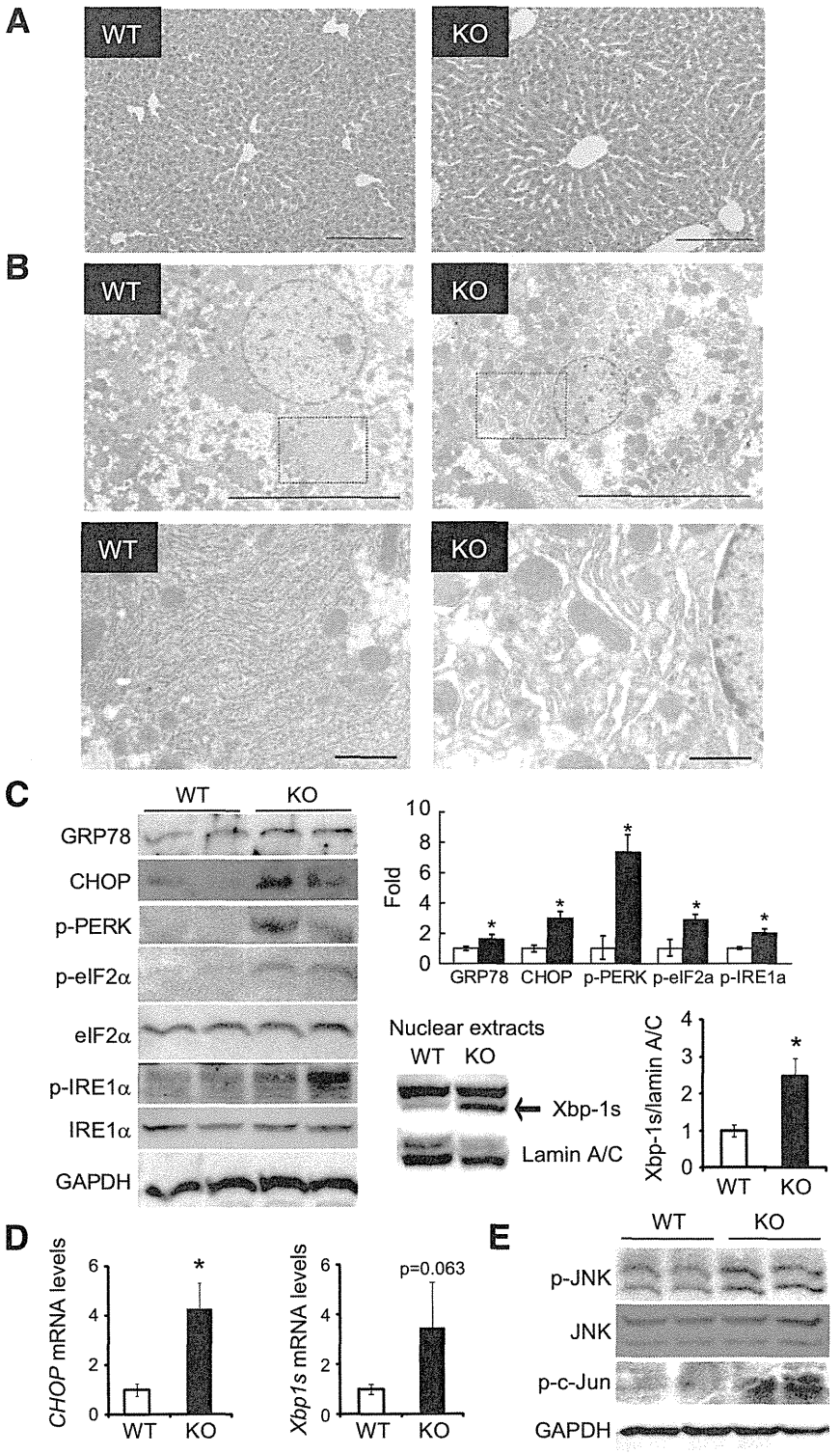

FIG. 5. Deletion of PA28 genes causes ER stress in the liver. *A*: Hematoxylin-eosin–stained liver sections from PA28 KO and WT mice. Scale bar, 200 μm. *B*: Electron microscopic analyses of the ER in livers from WT and PA28 KO mice. The highlighted region of the *upper panel* is shown at a higher magnification in the *lower panel*. Scale bars: 10 μm (*upper panel*) and 1 μm (*lower panel*). *C*: Western blotting for the ER stress–associated markers GRP78, CHOP, p-PERK, p-eIF2α, and p-IRE1α in the livers of 12-week-old male WT and PA28 KO mice. *Upper right panel*: Each expression level was quantified ($n = 3$ per group). Data represent means ± SE. $^*P < 0.05$. *Lower right panel*: Western blotting for nuclear spliced form of XBP-1s protein amounts in the livers of 12-week-old male WT ($n = 5$) and PA28 KO ($n = 5$) mice. Lamin A/C served as internal control. Data represent means ± SE. $^*P < 0.05$. *D*: Expression of mRNAs encoding CHOP and XBP-1s in the livers of WT (□) and PA28 KO (■) mice. Expression values were normalized to *Actb* mRNA. Data represent means ± SE ($n = 4$–7 per group). $^*P < 0.05$. *E*: Western blotting for p-JNK, p–c-Jun, and total JNK in the livers of WT and PA28 KO mice.

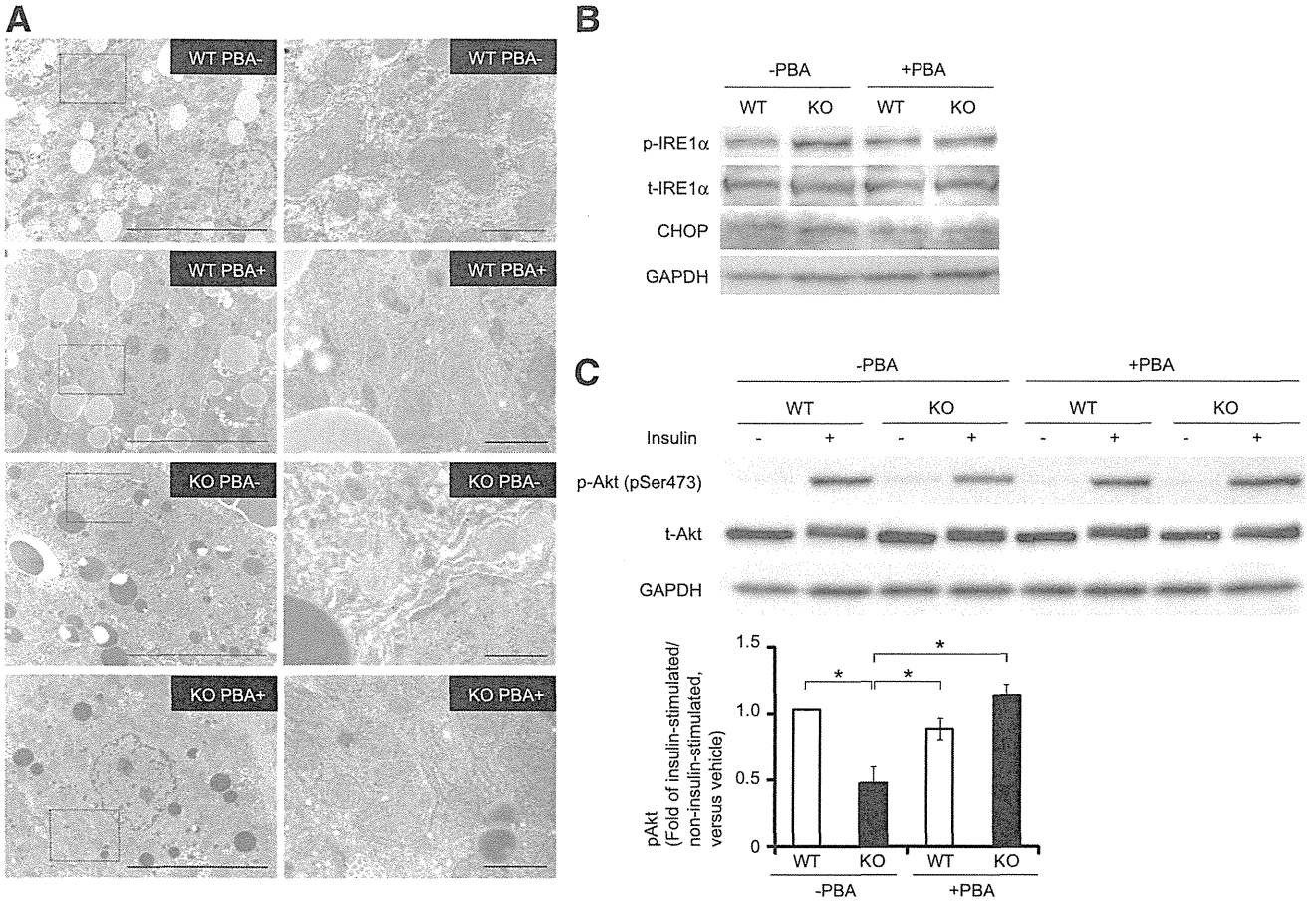

FIG. 6. Effects of a chemical chaperone, PBA, administration on proteasome dysfunction–induced ER stress and insulin resistance in PA28 KO mice. *A–C*: WT and PA28 KO mice were administered mixed PBA (4 mg/mL) through drinking water for 3 weeks. *A*: Electron microscopic analyses of the ER in livers from WT and PA28 KO mice administered orally with or without PBA. The highlighted region of the *left panel* (magnification ×5,000) is shown at a higher magnification in the *right panel* (magnification ×20,000). Scale bars: 10 μm (*left panel*) and 1 μm (*right panel*). *B*: IRE1α phosphorylation and CHOP levels in the livers of WT and PA28 KO mice. *C*: WT and PA28 KO mice fed STD were starved overnight and injected with insulin (10 IU/kg i.p.). Equal amounts of protein in total lysates of liver and muscle were immunoblotted with anti–p-Akt (Ser473) and anti-Akt antibodies. p-Akt values of insulin-injected fasted mice values were displayed relative to those of saline-injected mice. Data represent means ± SE (n = 3 per group). *P < 0.05. t-, total.

obesity and type 2 diabetes, accumulation of ubiquitinated proteins and damaged organelles, ER stress, JNK activation, and insulin resistance (Supplementary Fig. 2). A chemical chaperone PBA administration almost completely alleviated proteasome dysfunction–mediated insulin resistance, confirming the critical role of ER stress in the development of insulin resistance under proteasome dysfunction.

