Table 4 Differences between the baseline characteristics of 917 untreated patients in whom HBsAg persisted and 213 those who lost HBsAg

Features at the baseline	HBsAg persisted ($n = 917$)	HBsAg lost ($n = 213$)	Differences p value
Age (years)	37 (1–81)	44 (0–80)	<0.001
Men	553 (60.3 %)	152 (71.4 %)	0.003
HBV in family members	349 (38.1 %)	76 (35.7 %)	0.509
Chronic hepatitis	893 (97.4 %)	201 (94.4 %)	0.020
AST (IU/L)	27 (3–1,144)	25 (6–1,776)	0.283
ALT (IU/L)	28 (6–1,960)	27 (6–3,020)	0.389
γ-GTP (IU/L)	22 (1–1,494)	29 (4–1,092)	<0.001
Total bilirubin (mg/dL)	0.6 (0.2–20.1)	0.7 (0.1–4.0)	0.257
Albumin (g/dL)	4.3 (2.0–5.3)	4.4 (1.6–5.7)	0.004
Platelets ($\times 10^3$/mm^3)	203 (40–443)	203 (33–417)	0.473
α-Fetoprotein (μg/L)	3 (1–2,060)	1 (1–478)	0.373
Genotypes [A/B/C/others (%)]	5.7/19.0/73.3/1.9	5.5/24.7/69.2/0.7	<0.001
HBeAg-negative status	663 (72.3 %)	194 (91.1 %)	<0.001
HBV DNA (log copies/mL)	4.9 (<2.1 to >9.1)	3.8 (<2.1 to >9.1)	<0.001
HBsAg (IU/mL)	3,100 (1.94–141,000)	149 (0.06–88,800)	<0.001
HBcrAg (log U/mL)	3.9 (<3.0 to >6.8)	2.9 (<3.0 to >6.8)	<0.001
Wild-type precore sequence	441 (48.1 %)	160 (75.0 %)	<0.001
Wild-type core promoter sequence	320 (34.9 %)	47 (22.0 %)	0.001

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Table 5 Factors influencing the seroclearance of HBsAg in untreated patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	p value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	p value
Age ≥50 years	1.63 (1.19–2.23)	0.002	1.61 (1.09–2.37)	0.018
Male gender	1.08 (0.79–1.48)	0.618		
No HBV infection in family	1.38 (1.02–1.86)	0.037		
Cirrhosis	1.19 (0.73–1.93)	0.484		
AST ≥50 IU/L	1.01 (0.70–1.45)	0.979		
ALT ≥50 IU/L	0.93 (0.68–1.27)	0.633		
γ-GTP ≥20 IU/L	1.17 (0.85–1.61)	0.330		
Total bilirubin ≥1 mg/dL	1.41 (0.80–2.49)	0.239		
Albumin ≥4 g/dL	0.78 (0.51–1.18)	0.239		
Platelets >150 × 10^3/mm^3	0.99 (0.67–1.46)	0.946		
α-Fetoprotein ≤10 μg/L	0.84 (0.48–1.47)	0.543		
Genotype A or B	1.17 (0.81–1.69)	0.410		
HBeAg-negative status	0.78 (0.79–2.07)	0.314		
HBV DNA ≥5 log copies/mL	0.84 (0.58–1.24)	0.383		
HBsAg ≤2,000 IU/mL	1.87 (1.19–2.91)	0.006	1.77 (1.12–2.77)	0.014
HBcrAg ≥4 log U/mL	0.85 (0.50–1.45)	0.555		
Wild-type precore sequence	0.99 (0.60–1.52)	0.967		
Wild-type core promoter sequence	0.78 (0.35–1.73)	0.538		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

contributing to HBsAg seroclearance. The overall rate of HBsAg seroclearance was 1.75 % annually. The annual seroclearance rates of HBsAg are reported to be 1.7 % in Korea [14] and 1.6 % in Taiwan [15–17], as well as 2.5 % in Goto Islands of Japan, where HBV infections are very prevalent [18]. In 1,271 natives in Alaska, the rate of HBsAg seroclearance was 0.7 % annually [19]. These differences could be ascribed, in part, to HBV genotypes distinct among Asian countries and Alaska. Since treatment with IFN and/or nucleot(s)ide analogues has suppressive effects on the development of HCC [6, 20], they may influence HBsAg seroclearance.

