in combination with intra-arterial 5-FU. The eligibility criteria were as follows: age (18–80 years), Child–Pugh status A or B, leucocyte count > 2000/μL, neutrophil count > 1200/μL, haemoglobin > 8 g/dL, platelet count > 50 000/μL, total bilirubin < 3.0 mg/dL, serum creatinine < 1.5 mg/dL, unresectable or not suitable for local ablation therapy, main tumour size > 20 mm, tumour number > 2, presence of PVTT (in the second branch first branch or trunk), an Eastern Cooperative Oncology Group PS of 0–1 (14) and without extrahepatic metastases. All patients were asked to give their written informed consent to this study, which was approved by the Institutional Review Board of Hiroshima University.

From June 2003 to December 2006, 265 consecutive patients with unresectable HCC were admitted to our hospital. As a result of the progression of HCC (e.g. PVTT, extrahepatic metastases), these patients were not suitable candidates for either surgical resection or local ablation therapy, including RFA and PEI. Of the 265 patients with advanced HCC, 39 were considered to be suitable candidates for the intra-arterial 5-FU and IFN combination therapy. Eight patients could not be included because of refusal of enrolment. Thus, 31 patients with advanced HCC without extrahepatic metastases were enrolled in this prospective study. We treated the first 15 consecutive patients with recombinant IFN-α-2b (R group) and the second 16 consecutive patients with natural IFN-α (N group), combined with 5-FU.

Table 1 lists the baseline characteristics of the patients of the two groups (R group vs. N group). There were no differences between the two groups with respect to the sex ratio, age, proportion of patients with hepatitis B virus and hepatitis C virus infection, PVTT in the second branch (Vp2), major branch (Vp 3) and main trunk (Vp 4), median level of α-fetoprotein and des-γ-carboxy prothrombin, median leucocyte count, neutrophil count, haemoglobin, platelet count, total bilirubin and duration of the observation period [R: 10.5 (0.7–39.4 months), N: 6.3 (1.1–16.7 months)].

Treatment protocol

Patients received repeated arterial infusions of anticancer agents via an injection port. One course of

chemotherapy represented 4 weeks. 5-FU (500 mg/body weight/day; Kyowa Hakko, Tokyo, Japan) was administered within 5 h using a mechanical infusion pump on days 1–5 of the first and second weeks (5 g in one course). Recombinant IFN-α-2b (Intron A®; Schering-Plough Pharmaceuticals Co., Osaka, Japan) at 3×10^6 U (3 MU), or natural IFN-α (OIF®; Otsuka Pharmaceuticals Co., Tokyo, Japan) at 5×10^6 U (5 MU) was administered intramuscularly on days 1, 3 and 5 of each week (total dose of 36 and 60 MU respectively). In our hospitals, the minimum dose of recombinant IFN-α-2b for the treatment of chronic hepatitis C is 3×10^6 U and that of natural IFN-α is 5×10^6 U. Previous reports used 5×10^6 U as the minimum dose of natural IFN-α. With regard to recombinant IFN-α-2, we selected the above dose as the minimum dose in order to avoid potential adverse effects. In principle, treatment was repeated several courses unless PS changed to 3 or 4 during the treatment. A 2–4-week rest period of no treatment was allowed after each treatment course.

Implantation of arterial catheter

The catheter was inserted through the right femoral artery using the Seldinger method. After the detection of HCC, a 3-French heparin-coated catheter (Clinical Supply, Gifu, Japan) was inserted and its tip was advanced to the common hepatic artery or proper hepatic artery. The other end of the catheter was connected to the injection port, which was implanted in a subcutaneous pocket created in the right lower abdominal quadrant. The gastroduodenal and right gastric arteries were occluded using steel coils to prevent potential gastroduodenal injury from the anticancer agents.

Evaluation

The response to treatment was assessed in all patients enrolled in this study. The response was defined according to the criteria of the Response Evaluation Criteria in Solid Tumors (RECIST) (15). A complete response (CR) was defined as the complete disappearance of all target lesions. A partial response (PR) was defined as at least a 30% decrease in the sum of the longest diameter (LD) of target lesions with the baseline sum of the LD of target lesions as the reference. Progressive disease (PD) was defined as at least a 20% increase in the sum of the LD of target lesions. Stable disease (SD) was defined as neither PR nor PD criteria fulfilled. The duration of response was measured from the date of the start of treatment to the date of documented progression.

Adverse reactions were assessed every week during the treatment using the National Cancer Institute Common Toxicity Criteria (NCI-CTC) (version 3.0) (16).

Cost-effectiveness

The cost of each IFN with two courses of treatment was calculated. The effectiveness of treatment was reflected by the percentages of patients who achieved CR or PR. The cost-effectiveness ratio was calculated using the formula: cost/effectiveness.