Accumulating evidence suggests that obesity promotes ER stress, which is detected as enhanced UPR signaling, that activates JNK and impairs insulin signaling at the level of IRSs in adipose tissue and the liver (2,4). However, the link between obesity and ER stress has remained unclear. We propose that obesity-associated proteasome dysfunction induces ER stress in the liver, as PA28 KO mice showed accumulation of polyubiquitinated proteins and massive expansion of the ER in the liver, probably due to a reduced capacity for proteasome-mediated degradation of ubiquitinated proteins. This is the first in vivo evidence of proteasome dysfunction–induced insulin resistance mediated by ER stress in the liver. Furthermore, we showed that the selective proteasome inhibitor bortezomib increases ER stress and thereby activates JNK in a cultured hepatocyte cell line.

Yang et al. (38) recently reported that hepatic autophagy is downregulated in the livers of *ob/ob* mice and that defective autophagy in Atg7 KO mice causes ER stress and hepatic insulin resistance. Therefore, it is possible that both proteasome-mediated protein degradation and autophagy-mediated protein degradation are impaired in the livers of obese individuals, further exacerbating ER stress.

Proteasome function seems to be altered differently in different tissues. Streptozotocin-induced hyperglycemia impairs proteasome activity in the liver and kidney (39,40), whereas proteasome activity is enhanced in the wasted muscle of obese diabetic *db/db* mice (41). Taken together, these results indicate that obesity predominantly induces proteasome dysfunction in the liver. This clarifies the previous finding that ER stress causes insulin resistance in the liver together with the adipose tissue (2) and brain (42,43). Mechanisms underlying enhanced insulin sensitivity in the skeletal muscle of PA28 KO mice should be investigated in the future.

Why obesity impairs proteasome function in the liver remains unclear. One possible link may be overnutrition-induced oxidative stress. Visceral adiposity in obesity causes excessive flux of free fatty acids into the liver via

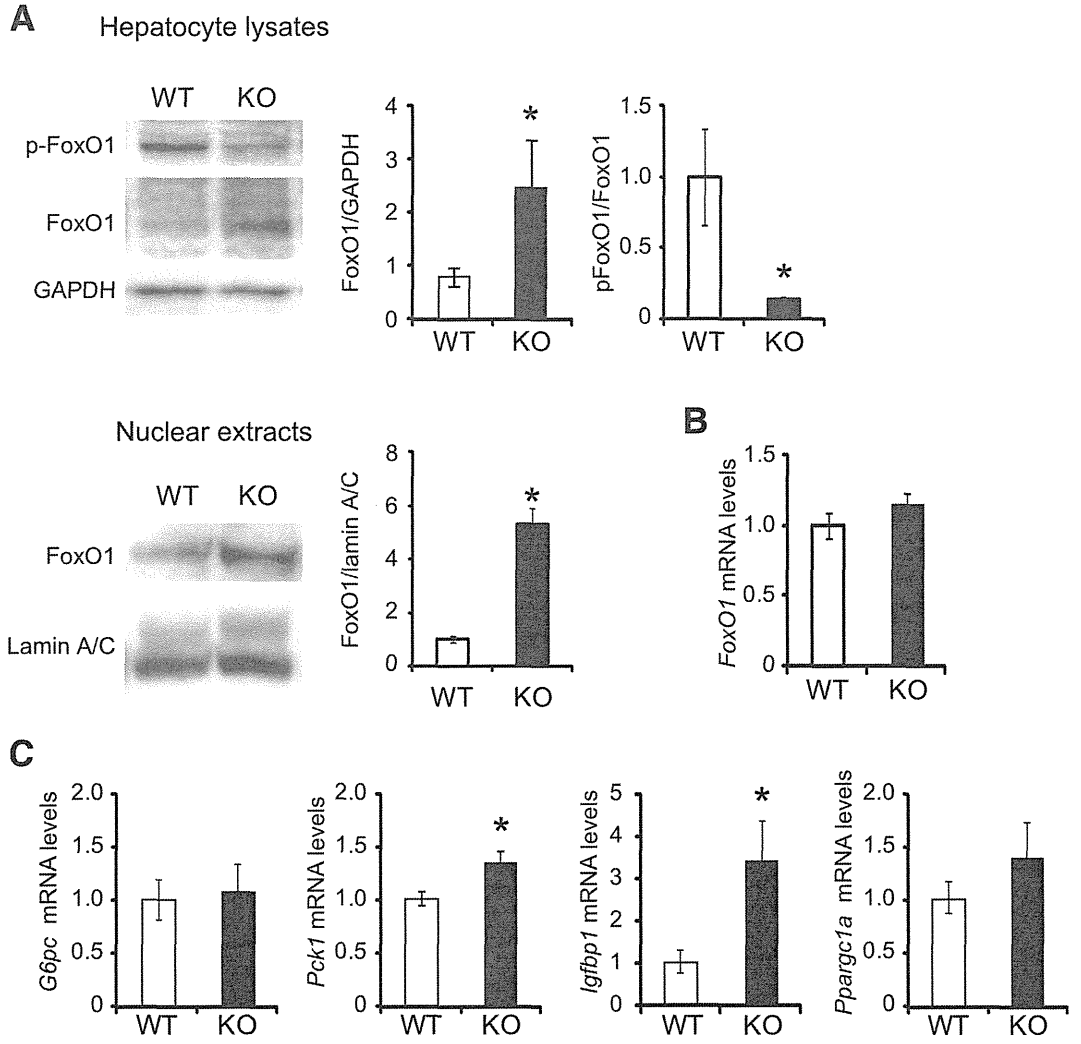

FIG. 7. Proteasome dysfunction upregulates FoxO1 protein amounts and gluconeogenic gene expression in the liver of PA28 KO mice. *A*: **Total liver extracts and nuclear fractions from the livers of WT and PA28 KO mice were analyzed by Western blotting for phosphorylated and total FoxO1. GAPDH served as internal control. Data represent means ± SE (n = 4–5 per group). *P < 0.05. Nuclear fractions from in the livers of WT and PA28 KO mice were analyzed by Western blotting for total FoxO1. Lamin A/C GAPDH served as internal control. Data represent means ± SE (n = 5 per group). *P < 0.05.** *B* **and** *C*: **Relative mRNA levels of *FoxO1*, *G6pc*, *Pck1*, *Igfbp1*, and *Ppargc1a* in the liver of WT and PA28 KO mice were analyzed by RT-PCR. Data were normalized according to GAPDH levels. Data represent means ± SE (n = 7 per group). *P < 0.05.**

the portal vein, resulting in oxidative stress in the liver (44). The saturated fatty acid palmitate induces excessive production of reactive oxygen species in mitochondria, activates JNK, and causes insulin resistance at the level of IRSs in hepatocytes (44,45). In addition, genes involved in oxidative phosphorylation are upregulated in parallel with insulin resistance in patients with type 2 diabetes who are obese compared with those who are not obese (5,16). Severe oxidative stress causes the covalent modification of 20S proteasome subunits, thereby reducing proteasome activity in the liver and in cultured hepatocytes (15). On the other hand, PA28α overexpression protects against oxidative stress in cultured cardiomyocytes, likely through enhancing the removal of oxidized proteins (46). PA28α and PA28β proteins interact with each other. The degradation rate of PA28β was also significantly decreased by PA28α overexpression in cultured cardiomyocytes (46). These findings suggest that obesity-associated mitochondrial reactive oxygen species and oxidative stress may impair proteasome function. Interestingly, expression of genes involved in the proteasome pathway, including PA28 genes, is increased in the liver tissues of obese mice compared with controls. A recent report compellingly demonstrated that upregulation of the immunoproteasome by interferons not only plays a previously recognized role in helping antigen presentation but also facilitates the removal of damaged proteins generated by interferon-induced oxidative stress (47). We speculate that these are compensatory mechanisms because the hybrid proteasome is better equipped to degrade misfolded proteins than is the conventional 26S proteasome. It was previously shown that the association of the 11S, also known as PA28 or REG, increases the peptidase activities of the 20S (48). PA28 KO mice may therefore be a suitable model for the development of therapies for proteasome dysfunction–associated diseases and metabolic abnormalities.