Table 6 Differences in baseline characteristics between the 833 treated patients in whom HBsAg persisted and 149 those who lost HBsAg

Features at the baseline	HBsAg persisted (n = 833)	HBsAg lost (n = 149)	Differences p value
Age (years)	41 (13–88)	43 (17–71)	0.285
Men	601 (72.2 %)	124 (83.2 %)	0.004
HBV in family members	496 (59.6 %)	72 (48.3 %)	0.010
Chronic hepatitis	802 (96.3 %)	134 (89.9 %)	0.001
AST (IU/L)	54 (6–2,192)	78 (7–888)	0.010
ALT (IU/L)	93 (8–2,740)	118 (8–1,700)	0.117
γ-GTP (IU/L)	44 (4–1,278)	46 (4–1,278)	0.023
Total bilirubin (mg/dL)	0.7 (0.2–21.2)	0.7 (0.3–8.4)	0.273
Albumin (g/dL)	4.3 (1.1–5.4)	4.5 (1.4–5.3)	0.281
Platelets ($\times 10^3$/mm^3)	182 (40–483)	171 (50–391)	<0.001
α-Fetoprotein (μg/L)	4 (1–1,610)	4 (1–765)	0.682
Genotypes [A/B/C/others (%)]	3.2/10.7/85.1/1.0	5.1/12.4/81.6/0.9	0.565
HBeAg-negative status	230 (27.6 %)	79 (53.0 %)	<0.001
HBV DNA (log copies/mL)	7.8 (<2.1 to >9.1)	8.3 (<2.1 to >9.1)	0.002
HBsAg (IU/mL)	7,880 (0.04–277,000)	1,380 (0.04–188,000)	<0.001
HBcrAg (log U/mL)	6.9 (<3.0 to >6.8)	5.9 (<3.0 to >6.8)	0.003
Wild-type precore sequence	554 (66.6 %)	61 (41.2 %)	0.013
Wild-type core promoter sequence	274 (32.9 %)	67 (45.0 %)	0.836

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Table 7 Factors influencing the seroclearance of HBsAg in treated patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	p value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	p value
Age ≥50 years	1.91 (1.32–2.77)	0.001		
Male gender	2.14 (1.37–3.33)	0.001		
No HBV infection in family	1.58 (1.15–2.19)	0.005	2.22 (2.32–3.94)	0.006
Treatments (interferon vs. others)	2.13 (1.53–2.98)	<0.001	3.15 (1.69–5.87)	<0.001
Chronic hepatitis	3.12 (2.05–4.74)	<0.001		
AST ≥50 IU/L	1.47 (1.04–2.09)	0.031		
ALT ≥50 IU/L	1.29 (0.82–1.92)	0.201		
γ-GTP ≥20 IU/L	1.87 (1.30–2.70)	0.001		
Total bilirubin ≥1 mg/dL	1.35 (0.87–2.08)	0.179		
Albumin ≥4 g/dL	1.11 (0.66–1.86)	0.688		
Platelets ≤150 × 10^3/mm^3	2.10 (1.49–2.96)	<0.001		
α-Fetoprotein ≤10 μg/L	1.33 (0.92–1.92)	0.136		
Genotype A or B vs. others	1.16 (0.74–1.82)	0.529		
HBeAg-negative status	2.53 (1.83–3.50)	<0.001	3.75 (2.09–6.74)	<0.001
HBV DNA ≤5 log copies/mL	2.07 (1.37–3.13)	0.001		
HBsAg ≤2,000 IU/mL	2.29 (1.52–3.47)	<0.001		
HBcrAg ≤4 log U/mL	2.28 (1.31–3.97)	0.003		
Wild-type precore sequence	2.04 (1.18–3.55)	0.011		
Wild-type core promoter sequence	1.18 (0.63–2.21)	0.608		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A176.2/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Therefore, we went on to extend our analysis to untreated patients and those treated with IFN or nucleotide analogues separately. Criteria for upper or lower levels of each parameter were set, taking into consideration the median value or a cutoff value with the lowest p value of the entire 2,112-patient cohort (Table 1), and unified for untreated and treated patients (Tables 5, 7).