Additional therapy

After two courses of the combination therapy, we assessed the response to therapy in all patients. According to the response, we provided various additional therapies such as RFA, TACE and radiotherapy (RT) for patients treated with the combination therapy. Patients assessed as PR continued to receive the combination therapy, in addition to local ablation therapy when further decrease of HCC was not expected. All patients assessed as SD or PD received RT for PVTT. Furthermore, one session of TACE using a cisplatin–lipiodol suspension was repeated before the initiation of each course of intra-arterial 5-FU and IFN combination therapy unless the Child–Pugh status changed to C.

Statistical analysis

Statistical analysis was performed on 31 December 2006. Differences between groups were examined for statistical significance using the Mann–Whitney test (*U*-test) and χ^2 test where appropriate. Cumulative survival rate and time to progression (TTP) were assessed by the Kaplan–Meier life-table method, and differences were evaluated by the log-rank test. All analyses described above were performed using the SPSS program (version 11; SPSS Inc., Chicago, IL, USA).

Results

Early response rate

We assessed all patients after two courses of treatment. Of all 31 patients, one (3.2%), eight (25.8%), eight (25.8%), 10 (32.3%) and four (12.9%) patients showed CR, PR, SD, PD and drop-out (DO) respectively (Table 2). For the R group, zero (0%), four (26.7%), three (20%), five (33.3%) and three (20%) patients showed CR, PR, SD, PD and DO respectively. The reasons for DO were confusion (one patient), refusal after initiation of therapy (one patient) and exanthema (one patient). The overall response rate

Table 2. Response to treatment after two courses

	CR	PR	SD	PD	DO	Response rate*
Recombinant IFN-α-2b ($n = 15$)	0	4	3	5	3	26.7%
Natural IFN-α ($n = 16$)	1	4	5	5	1	31.2%

*Response rate = CR+PR/CR+PR+SD+PD.
CR, complete response; DO, drop out; IFN, interferon; PD, progressive disease; PR, partial response; SD, stable disease.

was 26.7% for the R group. For the N group, one (6.3%), four (25%), five (31.2%), five (31.2%) and one (6.3%) patients showed CR, PR, SD, PD and DO. The reason for DO was infection around the catheter (one patient). The overall response rate for the N group was 31.2%. There was no statistically significant difference in the early response between the two groups.

Adverse reactions and complications

Table 3 summarizes the adverse reactions and complications encountered during and after the treatment. The most common adverse reactions were fever, nausea and loss of appetite, but these were mostly NCI-CTC Grade 1 or 2. The percentages of patients with various NCI-CTC Grade 3 adverse reactions of the two treatment groups were not significantly different. With regard to the two patients of the R group with leucopenia, the initial leucocyte counts were 2210 and 3980/μL and the lowest counts were 1214 and 1524/μL respectively. As for the four patients with thrombocytopenia, the initial platelet counts were 61 000, 114 000, 185 000 and 227 000/μL, and the lowest counts were 31 000, 48 000, 45 000 and 37 000/μL respectively. None required administration of granulocyte-colony-stimulating factor (G-CSF) or blood transfusion, and none developed depression. Complications associated with the indwelling catheter were infection (one patient each).

Additional therapy after two courses of combination treatment

For the R group, four patients received TACE, one patient received RT and two patients received both TACE and RT. In the four patients who achieved PR, three continued to receive the combination therapy while the fourth achieved CR after completing four courses of the combination therapy, and undergoing RFA for the remaining HCC. With regard to the N group, four patients received TACE, two patients underwent RT and one patient received both TACE and RT. With regard to the four patients who achieved PR, three continued the combination therapy, while the fourth patient achieved CR after receiving three courses of the combination therapy, followed by RT for PVTT.

Table 3. Adverse reactions (National Cancer Institute Common Toxicity Criteria Grade 3) and complications during and after the combination treatment

	Recombinant IFN-α-2b	Natural IFN-α	*P* value
Leucopenia	2 (13.3%)	0	NS
Thrombocytopenia	1 (6.7%)	3 (18.8%)	NS
Nausea	1 (6.7%)	0	NS
Exanthema	1 (6.7%)	0	NS
Confusion	1 (6.7%)	0	NS
Infection around the catheter	1 (6.7%)	1 (6.3%)	NS
Pseudoaneurysm of the femoral artery	1 (6.7%)	1 (6.3%)	NS

IFN, interferon; NS, not significant.

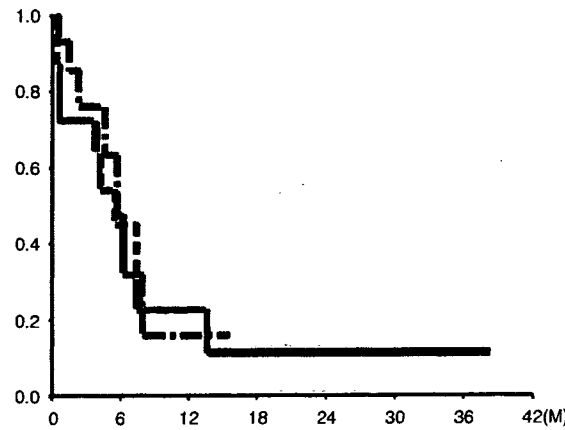

Fig. 1. Comparison of the time to progression of patients with hepatocellular carcinoma complicated with portal vein tumour thrombosis and treated with 5-fluorouracil (5-FU) and recombinant interferon (IFN)-α-2b (solid line) or with 5-FU combined with natural IFN-α (log-rank test: not significant).