In the current study, PA28 KO mice showed hepatic steatosis associated with upregulated *Srebf1* and *Acc1* and increased cleaved/active SREBP-1c (Supplementary Fig. 2). There may be a cross-talk between ER stress pathways

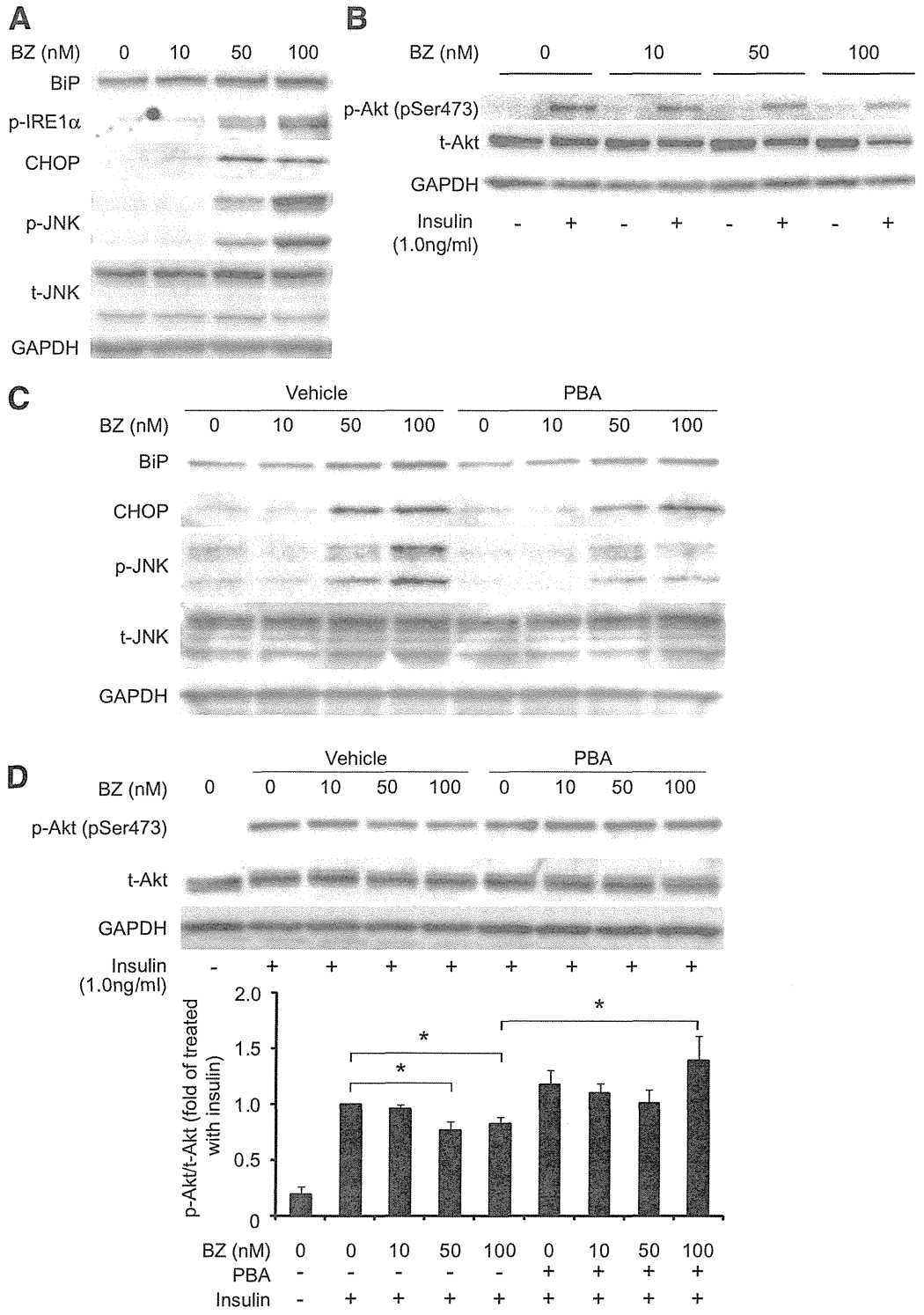

FIG. 8. The proteasome inhibitor bortezomib (BZ) induces ER stress and insulin resistance in H4IIEC3 cells. *A* and *B*: H4IIEC3 cells were treated with the indicated concentrations of bortezomib (in DMEM supplemented with 10% FBS) for 24 h. After washing, cells were serum starved for 16 h and then treated with insulin (1 nmol/L) or phosphate-buffered saline for 15 min. Cells were solubilized, and equal amounts of proteins were analyzed by Western blotting using BiP-, p-IRE1α–, CHOP-, p-JNK–, total JNK (*t*-JNK)-, p-Akt–, and total Akt (*t*-Akt)-specific antibodies. *C* and *D*: H4IIEC3 cells were pretreated or not for 24 h with 2 mmol/L PBA and then treated with the indicated concentrations of bortezomib (in DMEM supplemented with 10% FBS) for 24 h. Cells were washed, serum starved for 16 h, and treated with insulin (1 nmol/L) or phosphate-buffered saline for 15 min. Cells were solubilized, and equal amounts of proteins were analyzed by Western blotting using BiP-, CHOP-, p-JNK–, total JNK–, p-Akt–, and total Akt–specific antibodies. Blots of p-Akt were quantitated densitometrically and expressed as ratios to total Akt (n = 4 for each condition). Relative density is mean ± SE fold increase over control. *P = 0.05 vs. treatment with insulin alone.

and hepatic lipogenesis. Lee et al. (49) reported that the IRE1/XBP1 pathway induces expression of critical genes involved in fatty acid synthesis, such as *Acc1*. In addition, the PERK/eIF2α pathway decreases Insig1 protein translation, which increases cleaved/active SREBP-1c (50). In concert with these reports, we observed enhanced phosphorylation of IRE1α, PERK, and eIF2α and increased protein level of XBP-1s in the liver of PA28 KO mice. Indeed, our results are compatible with a model in which the ubiquitin-proteasome system degrades the amount of the endogenous nuclear SREBPs but not the precursors (51).

XBP-1s directly binds FoxO1 and promotes its protein degradation via the proteasome (52). In the current study, XBP-1s protein was increased in the liver of PA28 KO mice, probably owing to increased phosphorylation of IRE1α, an endonuclease for *XBP1* gene. Even in such condition, FoxO1 protein amounts dramatically increased in total cell lysates as well as in cytoplasmic and nuclear fractions, probably owing to proteasome dysfunction in the liver of PA28 KO mice. In addition, hepatic insulin resistance caused by ER stress/JNK pathway and increased SREBP-1c that downregulates IRS-2 further accumulates FoxO1 in the nucleus, leading to induction of genes involved in gluconeogenesis such as *Pepck1* (Supplementary Fig. 2).

A limitation of the current study is the fact that we cannot rule out the possibility of altered insulin secretion in the PA28 KO mice. In vitro studies suggest that the ubiquitin-proteasome system plays a role in insulin secretion by maintaining the normal function of voltage-dependent calcium channels (53). Recently, the ER-associated degradation (ERAD)/ubiquitin/proteasome system was reported to be compromised in β cells of obese patients with type 2 diabetes (54), which is compatible with our observation in the liver. Therefore, it might be possible that PBA ameliorated proteasome dysfunction–induced inhibition of glucose-stimulated insulin secretion in the current study (Supplementary Fig. 1). Indeed, PBA was reported to ameliorate β-cell dysfunction in nondiabetic obese humans infused with fatty acids (55). On the other hand, proteasome activity is rather enhanced in the skeletal muscle of obese diabetic *db/db* mice (41). Therefore, future research should involve the tissue-specific regulation of proteasome function in obesity and diabetes.

In conclusion, proteasome function is impaired in obesity, which contributes to the development of hepatic insulin resistance and steatosis via activating JNK and SREBP-1c by ER stress. Proteasome dysfunction also increases total and nuclear FoxO1 that enhances hepatic gluconeogenesis (Supplementary Fig. 2). Therefore, proteasome dysfunction may be a primary event linking obesity and ER stress–induced insulin resistance in the liver.

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T.Oto. researched the data, contributed to discussion, and wrote the manuscript. T.Taka. conceived and designed the experiments, researched the data, contributed to discussion, wrote the manuscript, and reviewed and edited the manuscript. H.M. and T. Ota contributed to discussion. S.M. contributed to discussion and reviewed and edited manuscript. H.H., H.T., A.K., T.K., K.R.S., F.L., T.Take., S.K., K.Is., Y.K., K.Iw., K.-i.K., M.U., Y.T., M.Y., and K.To. researched data. S.I. contributed to discussion. K.Ta. and S.K. contributed to discussion and reviewed and edited manuscript. T.Taka. is the guarantor of this work, had full access to all the data in the study, and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Special Report

A multicenter survey of re-treatment with pegylated interferon plus ribavirin combination therapy for patients with chronic hepatitis C in Japan

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Aim: This study aimed to clarify the factors associated the efficacy of re-treatment with pegylated interferon (PEG IFN) plus ribavirin combination therapy for patients with chronic hepatitis C who had failed to respond to previous treatment.

Methods: One hundred and forty-three patients who had previously shown relapse ($n = 79$), non-response ($n = 34$) or intolerance ($n = 30$) to PEG IFN plus ribavirin were re-treated with PEG IFN plus ribavirin.

Results: Twenty-five patients with intolerance to previous treatment completed re-treatment and the sustained virological response (SVR) rates were 55% and 80% for hepatitis C virus (HCV) genotype 1 and 2, respectively. On re-treatment of the 113 patients who completed the previous treatment, the SVR rates were 48% and 63% for genotype 1 and 2, respectively. Relapse after previous treatment and a low baseline HCV RNA level on re-treatment were associated with SVR in genotype 1 ($P < 0.001$). Patients with the interleukin-28B major genotype responded significantly better and earlier to re-treatment, but the difference in the SVR rate did not reach a significant level between the major and minor genotypes ($P = 0.09$). Extended treatment of 72 weeks raised the SVR rate among the patients who attained complete early virological response but not rapid virological response with re-treatment (72 weeks, 73%, 16/22, vs 48 weeks, 38%, 5/13, $P < 0.05$).

Conclusion: Relapse after previous treatment and a low baseline HCV RNA level have predictive values for a favorable response of PEG IFN plus ribavirin re-treatment for HCV genotype 1 patients. Re-treatment for 72 weeks may lead to clinical improvement for genotype 1 patients with complete early virological response and without rapid virological response on re-treatment.

Key words: chronic hepatitis C, pegylated interferon and ribavirin combination therapy, re-treatment

INTRODUCTION

PEGYLATED INTERFERON (PEG IFN) plus ribavirin combination therapy can show antiviral efficacy for patients with chronic hepatitis C (CH-C). However, a sustained virological response (SVR), which is defined as undetectable serum hepatitis C virus (HCV) RNA at 24 weeks after the treatment, remains at 50% for patients with HCV genotype 1 and 80% for those with HCV genotype 2 treated with PEG IFN plus ribavirin.[1–6] The number of patients who fail to achieve a SVR increases over time, requiring urgent action to eradicate HCV in them.