Firstly, in the univariate analysis, age, no family history of HBV infection in third-degree or closer relatives, and decreased HBsAg levels lowered the annual rate of HBsAg seroclearance significantly. In multivariate analysis, age $\geq$50 years (RR 1.61, $p = 0.018$) and HBsAg $\leq$2,000 IU/mL (RR 1.77, $p = 0.014$) decreased the annual rate of HBsAg seroclearance significantly. Kato et al. [18] reported high HBsAg seroclearance rates in patients over 40 or over 50 years; in our patients, also, age $\geq$50 years increased RR to 1.61 ($p = 0.018$). As for HBsAg and HBV DNA, low HBsAg and HBV DNA levels increased the HBsAg seroclearance rate to 37.7 %, and therefore, low HBsAg levels are an important factor. In actuality, HBsAg levels $\leq$2,000 IU/mL increased the rate of HBsAg seroclearance with RR 1.77 ($p = 0.014$).

In treated patients, by contrast, age, the male gender, no HBV infections in third-degree or closer relatives, treatment with IFN, chronic hepatitis, high AST levels, high γ-GTP levels, low platelet counts, HBeAg-negative status, low HBsAg levels, low HBcrAg levels and the wild-type precore sequence were significant factors in univariate analysis. In multivariate analysis, no HBV infections in third-degree or closer relatives (RR 2.22, $p = 0.006$), interferon treatments (RR 3.15, $p < 0.001$), and HBeAg-negative status (RR 3.75, $p < 0.001$) were significant factors.

Thus, there were differences in factors predictive of the HBsAg loss between untreated and treated patients. Remarkably, age and HBsAg titer were independent factors in untreated patients, whereas family history and negative HBeAg were independent factors in treated patients. Since this work studied patients who were followed for a long time (>15 years), age and HBsAg titer were factors for clearance of HBsAg in untreated patients. Treated patients, in contrast, would have included more patients with HBeAg, with a good response to antiviral treatment, as well as those without family history who would have been infected with HBV with a sorter duration than those with family history. In other words, most untreated patients were those with favorable clinical course, in whom HBsAg titer gradually decreased and eventually lost it with time. In fact, there would be many such patients, the majority of whom do not visit hospitals and are unaware of HBV infection, who may have unapparent liver disease. Treated patients, on the other hand, would have had higher risks for cirrhosis and HCC, owing to elevated ALT/AST levels; this risk is especially high for patients with a family history of HBV [21]. Therefore, patients with family history would not be able to easily lose HBsAg.

In treated patients, IFN led to HBsAg loss more effectively than other treatments [RR 2.13, $p < 0.001$ (Table 7)]. The immunomodulatory activity of IFN, which is not shared by nucleot(s)ide analogues, would have accelerated the immune response to HBV required for the seroclearance of HBsAg. Of the 333 patients who received IFN, 190 (57 %) were treated with IFN multiply. In them, seroclearance of HBsAg was achieved in 49 of the 190 (26 %) patients with multiple IFN treatments in comparison with 41 of the 143 (29 %) with single IFN treatment. Owing to indications for IFN, patients who received IFN tended to be younger, without previous treatments and higher HBV DNA as well as ALT levels. They might have increased the rate of HBsAg loss that was higher with IFN than other treatments.

Since this is a retrospective cohort study of patients visiting our hospital for more than 15 years, and there has been so much innovation in the treatment of chronic hepatitis B during that period, treated and untreated patients have different backgrounds at the baseline. Hence, treated patients had higher ALT and HBV DNA levels with severer liver disease than untreated patients (Table 3). This might have been responsible, at least in part, for the failure in finding differences in the rate of HBsAg loss between untreated and treated patients (Fig. 2). Future studies will be aimed at analyzing contributing factors in treated and matched controls. This will allow us to analyze factors contributing to HBsAg seroclearance in the treatment of patients with chronic hepatitis B.

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Conflict of interest These authors disclose the following: Dr. Kumada reports having received investigator, lecture, and consulting fees from Dainippon Sumitomo Pharma Co., MSD KK, Bristol-Myers Squibb, Pharma International, Dentsu Sudler, and Hennessey Inc. Dr. Ikeda reports having received investigator, lecture, and consulting fees from Dainippon Sumitomo Pharma Co. No other potential conflicts of interest relevant to this article were reported.