Time to progression

The median TTP for all 31 patients was 5.8 months [95% confidence interval (CI), 3.9–7.7 months]. For the R and N groups, the median TTP was 5.6 months (95% CI, 3.0–8.2 months) and 5.8 months (95% CI,

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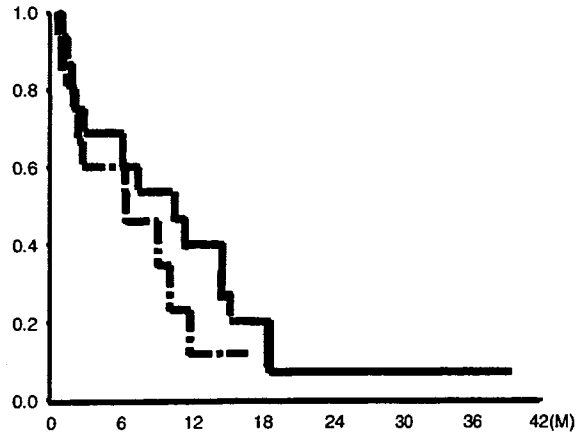

Fig. 2. Comparison of the survival rate of patients with hepatocellular carcinoma complicated with portal vein tumour thrombosis and treated with 5-fluorouracil (5-FU) and recombinant interferon (IFN)-α-2b (solid line) or with 5-FU combined with natural IFN-α (log-rank test: not significant).

4.0–7.6 months) respectively. There was no significant difference in TTP between the two groups (Fig. 1).

Survival rates

Of all 31 patients, the median survival time was 7.5 months (95% CI, 3.3–11.7 months) and the cumulative survival rates at 6, 12, 18 and 24 months were 64.5, 29.0, 16.5 and 5.6% respectively. The median survival time of the R group [10.5 months (95% CI, 0.0–21.4 months)] was not significantly different from that of the N group [6.5 months (95% CI, 3.7–9.3 months)] (Fig. 2).

Cost-effectiveness

The cost of IFN with two course of treatment was $US1052 for the R group and $US3060 for the N group. Four (26.7%) patients of the R group showed PR, while one (6.3%) and four (25%) patients of the N group showed CR and PR respectively. Thus, the estimated effectiveness was 26.7 for the R group and 31.3 for the N group. The cost-effectiveness ratios were 39.4 for the R group and 97.8 for the N groups, indicating that the R combination therapy was about three times more cost-efficient than the N combination therapy (Table 4).

Causes of death

Seven patients were still alive at the end of the observation period while 24 patients had died. All the 24 patients died of cancer-related disease.

Table 4. Cost-effectiveness of each interferon with two courses of the treatment

	Cost ($)	Effectiveness* (%)	Cost-effectiveness ratio†
Recombinant IFN-α-2b	1052	26.7	39.4
Natural IFN-α	3060	31.3	97.8

*Effectiveness; percentage of patients who showed CR or PR.
†Cost-effectiveness ratio = cost/effectiveness.
CR, complete response; IFN, interferon; PR, partial response.

Discussion

The prognosis of patients with advanced HCC complicated with PVTT remains poor, particularly in those patients with PVTT in the first branches or the portal trunk. The median survival time of HCC patients with PVTT in the portal trunk is reported to be about 90 days with supportive care (17). In this regard, Patt *et al.* (18) reported the efficiency of intravenous 5-FU combined with recombinant IFN-α-2b. Recently, several studies assessed the efficacy of combination therapy of intra-arterial 5-FU and IFN (9–11). However, most of the reports that analysed the effects of combination therapy of intra-arterial 5-FU and IFN used natural IFN-α. The use of recombinant IFN-α-2b has been reported in only one study (9). The response rates (CR and PR patients/all patients) reported in the two studies by Sakon *et al.* (10) and Obi *et al.* (11) were 73% (8/11 patients) and 52.6% (61/116 patients) respectively. In this study, for patients of the two groups, the objective response rate according to the early response was 29% (9/31 patients). The discrepancy between the studies may be because of the following reasons. First, the early response to our protocol was assessed after two courses of the treatment, while others evaluated the maximum response. Second, the method of evaluation of the response was different. The above two studies used the Eastern Cooperative Oncology Group criteria but we used the RECIST criteria. Third, the sample size was very small in the report by Sakon *et al.* (10). In our study, the survival rates of the patients of the two groups were almost identical to those reported by Obi *et al.* (11) (the survival rates at 6 and 12 months were 53 and 34% respectively). Thus, the protocol used in our study was considered to be suitable for patients with advanced HCC.

Both IFN-α and IFN-β induce the transcription of the p53 gene and contribute in boosting the responses to p53 activation, which suppresses cancer (19). IFN-α is also known to inhibit cancer cells directly as well as indirectly (20–26) and to have anti-angiogenic and