Recently, addition of the first-wave protease inhibitor telaprevir to PEG IFN plus ribavirin combination therapy, which has been reported to improve antiviral efficacy, has become commercially available, but this

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triple therapy increases side-effects, especially severe anemia and skin rash.[7–11] Second-wave protease inhibitors, such as TMC435, which not only improve antiviral efficacy but also decrease side-effects, have been developed and are undergoing clinical trials.[12] Also, IFN-free regimens, such as protease inhibitor and polymerase inhibitor combination therapy, have been developed.[13,14] In Japan, HCV carriers are increasing in an aging population, and large numbers of patients are ineligible for triple therapy with telaprevir due to potential anemia. That is why re-treatment with PEG IFN plus ribavirin is a possible choice for patients who failed to achieve SVR to previous antiviral therapy or patients ineligible for triple therapy with telaprevir who must wait until next-generation antiviral therapies, such as triple therapy with second-wave protease inhibitors or IFN-free regimens, become commercially available.

As for re-treatment with PEG IFN plus ribavirin, some studies have been reported but the subjects and treatment protocols were varied.[15–20] According to past reports, the previous treatment response is associated with the efficacy of the re-treatment[17,20] and the SVR rates in re-treatment ranged 4–23%.[16–18] Recently, host factors, such as single nucleotide polymorphisms (SNP) located near the interleukin (IL)-28B gene, and virus factors, such as the amino acid substitutions in the HCV core region, were revealed to have a strong impact on SVR in PEG IFN plus ribavirin combination therapy for naïve CH-C patients.[21–26] Moreover, response-guided therapy which extends treatment duration until 72 weeks for patients with a slow virological response can raise the SVR rate for naïve CH-C patients.[27–29] However, the value of IL-28B SNP has been uncertain in re-treatment and the most appropriate treatment duration in re-treatment is still unclear. Although it remains obscure which factors are associated with SVR in re-treatment with standard PEG IFN plus ribavirin therapy as pointed out above, some patients do respond to re-treatment and it is very important to be able to identify them. Such findings will be valuable for optimizing the antiviral treatment for CH-C patients by making it possible to decide which patients should be considered for re-treatment with PEG IFN plus ribavirin therapy and which should wait for next-generation antiviral treatment.

In the present study, we tried to determine which patients could benefit from re-treatment and to identify the factors associated with SVR in re-treatment, including the host genome SNP and treatment duration.

METHODS

Patients

THIS RETROSPECTIVE, MULTICENTER study was conducted by the Study Group of Antiviral Therapy for Difficult-to-Treat Chronic Hepatitis C supported by the Ministry of Health, Labor and Welfare, Japan. This study was conducted with 143 CH-C patients, 113 patients (genotype 1, $n = 86$; genotype 2, $n = 27$) who had previously completed PEG IFN-α-2b plus ribavirin combination therapy but had failed to attain SVR, and 30 patients (genotype 1, $n = 22$; genotype 2, $n = 8$) who had previously discontinued this combination therapy due to adverse events.

Treatment

For the previous treatment, patients had been treated with PEG IFN-α-2b (PEGINTRON; MSD, Whitehouse Station, NJ, USA) plus ribavirin (REBETOL; MSD). For re-treatment with PEG IFN plus ribavirin, patients were treated PEG IFN-α-2a (PEGASYS; Roche, Basel, Switzerland) plus ribavirin (COPEGUS; Roche) or PEG IFN-α-2b plus ribavirin. In principle, as a starting dose, PEG IFN was given once weekly at a dose of 180 μg of PEG IFN-α-2a and 1.5 μg/kg of PEG IFN-α-2b and ribavirin was given at a total dose of 600–1000 mg/day based on bodyweight (bodyweight, ≤60 kg, 600 mg; 60–80 kg, 800 mg; ≥80 kg, 1000 mg), according to the standard treatment protocol for Japanese patients and the decision of the investigator at the participating clinical center. Dose modification followed, as a rule, the manufacturer's drug information on the intensity of the hematological adverse effects.

Laboratory tests and virological assessment

Examination of peripheral blood, transaminase and the serum HCV RNA level were tested at the start of treatment, weeks 4, 12 and 24, end of treatment (EOT), and 24 weeks after the treatment. Sequences of the IFN-sensitivity determining region (ISDR) and the core region of HCV were determined at start of the previous treatment, and the number of mutations in the ISDR, the amino acid substitutions at core 70 and 91, glutamine (Gln) or histidine (His) at core 70 and methionine (Met) at core 91, were analyzed. Genetic polymorphisms located near the IL-28B gene (rs8099917) and ITPA gene (rs1127354) were determined. As for the IL-28B gene, homozygosity for the major sequence (TT) was defined as having the IL-28B major allele, whereas homozygosity (GG) or heterozygosity (TG) of the minor sequence was defined as having

the IL-28B minor allele. As for the ITPA gene, homozygosity for the major sequence (CC) was defined as having the ITPA major allele, whereas homozygosity (AA) or heterozygosity (CA) of the minor sequence was defined as having the ITPA minor allele. The serum HCV RNA level was quantified using the COBAS AMPLICOR HCV MONITOR test ver. 2.0 (detection range, 6–5000 KIU/mL; Roche Diagnostics, Branchburg, NJ, USA) or COBAS TaqMan HCV test (detection range, 1.2–7.8 $\log_{10}$ IU/mL) and qualitatively analyzed using the COBAS AMPLICOR HCV test ver. 2.0 (lower limit of detection, 50 IU/mL). When the serum HCV RNA level quantified by the COBAS TaqMan HCV test was less than 1.7 $\log_{10}$ IU/mL, which was equivalent to 50 IU/mL of HCV RNA, that case was judged as HCV RNA negativiation against the lower limit of detection of the COBAS AMPLICOR HCV test.

Definition of virological response

A rapid virological response (RVR) was defined as undetectable serum HCV RNA level at week 4, partial early virological response (p-EVR) as a more than 2-log decrease in the HCV RNA level at week 12 compared with the baseline, complete EVR (c-EVR) as undetectable serum HCV RNA at week 12, late virological response (LVR) as detectable serum HCV RNA at week 12 and undetectable at week 24, and SVR as undetectable serum HCV RNA at 24 weeks after the treatment. Relapse was defined as undetectable serum HCV RNA at the EOT but a detectable amount after the treatment. Patients without p-EVR or without clearance of HCV RNA at week 24 were considered to be showing non-response (NR), and treatment was stopped in both the previous treatment and this re-treatment. A patient who attained HCV RNA negativiation during the re-treatment continued to be treated for 48 weeks or 72 weeks according to response-guided therapy or the decision of the investigator at the participating clinical center.

Statistical analysis

Baseline data of the patients are expressed as means ± standard deviation or median values. In order to analyze the difference between baseline data or the factors associated with SVR, univariate analysis using the Mann–Whitney *U*-test or χ^2-test and multivariate analysis using logistic regression analysis were performed. A two-tailed *P*-value of less than 0.05 was considered significant. The analysis was conducted with SPSS ver. 17.0J (IBM, Armonk, NY, USA).

RESULTS

THE PATIENT FLOW in this study is shown in Figure 1. Among the patients who had previously discontinued PEG IFN-α-2b plus ribavirin combination therapy, two patients underwent splenectomy to increase platelet count prior to re-treatment, 25 completed re-treatment of PEG IFN plus ribavirin combination therapy and 15 achieved SVR (genotype 1, $n = 11$; genotype 2, $n = 4$).

All of the patients who completed previous treatment also completed re-treatment and the baseline characteristics of those patients are shown in Table 1. Of the 86 genotype 1 patients, 54 were relapsers and 32 had shown NR to previous treatment. Of the 27 patients with genotype 2, 25 were relapsers and two had shown NR to previous treatment. Thirty-seven patients with genotype 1 and 14 patients with genotype 2 were assessed as IL-28B genotype, and 27 patients with genotype 1 and 10 patients with genotype 2 were assessed as ITPA genotype. There was no significant difference in the baseline characteristics between the previous treatment and the re-treatment with respect to peripheral blood cell counts, amino transaminase level and serum HCV RNA at the start of treatment (Table 1).

The baseline characteristics of patients with genotype 1 according to antiviral efficacy of the previous treatment are shown in Table 2. Among those with NR in the previous treatment, the rate of the minor allele of IL-28B was significantly higher than those with relapse in the previous treatment ($P < 0.01$). For genotype 1, the HCV RNA negative rate on re-treatment was 20% (17/86) at week 4, 61% (52/85) at week 12 and 76% (65/86) at week 24, and the SVR rate was 48% (41/86). The factors associated with SVR were assessed by univariate analysis and the factors of relapse after previous treatment and the serum HCV RNA level at the start of re-treatment were selected as being significant (Table 3). The SVR

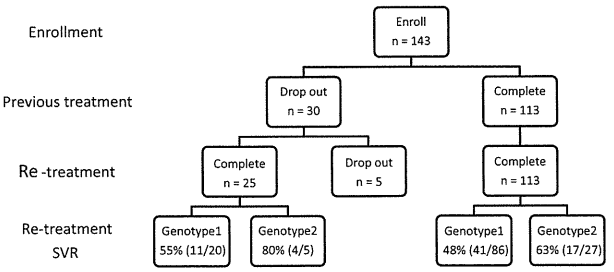

Figure 1 Patient flow for this study. SVR, sustained virological response.