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Original article

Telaprevir is effective given every 12 h at 750 mg with pegylated interferon-α2b and ribavirin to Japanese patients with HCV-1b IL28B rs8099917 TT

*Yoshiiku Kawakami[1,2], Fumitaka Suzuki[3], Yoshiyasu Karino[4], Joji Toyota[4], Hiromitsu Kumada[3], Kazuaki Chayama[1,2,5]**

[1]Department of Gastroenterology and Metabolism, Applied Life Sciences, Institute of Biomedical & Health Sciences, Hiroshima University, Hiroshima, Japan
[2]Liver Research Project Center, Hiroshima University, Hiroshima, Japan
[3]Department of Hepatology, Toranomon Hospital, Tokyo, Japan
[4]Department of Gastroenterology, Sapporo Kosei General Hospital, Hokkaido, Japan
[5]Laboratory for Digestive Diseases, RIKEN Center for Integrative Medical Sciences, The Institute of Physical and Chemical Research (RIKEN), Hiroshima, Japan

*Corresponding author e-mail: chayama@hiroshima-u.ac.jp

Background: The aim of this study is to explore the efficacy, safety and pharmacokinetics of 750 mg telaprevir (TVR) given at 8 or 12 h intervals during triple therapy with pegylated interferon-α2b (PEG-IFN) and ribavirin (RBV) for patients with chronic HCV infection.

Methods: A total of 52 patients with high viral loads of HCV genotype 1b who were expected to respond well to therapy (rs8099917 TT genotype or relapse to previous therapy) were randomly assigned to two groups who were given 750 mg TVR at either 8 h (q8h) or 12 h (q12h) intervals in combination with PEG-IFN and RBV for 12 weeks, followed by 12 additional weeks of treatment with PEG-IFN and RBV alone. The primary end point of the study was undetectable HCV RNA at 12 weeks after the end of treatment (sustained virological response $[SVR]_{12}$).

Results: SVR_{12} rates were 92.3% (24/26) for both q8h and q12h. The changes in mean $\log_{10}$ HCV RNA levels and viral response were also similar in q8h compared to q12h, whereas pharmacokinetic properties such as maximum plasma concentration, area under the concentration-time curve at 24 h and trough plasma concentration of TVR were slightly higher in q8h than in q12h ($P>0.2$). The frequency of TVR discontinuation due to anaemia or renal damage was significantly higher in q12h than in q8h (6/26 [23%] versus 0/20 [0%], respectively; $P=0.02$).

Conclusions: TVR given at 12 h intervals should be considered for patients with lower body weight, especially patients with prior relapse and with IL28B polymorphisms at rs8099917 TT (interferon-λ 4 ss469415590 polymorphism TT/TT) genotype in patients with genotype 1b HCV infection.

Introduction

There are estimated to be 170 million HCV carriers worldwide [1,2]. Approximately 30% of carriers develop serious liver diseases, such as decompensated cirrhosis and hepatocellular carcinoma [3,4]. Eradication of the virus is necessary to prevent the development of severe liver damage in these patients.

Telaprevir (TVR), an HCV NS3/4A serine protease inhibitor, has recently been approved in the US, Canada, the European Union and Japan for treatment of patients with chronic HCV genotype 1 infection. In Phase III studies, sustained virological response (SVR) rates increased significantly in both treatment-naive as well as previously treated patients when TVR was administered in combination with pegylated interferon (PEG-IFN) and ribavirin (RBV) compared to PEG-IFN and RBV alone [5–7]. High SVR rates were also observed in Phase III studies in Japan [8,9]; however, side effects of triple therapy in the Japanese studies were so severe that many patients were forced to discontinue therapy due to adverse events, such as anaemia and fatigue [5–9]. Anaemia, in particular, is commonly associated with triple therapy. The frequency of anaemia ranged from 15% to 19% [5,6] in patients treated with PEG-IFN and RBV alone, whereas in patients treated with triple

therapy, the frequency of anaemia increased to between 30% and 37% [5,6]. In addition, RBV dose-reduction rates and discontinuation rates of TVR treatment due to severe adverse events are higher in Japan than in the US and European Union [5–9]. The higher discontinuation rate may result from taking the same standard prescription dosage of TVR despite the lighter body weight of Japanese patients compared with patients in other countries. Japanese patients also tend to be relatively older, and may therefore be at greater risk of severe side effects due to poorer drug metabolism rates. The aim of this study is thus to compare effects and safety of triple therapy with TVR administered at 12 h intervals compared with the standard 8 h interval regimen. We also studied pharmacokinetics of TVR in both groups of patients to see how the reduction of TVR affects the concentration of TVR.