Table 1 Baseline characteristics of patients and treatment factors in previous treatment and re-treatment

Factor	Genotype 1		Genotype 2	
No.	86		27	
Sex: male/female	46/40		15/12	
Effect of previous treatment: relapse/NR	54/32		25/2	
	Previous treatment	Re-treatment	Previous treatment	Re-treatment
PEG IFN type: α-2a/α-2b	0/86	41/45	0/27	6/21
Age (years)	58.1 ± 8.3	60.0 ± 8.5	58.9 ± 8.2	60.0 ± 8.1
White blood cells (/mm^3)	4779 ± 1383	4610 ± 1443	5195 ± 1473	4724 ± 1266
Neutrophils (/mm^3)	2478 ± 930	2355 ± 1071	2561 ± 827	2389 ± 941
Hemoglobin (g/dL)	13.7 ± 1.2	13.5 ± 1.7	14.4 ± 1.3	14.0 ± 1.2
Platelets (×10^4/mm^3)	16.0 ± 5.9	16.6 ± 6.2	18.0 ± 5.7	16.8 ± 5.2
ALT (IU/L)	75 ± 51	73 ± 72	57 ± 46	42 ± 32
Histology: activity, 0–1/2–3	29/29		11/7	
Fibrosis, 0–2/3–4	45/14		17/1	
Serum HCV RNA (KIU/mL)	1600	850	1500	700
IL-28B SNP: rs8099917; TT/TG	26/11		10/4	
ITPA SNP: rs1127354; CC/CA	20/7		9/1	
Core 70: wild/mutant	11/11			
Core 91: wild/mutant	15/7			
ISDR: 0–1/≥2	15/1			

ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, IFN-sensitivity determining region; NR, non-response; PEG, pegylated; SNP, single nucleotide polymorphism.

rates of relapsers were significantly higher than those of patients with NR in the previous treatment (relapse, 67%, 36/54 vs NR, 16%, 5/32, $P < 0.0001$). As for the serum HCV RNA level at the start of re-treatment, although the SVR rate of those patients with 5 $\log_{10}$ IU/mL or more of HCV RNA was 38% (26/69), all patients with less than 5 $\log_{10}$ IU/mL of HCV RNA attained SVR (11/11) ($P = 0.0001$). As for the IL-28B genotype, among the patients with the major allele, the p-EVR rate was significantly higher and the EOT response rate showed marginal significance compared to that with the minor allele (p-EVR rate, 100%, 23/23 vs 30%, 3/10, $P < 0.0001$, EOT rate, 92%, 24/26 vs 64%, 7/11, $P = 0.05$). There was no significant difference of the SVR rate between major and minor alleles (major, 65%, 17/26 vs minor, 36%, 4/11, $P = 0.15$).

Figure 2(a) shows the result of stratified analysis according to the previous treatment response and HCV RNA at the start of re-treatment. The significant difference in SVR observed between high (≥5 $\log_{10}$ IU/mL) and low (<5 $\log_{10}$ IU/mL) baseline viral loads was still found in both previous relapsers ($P = 0.02$) and previous non-responders ($P = 0.02$). In patients with a high baseline viral load, previous relapsers achieved a higher SVR rate than previous non-responders ($P < 0.0001$). Next, the results of stratified analyses according to IL-28B genotype and previous treatment response or HCV RNA at the start of re-treatment showed no significant difference in SVR rates between the IL-28B genotype in patients with relapse after previous treatment ($P = 0.63$) (Fig. 2b). All patients with less than 5 $\log_{10}$ IU/mL of HCV RNA achieved SVR despite their IL-28B genotype and the SVR rates of patients with 5 $\log_{10}$ IU/mL or more of HCV RNA did not differ between IL-28B genotypes (Fig. 2c). Multivariate analysis among the factors of relapse to previous treatment response, HCV RNA at the start of re-treatment and IL-28B genotype showed that relapse after previous treatment response bore the most predictable relationship to SVR in re-treatment ($P = 0.074$).

As for the efficacy of re-treatment according to treatment duration among patients with HCV RNA negativity during re-treatment, the SVR rate of 72-week treatment was significantly higher than that of 48-week treatment (72 weeks, 73%, 29/40, vs 48 weeks, 52%, 12/25, $P < 0.05$). This significant difference was especially found in patients who attained c-EVR but not RVR on re-treatment (72 weeks, 73%, 16/22, vs 48 weeks,

Table 2 Baseline characteristics of patients and treatment factors according to the virological response in previous treatment among patients with genotype 1

Factor	Relapser in previous treatment		NR in previous treatment	
No.	54		32	
Sex: male/female	28/26		18/14	
	Previous treatment	Re-treatment	Previous treatment	Re-treatment
PEG IFN type: α-2a/α-2b	0/54	29/25	0/32	12/20
Age (years)	58.1 ± 8.1	60.3 ± 8.4	57.9 ± 8.9	59.6 ± 8.8
White blood cells (/mm^3)	4917 ± 1290	4692 ± 1035	4546 ± 1520	4462 ± 1993
Neutrophils (/mm^3)	2618 ± 846	2479 ± 805	2225 ± 1033	2105 ± 1454
Hemoglobin (g/dL)	13.9 ± 1.2	13.7 ± 1.6	13.5 ± 1.3	13.1 ± 1.9
Platelets (×10^4/mm^3)	17.1 ± 6.3	17.7 ± 6.1	14.1 ± 4.7	14.7 ± 6.2
ALT (IU/L)	75 ± 57	70 ± 76	75 ± 39	78 ± 64
Histology: activity, 0–1/2–3	20/18		9/11	
Fibrosis, 0–2/3–4	31/8		14/6	
Serum HCV RNA (KIU/mL)	1600	980	1550	800
IL-28B SNP: rs8099917; TT/TG	24/5		2/6	
ITPA SNP: rs1127354; CC/CA	15/6		5/1	
Core 70: wild/mutant	6/6		5/5	
Core 91: wild/mutant	9/3		6/4	
ISDR: 0–1/≥2	9/0		6/1	

ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, IFN-sensitivity determining region; NR, non-response; PEG, pegylated; SNP, single nucleotide polymorphism.

38%, 5/13, $P < 0.05$) but not in patients who attained RVR or LVR (Fig. 3).

In genotype 2, the HCV RNA negative rate on re-treatment was 59% (16/27) at week 4, 85% (23/27) at week 12 and 93% (25/27) at week 24, and the SVR rate was 63% (17/27). The two patients with NR in previous treatment did not attain SVR with re-treatment. The factors associated with SVR were assessed by univariate analysis and only the factor of younger age at the start of re-treatment showed marginal significance ($P = 0.06$) (Table 4). Among the patients with RVR on re-treatment, the SVR rates were similar at 75% (6/8) to those with 24-week and 48-week treatment.

DISCUSSION

PAST STUDIES HAVE revealed that the factors of age, sex, progression of liver fibrosis, value of HCV RNA, number of mutations in the ISDR, amino acid substitutions in the core region, drug adherence and treatment duration show association with HCV eradication in PEG IFN plus ribavirin combination for naïve patients with CH-C.[3–5,25–33] Recently, the IL-28B genotype has been reported to be the most powerful factor associated with the antiviral effect of this combination therapy.[21–25] While the predictive factors for SVR in PEG IFN plus ribavirin combination therapy for naïve patients have been actively analyzed, those factors for patients who had already experienced this therapy are still unclear. Especially needing assessment is the correlation between IL-28B SNP or the previous treatment response and the antiviral effect in re-treatment. In this study, we tried to determine which factors could most effectively predict the antiviral effect in re-treatment.

In the present study, patients with relapse after the previous treatment and patients with a low serum HCV RNA level at the start of re-treatment showed significantly different results in this study of re-treatment of CH-C patients who had previously failed to attain SVR with PEG IFN plus ribavirin therapy. This result was similar to those of the EPIC[3] study on relapse and NR[17] and the SYREN trial of NR.[18] On the other hand, there was no significant difference between the influence of the IL-28B genotype and SVR. More specifically, if the previous treatment response was the same, there was no difference regardless of the IL-28B genotype. Considering this result, in re-treatment, the previous treatment response was a more effective predictive factor than IL-28B genotype. However, further investigation is needed to clarify the association between IL-28B

Table 3 Factors associated with a sustained virological response in re-treatment with PEG IFN plus ribavirin in patients with genotype 1

Factor		SVR	Non-SVR	*P*-value
No. of patients		41	45	
Age (years)		60.2 ± 7.1	59.9 ± 9.6	0.71
Sex: male/female		24/17	22/23	0.40
Serum HCV RNA (log IU/mL)		5.8 ± 1.4	6.4 ± 0.6	0.11
Serum HCV RNA: <5 log/≥5 log		11/28	0/43	<0.001
White blood cells (/mm^3)		4656 ± 1029	4566 ± 1763	0.42
Neutrophils (/mm^3)		2443 ± 804	2259 ± 1301	0.16
Hemoglobin (g/dL)		13.5 ± 1.6	13.4 ± 1.8	0.80
Platelets (×10^4/mm^3)		16.9 ± 5.7	16.3 ± 6.7	0.36
ALT (IU/L)		68 ± 69	78 ± 75	0.43
IL-28B SNP: TT/TG		17/4	9/7	0.15
ITPA SNP: CC/CA		13/3	7/4	0.39
Core 70: wild/mutant		5/4	6/7	1.00
Core 91: wild/mutant		7/3	8/5	1.00
ISDR: 0–1/≥2		9/0	6/1	0.44
PEG IFN: α-2a/α-2b		16/25	25/20	0.14
PEG IFN dose (μg/kg per week)	α-2a	2.91 ± 0.77	2.74 ± 0.69	0.61
	α-2b	1.25 ± 0.39	1.20 ± 0.32	0.59
Ribavirin dose (mg/kg per day)		9.34 ± 2.72	9.64 ± 3.20	0.51
1st treatment virological response	Relapse/NR	36/5	18/27	<0.001

ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, IFN-sensitivity determining region; NR, non-response; PEG, pegylated; SNP, single nucleotide polymorphism; SVR, sustained virological response.