Methods

Patients

We enrolled patients at Hiroshima University Hospital (Hiroshima, Japan), Toranomon Hospital (Tokyo, Japan) and Sapporo Kosei General Hospital (Hokkaido, Japan). Patients were enrolled from August 2012, and the last patient completed follow-up in May 2013. Criteria for inclusion were age between 20 and 70 years, chronic infection with HCV genotype 1b, and plasma HCV RNA level ≥100,000 IU/ml. We selected patients who were expected to respond well to triple therapy based on one of the following criteria: patients with the treatment-favourable rs8099917 TT genotype in the IFN-λ 3 (IL28B) locus or patients who experienced relapse during prior treatment with PEG-IFN and RBV combination therapy. In order to avoid poor response to reduction of TVR, we excluded patients who were expected to have poor response to the therapy, including prior non-responders to PEG-IFN and RBV therapy (that is, patients who failed to become negative for HCV RNA) and patients with rs8099917 T/G or G/G genotypes. Exclusion criteria also included liver disease due to other causes, decompensated cirrhosis, presence of liver cancer, HBV or HIV infection, renal insufficiency, history of heart disease or cerebral infarction, and pregnancy or current breastfeeding. IL28B rs8099917, IFN-λ 4 (IFNL4) ss469415590 and inosine triphosphate pyrophosphatase (ITPA) polymorphism (rs1127354) were genotyped using the Invader assay (Third Wave Technologies, Madison, WI, USA), TaqMan assay (Life Technologies, Carlsbad, CA, USA) or by direct sequencing, as described elsewhere [10–12]. Amino acid substitutions in the HCV core were determined using direct sequencing of PCR products after extraction and reverse transcription of HCV RNA. Core amino acid substitutions at positions 70 and 91 (core 70 and core 91, respectively) were determined as reported by Akuta *et al.* [13,14]. The demographic and baseline characteristics of patients are shown in Table 1. Median body weight was 62.3 kg and 25 (48%) patients had body weight <60 kg. IFNL4 ss469415590 and IL28B rs8099917 genotypes were completely linked, except in one patient (Additional file 1).

Study design and randomization

This was an exploratory prospective multicenter randomized study. Experimental procedures were approved by the institutional review boards at participating hospitals, and informed consent was obtained from all participants. Sample size was not based on hypothesis testing other than the precision estimate of SVR. If we assume that 80%, 85% and 90% of subjects will have undetectable HCV RNA 12 weeks after the end of therapy (SVR_{12}), then 25 subjects per arm would yield two-sided 95% confidence intervals of 64.3% to 95.7%, 71.0% to 99.0% and 78.0% to 100%, respectively. The study was conducted in accordance with the Declaration of Helsinki, and the trial was registered with UMIN Clinical Trials (UMIN000006758). Randomization was stratified according to the combination of prior treatment experience and amino acid substitution at HCV core amino acid 70 (treatment-naive and wild type, naive and mutant, transient response and wild type, transient response and mutant, non-response and wild type, or non-response and mutant), age (<60 or ≥60 years), gender (male or female) and baseline haemoglobin level (<13 or ≥13 g/dl). As shown in Table 1, the demographic and baseline characteristics were well balanced in the two groups of patients.

Mythos (Osaka, Japan), a third party institute that was not involved in the conduct of the study, randomly allocated the two groups of patients to different doses of TVR by means of computer-generated randomization codes.

Study procedures

TVR was administrated at a randomized dose of 750 mg after meals at 8 h (q8h) or 12 h (q12h) intervals. PEG-IFN-α2b (PegIntron; MSD, Tokyo, Japan) was administered subcutaneously at a dose of 1.5 μg/kg of body weight once weekly, and oral RBV (Rebetol; MSD) was administered at a total dose of 600 to 1,200 mg/day based on body weight. Patients received 12 weeks of treatment with TVR plus PEG-IFN/RBV followed by PEG-IFN/RBV alone for an additional 12 weeks. Follow-up observation was performed for 12 weeks (Additional file 2). RBV dosage was reduced or discontinued as required, based on reduction of haemoglobin levels or the development of adverse events. When haemoglobin decreased

Table 1. Baseline characteristics of patients

Characteristic	TVR 750 mg q8h (*n*=26)	TVR 750 mg q12h (*n*=26)	*P*-value
Gender	-	-	0.77
Male	17	18	-
Female	9	8	-
Age, years	61 (24–68)	61 (37–70)	0.99
Body weight, kg	61.4 (39–82)	63.3 (40–81)	0.76
Body mass index, kg/m^2	23.5 (16.8–32.0)	22.5 (17.8–27.7)	0.61
White blood cell count, cells/mm^3	4,890 (3,500–8,920)	4,995 (2,970–11,830)	0.67
Haemoglobin, g/dl	14.2 (12.2–16.5)	15.2 (11.4–17.4)	0.17
Platelet count, $\times 10^4$ cells/μl	15.9 (5.7–25.3)	16.9 (5.2–25.6)	0.74
ALT, IU/l	36 (16–292)	40 (14–117)	0.62
γ-GTP, IU/l	26 (13–125)	20 (10–192)	0.25
eGFR, ml/min	80 (62–105)	80 (60–120)	0.61
HCV RNA, log IU/ml	6.8 (5.3–7.4)	6.9 (5.2–7.8)	0.26
Previous IFN therapy	-	-	0.42
Treatment-naive	14	11	-
Relapse	9	11	-
Non-response	3	4	-
rs8099917	-	-	0.32
TT	25	26	-
TG	1	0	-
ss469415590	-	-	0.49
TT/TT	24	26	-
TT/ΔG	2	0	-
rs1127354	-	-	0.39
CC	18	20	-
Non-CC	8	5	-
ND	0	1	-
HCV core 70	-	-	0.67
Wild type	17	20	-
Mutant	6	4	-
ND	3	2	-

Data are median (range) or *n*. ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate; IFN, interferon; ND, not done; q8h, every 8 h; q12h, every 12 h; TVR, telaprevir; γ-GTP, γ-glutamyl transpeptidase.

<10 g/dl, the daily dose of RBV was reduced from 600 to 400 mg, from 800 to 600 mg and from 1,000 to 600 mg, depending on the initial dose of each patient. RBV was withdrawn when haemoglobin decreased <8.5 g/dl. Decrease of TVR dose was not permitted, but administration was stopped if necessary due to the development of adverse events.

Efficacy assessments

Serum HCV RNA levels were measured using COBAS TaqMan HCV RNA 2.0 assay (Roche Diagnostics, Tokyo, Japan), with a lower limit of quantification of 25 IU/ml and a lower limit of detection of 10 IU/ml. The lower limit of detection was used in the determination of undetectable HCV RNA at week 4. HCV RNA levels were measured on day 1 and at the following times: weeks 2, 4, 8, 12, 16, 20, 24 and every 4 weeks until 12 weeks after the end of treatment.

End points

The primary end point was the proportion of patients who had undetectable plasma HCV RNA 12 weeks after the end of treatment (SVR_{12}). The secondary end point was the rate of discontinuation of the therapy due to adverse events.

Pharmacokinetic assessments

Blood samples were collected immediately prior to administering the first morning dose, and at week 2 at 1, 2.5, 4, 6, 8 and 12 h after the first dose to determine the concentration of TVR (750 mg q8h or 750 mg q12h) in the plasma. Plasma concentrations of TVR were determined using a HPLC apparatus fitted with a mass spectrometer. Area under the concentration-time curve (AUC) at 24 h ($AUC_{24\ h}$) was calculated by multiplying $AUC_{8\ h}$ by 3 or $AUC_{8\ h}$ by 2. The maximum plasma concentration (C_{max}) and trough plasma concentration (C_{trough}) were

directly determined from the observed values at week 2. RBV concentration was measured prior to the morning dose at week 2.

Safety assessments

Safety assessments including physical examinations, clinical laboratory tests and evaluation of adverse events were performed at each hospital visit during and after treatment at least every 4 weeks until 12 weeks after cessation of the therapy.

Statistical analyses

Analysis was performed on the intention-to-treat population, defined as all randomly assigned patients who received one dose of the study medication. Categorical variables between groups were compared using Fisher's exact test and continuous variables using the Mann-Whitney test. All analyses were performed using R version 2.15.3.