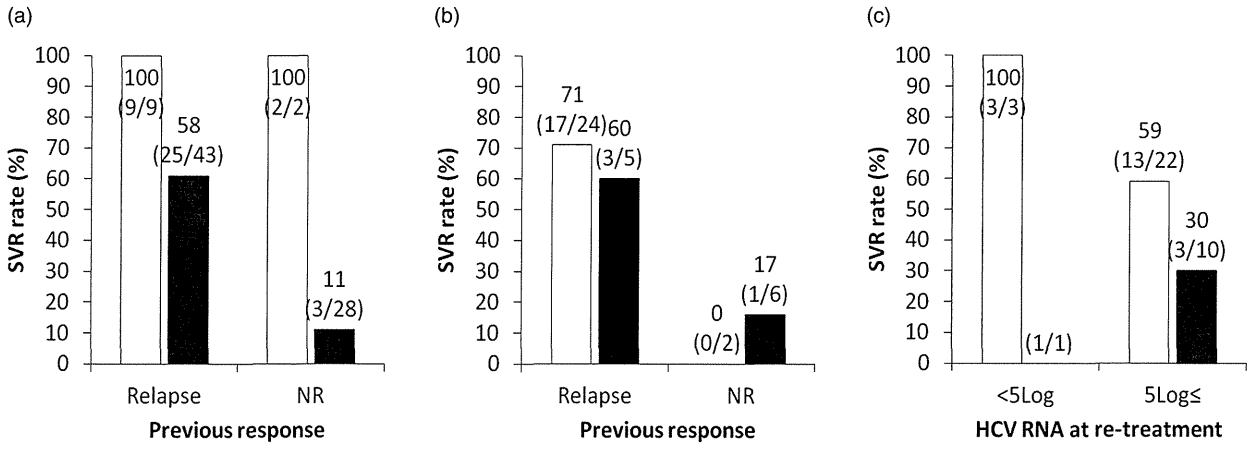

Figure 2 Sustained virological response (SVR) rates according to previous virological response, hepatitis C virus (HCV) RNA at start of re-treatment and genotype of interleukin (IL)-28B single nucleotide polymorphism (SNP) in patients with genotype 1. (a) Stratified analysis of previous virological response and HCV RNA at start of re-treatment. □, HCV RNA <5 log IU/mL at start of re-treatment; ■, HCV RNA ≥5 log IU/mL at start of re-treatment. (b) Stratified analysis of previous virological response and genotype of IL-28B SNP. □, Patients with major allele of IL-28B SNP; ■, patients with minor allele of IL-28B SNP. (c) Stratified analysis of HCV RNA at start of re-treatment and genotype of IL-28B SNP. □, Patients with major allele of IL28B SNP; ■, patients with minor allele of IL-28B SNP.

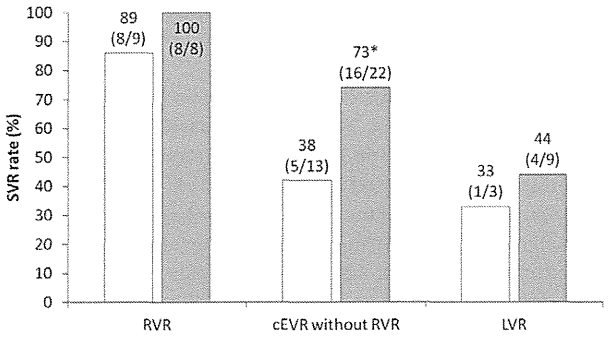

Figure 3 Sustained virological response (SVR) rates according to virological response in re-treatment and treatment duration in patients with genotype 1. □, Patients treated for 48 weeks; ▩, patients treated for 72 weeks. RVR, rapid virological response; cEVR, complete early virological response; LVR, late virological response. *$P < 0.05$; compared to 48 weeks of treatment.

genotype and antiviral effect of re-treatment because of their small number in this study. In this study, only one patient with the minor allele of IL-28B and NR in previous treatment could start and continue with the increased dose of PEG IFN (from 1.37 μg/kg in the previous treatment to 1.79 μg/kg in re-treatment) and ribavirin (from 10.3 mg/kg per day in the previous treatment to 11.1 mg/kg per day in re-treatment) and attained SVR by extended treatment. If the drug adherence does not improve, patients with the minor allele of IL-28B who show NR in the previous treatment should be treated with new drugs.

The next question is how the patients should be re-treated in order to attain SVR on re-treatment. In this study, the patients with a low serum HCV RNA level (<5 $\log_{10}$ IU/mL) at the start of re-treatment showed a significant rate of cure on re-treatment, and this is almost the same result as that previously reported.[16,17] In this study, the two patients with NR in the previous treatment and with less than 5 $\log_{10}$ IU/mL of HCV RNA level (20 KIU/mL and 52 KIU/mL of HCV RNA) at the start of re-treatment attained SVR. On the other hand, even if the previous treatment response was a relapse, the SVR rates were 58% (25/43) among the patients with 5 $\log_{10}$ IU/mL or more of HCV RNA. Because the HCV RNA level changed after the antiviral treatment, it is important to not miss the timing of when the HCV RNA level is low.

With respect to treatment duration among patients with HCV RNA negativiation during re-treatment, 72 weeks of treatment significantly increased the SVR rate compared to 48 weeks. This result was almost the same as that of the REPEAT study.[16] In our present study, the SVR rate among the patients with c-EVR but not RVR in re-treatment was significantly high by 72 weeks of treatment. On the other hand, the SVR rates among the

Table 4 Factors associated with a sustained virological response in re-treatment with PEG IFN plus ribavirin in patients with genotype 2

Factor		SVR	Non-SVR	*P*-value
No. of patients		17	10	
Age (years)		57.7 ± 8.8	63.7 ± 5.1	0.06
Sex: male/female		7/10	8/2	0.11
Serum HCV RNA (log IU/mL)		5.4 ± 1.4	6.1 ± 0.8	0.15
Serum HCV RNA: <5 log/≥5 log		5/11	1/9	0.35
White blood cells (/mm^3)		5049 ± 1355	4171 ± 910	0.10
Neutrophils (/mm^3)		2556 ± 1064	1999 ± 404	0.24
Hemoglobin (g/dL)		14.1 ± 1.3	13.8 ± 1.6	0.51
Platelets (×10^4/mm^3)		17.9 ± 5.4	14.8 ± 4.3	0.17
ALT (IU/L)		38 ± 19	48 ± 47	0.71
IL-28B SNP: TT/TG		6/2	4/2	1.00
ITPA SNP: CC/CA		5/1	4/0	1.00
PEG IFN: α-2a/α-2b		4/13	2/8	1.00
PEG IFN dose (μg/kg per week)	α-2a	3.23 ± 0.34	2.24 ± 2.25	1.00
	α-2b	1.32 ± 0.28	1.18 ± 0.23	0.21
Ribavirin dose (mg/kg per day)		10.4 ± 2.21	10.1 ± 1.31	0.44
1st treatment virological response	RVR/non-RVR	4/13	3/7	1.00

ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, IFN-sensitivity determining region; PEG, pegylated; RVR, rapid virological response; SNP, single nucleotide polymorphism; SVR, sustained virological response.

patients with RVR in re-treatment were similar between the patients with 48 weeks and 72 weeks of treatment. Thus, patients with c-EVR but not RVR in re-treatment should be re-treated for a longer period. In order to attain better SVR, extended treatment duration is generally recommended for patients with on-treatment LVR, whereas standard treatment duration is considered to be sufficient for patients with on-treatment c-EVR. However, the present study revealed that, even if patients achieved c-EVR on re-treatment, 72 weeks of treatment seems to be better than 48 weeks for treatment-experienced patients. The majority of naïve patients showing on-treatment c-EVR could eradicate HCV with 48 weeks of treatment while some could not. In a treatment-experienced setting, patients who are able to respond early but not eradicate HCV would be selected, and therefore extended treatment may be needed.

With genotype 2, the SVR rate was relatively high (63%). The patients who could not attain SVR in re-treatment (two patients) showed NR in the previous treatment. Thus, the patients with genotype 2 and showing NR in previous treatment seemed to be difficult to treat and could be treated with other drugs. Among the patients with RVR in re-treatment, the SVR rates were similar among those with RVR in re-treatment between 24 weeks and 48 weeks of treatment. The effectiveness of extended treatment for the patients with genotype 2 in re-treatment could not be demonstrated because of their small number in this study. Further investigation is needed to clarify this.

In conclusion, this study shows that the efficacy of re-treatment for genotype 1 patients who failed to show SVR to previous treatment with PEG IFN plus ribavirin could be predicted from the previous treatment response and a low HCV RNA level at the start of re-treatment. Re-treatment for 72 weeks led to clinical improvement for genotype 1 patients with c-EVR and without RVR on re-treatment.

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Review Article

Treatment strategies for hepatocellular carcinoma in Japan

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The main methods of treatment for hepatocellular carcinoma (HCC) in Japan are hepatic resection, radiofrequency ablation (RFA) and transcatheter arterial chemoembolization (TACE). Meticulous follow up is then undertaken to check for recurrence, which is treated using repeated RFA or TACE. Hepatic arterial infusion chemotherapy has been introduced as treatment for advanced HCC, and the molecular-targeted drug sorafenib is also now available. Rigorous medical care using these treatment methods and early diagnosis mean that the prognosis for HCC in Japan is the best in the world. This paper reviews the treatment strategies for HCC in Japan.

Key words: hepatocellular carcinoma, treatment algorithm, treatment strategies

INTRODUCTION

TREATMENT FOR HEPATOCELLULAR carcinoma (HCC) is peculiar in that, unlike other solid carcinomas, the treatment methods must be selected in consideration of the underlying clinical condition of the liver. A wide range of treatment methods is available, including hepatectomy, liver transplant, radiofrequency ablation (RFA), transcatheter arterial chemoembolization (TACE), sorafenib therapy, hepatic arterial infusion chemotherapy (HAIC) and radiotherapy. These treatment methods can also be used in combination. This paper reviews the treatment strategies for HCC in Japan.

CHOICE OF TREATMENT METHOD

MANY CASES OF HCC arise from liver cirrhosis, and are associated with deterioration in liver function. This means that in addition to cancer stage, hepatic reserve is also an important prognostic factor. This balance must be taken into account when choosing between different types of treatment. In Japan, the Japan Society of Hepatology issued consensus-based HCC treatment guidelines in 2010, which include a HCC treatment algorithm that offers the closest method of selecting treatment to current clinical practice (Fig. 1).[1]

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In this algorithm, the treatment method is guided by five factors: extrahepatic lesions; hepatic reserve (Child–Pugh class); vascular invasion; number of tumors; and tumor diameter. This algorithm was prepared on the basis of another algorithm compiled in evidence-based clinical practice guidelines for HCC – the Japan Society of Hepatology 2009 update[2] – and reflects the consensus reached among HCC treatment specialists in Japan. This algorithm is somewhat complex, listing multiple methods of treatment with the addition of numerous comments, but reflects the current Japanese choices of treatment for HCC almost in their entirety.[1]

This treatment algorithm was basically prepared for the treatment of primary HCC, but also provides a reference for recurrent HCC, for which the treatment method is determined by taking into account the time to recurrence, type of recurrence, anticipated tumor malignancy according to tumor markers and pathology, age at recurrence, degree of deterioration in liver function between primary occurrence and recurrence, and the adverse effects of initial treatment.

HEPATIC RESECTION

ALONG WITH LIVER transplantation, this offers the most radical treatment, but the degree of surgical invasiveness, complications and the deterioration of hepatic reserve after resection must be taken into account.

Hepatic resection procedures include partial resection, subsegmental resection, segmental resection, two-segment resection, extended two-segment resection and three-segment resection. As HCC frequently metastasizes

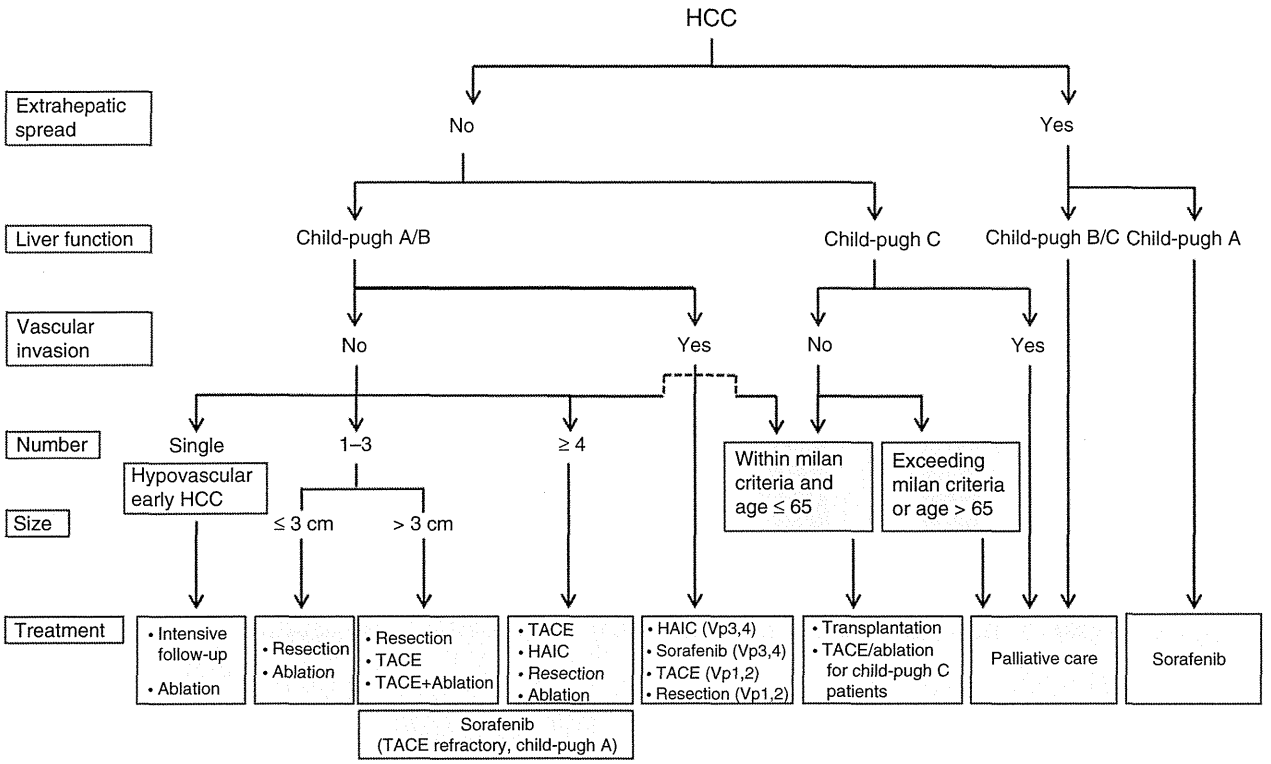

Figure 1 Consensus-based treatment algorithm for HCC proposed by Japan Society of Hepatology 2009 revised in 2010 (modified from ref. [1]). HAIC, hepatic arterial infusion chemotherapy; HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

within the liver via the portal vein, anatomical resection of the entire portal segment where the cancer is located increases the curative nature of the procedure, and anatomical resection is therefore commonly performed provided hepatic reserve is sufficient. The standard procedure is to inject dye under guidance of ultrasonography (USG) into the portal vein in the segment containing the cancer, and to perform systematic subsegmental resection to remove all areas stained by the dye.[3,4]

It is important to evaluate hepatic reserve prior to hepatic resection, and the permissible extent of resection is considered on the basis of presence or absence of ascites, jaundice and the indocyanine green (ICG) retention rate at 15 min when determining the type of resection procedure.[5] If necessary, technetium-99*m* diethylenetriamine pentaacetic acid galactosyl human serum albumin single photon emission computed tomography (CT) is used to evaluate patients who cannot be adequately evaluated by means of an ICG load test.[6,7]

According to the report of the 18th follow-up survey of primary liver cancer in Japan, hepatic resection was performed in 31.7% of all cases of HCC, with operative mortality of 1.4% (Fig. 2).[9] Three-, 5- and 10-year survival rates after hepatic resection were 69.5%, 54.2% and 29.0%, respectively.[9]

As a recent trend in surgery, minimally invasive resection methods such as laparoscopic hepatectomy[10–12] and robot surgery[13] have been developed for some cases of HCC. Percutaneous isolated hepatic perfusion chemotherapy following debulking hepatectomy is reportedly useful in treating patients with severe advanced HCC with tumor thrombus of major vessels.[14]

LIVER TRANSPLANTATION

LIVER TRANSPLANTATION IS the best treatment method for removing metastatic foci in the liver together with the cirrhotic liver from which the cancer develops. In Japan, living-donor liver transplantation has been covered by health insurance since January 2004.

According to reports published up to the end of 2009, almost all liver transplantations for HCC in Japan

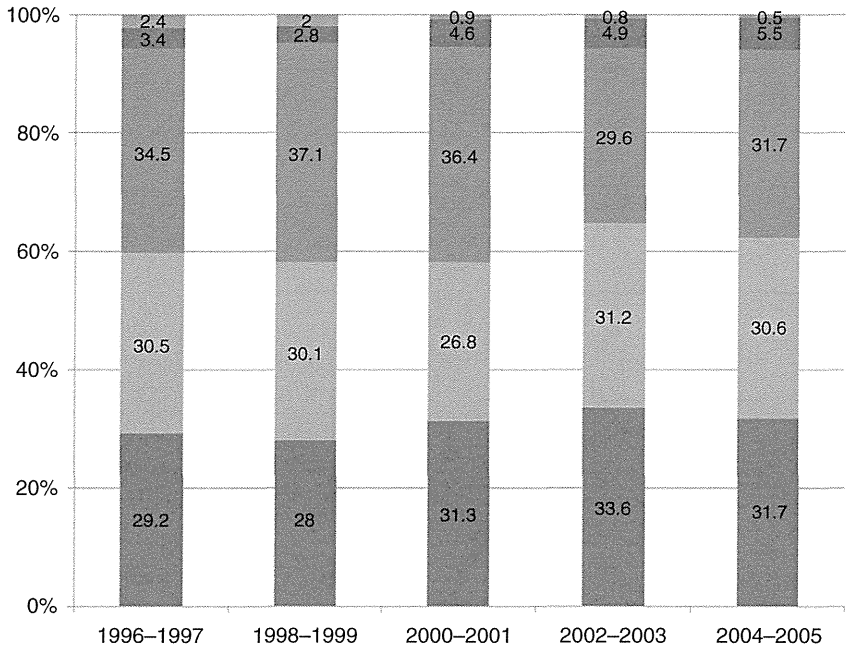

Figure 2 Changes in treatment methods for primary hepatocellular carcinoma in Japan between 1996 and 2005 (modified from ref. [8]). ■, Others; ■, chemotherapy; ■, transcatheter arterial chemoembolization; ■, ablation; ■, resection.

involved living donors, with 1131 transplantations from living donors and seven from deceased donors.[15] As liver transplantations are taken from living donors, indications for liver transplantation in Japan only cover those patients who meet the Milan criteria (≤3 tumors with tumor diameter ≤3 cm or a single tumor ≤5 cm in diameter), but whose hepatic reserve has deteriorated severely (Child–Pugh class C),[1,2] meaning that liver transplantations are regarded very differently in comparison with other countries where the majority of transplantations are from deceased donors.[15]

However, because most liver transplantations are from living donors, issues of the appropriate distribution of liver grafts and waiting times involved in transplantations from deceased donors are almost non-existent. Recently, tumor markers have also been included in the criteria, and attempts are being made to extend indications beyond those of the Milan criteria.[16,17] In addition, donors are restricted to close relatives. As a result, blood groups are frequently mismatched, although in almost all cases this can be managed by the preoperative administration of anti-CD20 antibodies and plasmapheresis.[18]

According to a report by the Japanese Liver Transplantation Society, 1-, 3-, 5- and 10-year survival rates following liver transplantation from a living donor were 84.4%, 73.9%, 68.5% and 58.8%, respectively.[15]

The Act on Organ Transplantation was revised in July 2010 to enable organ donation with the family's permission even if the donor's own intentions had not been made clear, and since then the number of liver transplants from deceased donors has gradually been increasing.

LOCAL ABLATION THERAPY

LOCAL ABLATION THERAPY constitutes the main medical therapy for HCC in Japan. According to the report of the 18th follow-up survey, local ablation therapies were used in 30.6% of cases, administrated percutaneously in approximately 90% of those cases. RFA was used in 72.1% of cases (Fig. 2).[9]

Radiofrequency ablation has been covered by health insurance in Japan since April 2004, and its efficacy has been demonstrated in several subsequent randomized comparative trials,[19–22] making this the first choice in percutaneous local therapy today.[2] Percutaneous ethanol injection therapy, the therapy previously used, is still performed in rare cases for sites where insertion of an electrode for RFA is regarded as dangerous.

Indications for RFA are generally considered to be three or less tumors with a tumor diameter of 3 cm or less, with Child–Pugh class A or B liver function, no uncontrollable ascites and no hemorrhagic tendencies. In practice, commonly used criteria comprise platelet count of 50 000/μL or more, prothrombin time of 50% or more and serum bilirubin of 3 mg/dL or less. For

tumors more than 3 cm in diameter, TACE is frequently performed first, followed by additional RFA.[8]

According to the report of the 18th follow-up survey, 1-, 3- and 5-year survival rates for RFA were 95.0%, 76.7% and 56.3%, respectively.[9]

Radiofrequency ablation is usually performed percutaneously; however, this method can be adapted by performing RFA laparoscopically for lesions on the liver surface or touching neighboring organs such as the intestines or diaphragm,[23] and can also be carried out with artificial pleural effusion for lesions under the diaphragm or when the lungs intrude on the puncture route.[24,25] Artificial ascites can also be used to prevent perforation of the digestive tract for lesions touching the intestines,[24–28] and an endoscopic nasobiliary drainage tube can be used to cool the bile duct before treatment when the lesion is close to the bile duct and the latter is at risk of damage.[24,29] For lesions in which the tumor boundaries are not clearly demarcated and that are difficult to visualize under b-mode USG, or when performing additional treatment to secure ablative margins around the target lesion, treatment can be assisted using contrast USG using Sonazoid[24,30,31] or a real-time virtual sonography system that synchronizes image data from or multidetector-row computed tomography with the position of the USG probe, and simultaneously displays the USG images and virtual images from CT data.[32]

TACE

TRANSCATHETER ARTERIAL CHEMOEMBOLIZATION is widely used in Japan to treat HCC.[9] Usually, an adequate amount of emulsion containing oil-based contrast agent Lipiodol and anticancer agents is injected through a catheter then the selected arteries are embolized by embolic agents. Formerly, the embolic agents used in Japan were the absorbent gelatin sponge materials Gelfoam or Spongel treated to create fine fragments, but Gelpart porous gelatin granules were approved for health insurance coverage in 2006 and are now in common use.

Superselective TACE is generally used in Japan to minimize damage to non-tumorous areas by using a microcatheter to embolize only the cancerous subsegment.[33–35] Epirubicin and cisplatin are commonly used as anticancer agents, and miriplatin, a new platinum drug, came into use in 2010.[36,37]

Indications for TACE are wide-ranging, and the procedure is generally performed in patients with hypervascular HCC who are not indicated for surgery or local therapy for reasons such as multiple bilobar HCC, liver dysfunction, old age or comorbidity, and in whom the first branch from the main portal vein is not occluded. In practice, this technique is commonly indicated for patients who are Child–Pugh class A or B with multiple tumors with a diameter of 3 cm or more or with four or more HCC (Fig. 1).[1,2]

According to the report of the 18th follow-up survey, 3-, 5- and 10-year survival rates for TACE (including chemolipiodolization) used to treat HCC were all poor, at 43.2%, 24.1% and 6.6%, respectively.[9] These outcomes are due to the inclusion of patients in poor condition with hepatic reserve or tumor stage that contraindicates hepatic resection or RFA. The same Japanese follow-up survey of outcomes for TACE as initial therapy for Child–Pugh class A patients with a single tumor found that 1-, 3- and 5-year survival rates were good, at 93%, 73% and 52%, respectively.[35,38]

Transcatheter arterial chemoembolization is performed as initial treatment in 31.7% of cases,[9] but is the most frequently used treatment for recurrence, and it is no exaggeration to say that most HCC patients undergo this therapy at some point (Fig. 2). TACE is periodically repeated in Europe and the USA, but this situation rarely arises in Japan. When one to three intrahepatic lesions are present, TACE is followed by additional RFA with the aim of improving local control. With the advent of sorafenib, definitions of TACE failure/refractory HCC have now been proposed to prevent liver dysfunction from decreasing after excursively repeating TACE and to maintain opportunities to administrate sorafenib.[1]

MOLECULAR-TARGETED DRUGS

SORAFENIB WAS APPROVED as a molecular-targeted drug for the treatment of HCC in Japan from May 2009. This agent was approved based on the results of two randomized control trials from outside of Japan[39,40] and a phase I clinical trial carried out in Japan.[41] However, studies continued after sorafenib entered the market due to a lack of experience with administration in Japan. A safety alert was initially issued due to early deaths resulting from liver failure and hepatic encephalopathy, but it has since been used correctly. The median survival period in Japan is 11.0 months and the response rate is 4%, almost the same outcomes as those of the SHARP trial, but reports to date have shown a tendency for a greater number of side-effects, including hand–foot skin reaction, diarrhea, hypertension, loss of appetite and fatigue.[42]

Sorafenib is used to treat Child–Pugh class A patients who have extrahepatic lesions or multiple intrahepatic

lesions who are unable to undergo TACE or HAIC, and patients with vascular invasion.[1]

Measures taken in Japan to reduce side-effects include a low initial dose of 400 mg/day,[42] but drug effectiveness at half dose has yet to be fully investigated. Sorafenib has also not been compared with HAIC, which was already being performed in Japan, and there is debate on its positioning in the treatment of advanced intrahepatic cancer. A study is currently underway to verify the effects of combining sorafenib therapy and HAIC.

HAIC

HEPATIC ARTERIAL INFUSION chemotherapy has been used in Japan for some time to treat intrahepatic advanced HCC that is not expected to respond to other existing treatment methods. According to the report of the 18th follow-up survey, chemotherapy is used in approximately 5% of cases of primary HCC, and is administrated arterially in 87% of cases (Fig. 2).[9] HAIC enables high-concentration anticancer agents to be administrated directly into the carcinoma, and is also used as a treatment method to keep systemic concentrations of anticancer agent low due to the first-pass effect, with the aim of reducing systemic side-effects. There is little evidence for the efficacy of this approach, with randomized control trials showing no effect in improving survival prognosis. In addition, the therapeutic regimen has not been standardized, and the treatment is associated with many side-effects including hematological toxicities (neutropenia and thrombopenia) and non-hematological toxicities (nausea, vomiting, peptic ulcers, reservoir infection, catheter dislocation and vasculitis along injection site).

In general, HAIC is indicated for patients with multiple intrahepatic lesions or vascular invasion who are excluded from the indications for TACE and other existing treatments or for whom these are not expected to be effective, other than Child–Pugh class C patients with severe liver dysfunction.[1]

In Japan, the main forms used are interferon-combined 5-fluorouracil (5-FU) HAIC,[39,40,43–45] low-dose cisplatin-combined 5-FU HAIC[43,46–48] and HAIC with cisplatin alone.[43,49] All of these have a response rate of approximately 30–40%, and the addition of more curative therapy is known to dramatically improve prognosis in responders. Use of a subcutaneous implantable HAIC reservoir enables HAIC to be administrated in outpatient clinics.[44,45] In terms of side-effects, attention must be paid not only to the side-effects of the anticancer agents used in treatment, but also to complications such as catheter displacement, reservoir infection and peptic ulcer that are specific to hepatic arterial infusion, and the management techniques affect treatment response.[45]

RADIOTHERAPY

RADIOTHERAPY IS ANOTHER treatment option. According to the report of the 18th follow-up survey, this treatment is administrated to only 1.5% of cases,[9] but reports in recent years have described the efficacy of stereotactic radiotherapy, which enables selective irradiation of the tumor alone while avoiding the background liver (which has a low tolerance for radiation), and of intensity-modulated radiotherapy,[50] as well as of good outcomes from particle beam therapies such as proton-beam and carbon-beam therapy.[51,52]

PREVENTION OF RECURRENCE

HEPATOCELLULAR CARCINOMA HAS two mechanisms of recurrence – multicentric carcinogenesis and intrahepatic metastasis – and a high annual recurrence rate of 20–30% even after treatment.[53] Aiming for long-term survival is thus impossible without suppressing this recurrence, even if curative treatment is performed. If the underlying condition is viral hepatitis, interferon therapy is administrated proactively with the aim of viral elimination in the case of hepatitis C, whereas the nucleoside analog entecavir is given for hepatitis B. Even if this cannot be administrated, alanine transferase levels are kept as low as possible and hepatitis proactively suppressed by means of glycyrrhizin, ursodeoxycholic acid, phlebotomy or low-dose long-term interferon therapy, and branched-chain amino acids are administrated and nutritional management implemented with the aim of preventing reduced hepatic reserve at the time of recurrence.

CONCLUSION

IN ADDITION TO the so-called three major cancer treatments of surgery, chemotherapy and radiotherapy, methods of treatment for HCC also include RFA, TACE and liver transplantation. These treatment methods are all major interventions that depend on therapeutic techniques, and it must be understood that treatment procedures vary greatly not only between Japan, Europe and the USA, but also between