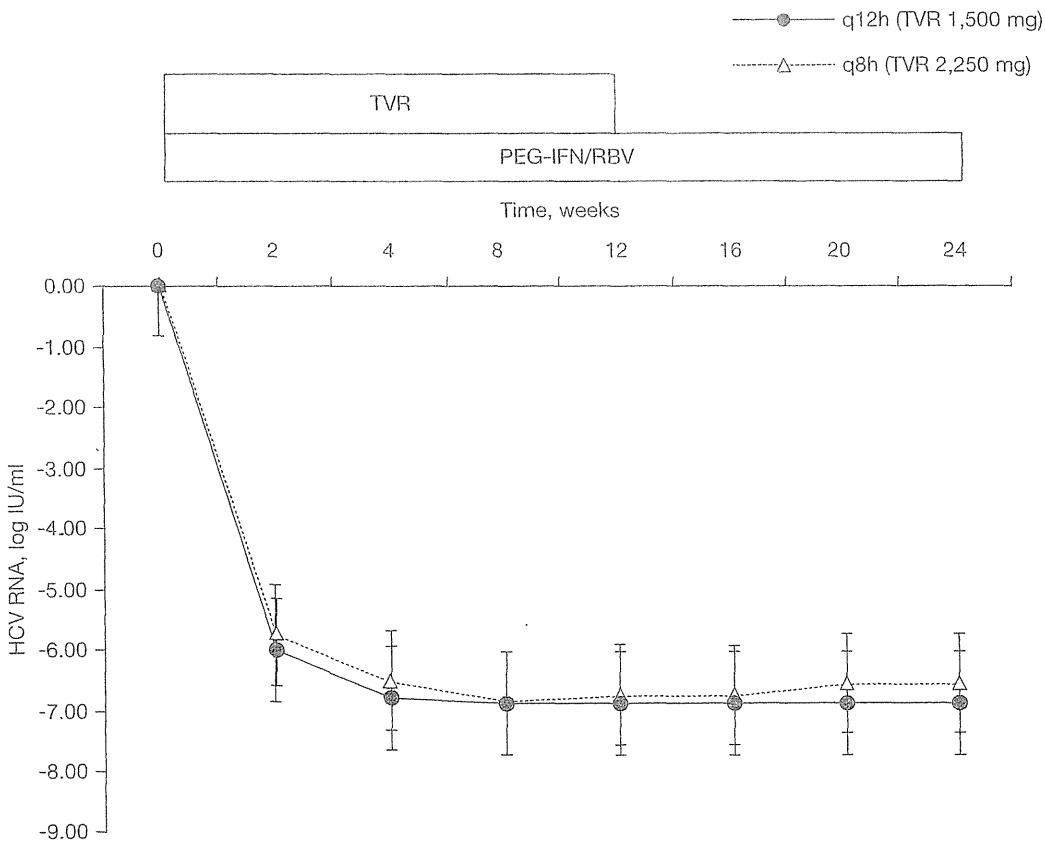
Results

Efficacy

SVR_{12} rates were 92.3% (24/26) for both q8h and q12h (Additonal file 2). The percentage of patients with undetectable HCV RNA at weeks 2, 4, 12, 24 and at 12 weeks after the end of treatment (SVR_{12}) was not statistically different between the two groups of patients (Additional file 2). Similar decreases in mean $\log_{10}$ HCV RNA levels were observed in both groups of patients (Figure 1). The SVR_{12} rate did not differ when the patients were divided by response to previous therapy, age, gender and platelet count (Additional file 1). These results show that the antiviral effect of triple therapy was nearly equivalent between the two patient groups.

Four patients did not achieve SVR_{12}. The characteristics of these four patients were as follows: median 64 years (range 62–65), male/female gender n=3/1, median viral

Figure 1. Decrease of HCV RNA during therapy



Data are shown as mean (SD). PEG-IFN, pegylated interferon; RBV, ribavirin; q8h, every 8 h; q12h, every 12 h; TVR, telaprevir.

load 6.9 log IU/ml (range 5.8–7.2) and median platelet count 17×10^4 cells/μl (range 12–22).

Pharmacokinetics

Mean pharmacokinetic parameters of TVR are shown in Table 2. C_{trough} was slightly lower in the q12h group than in the q8h group. $AUC_{24\ h}$ was also slightly higher in the q8h group than in the q12h group. However, these differences were not statistically significant. C_{max} was similar in both groups of patients.

The mean (SD) of RBV concentration (C_{trough}) at week 2 in the q8h and q12h groups was 1,706 (221) and 1,562 (222) ng/ml, respectively. Although the concentration was slightly higher in the q8h group than in the q12h group, the difference was not statistically significant (P=0.515).

Safety

There were no deaths or serious adverse effects. Adverse events with a frequency of >5% in total patients are listed in Table 3. The overall safety profile was similar in both groups of patients except for the frequency of renal damage. The ratios of discontinuation of all treatment due to adverse events were 12% (3/26) in the q8h group and 15% (4/26) in the q12h group (Additional file 1). Discontinuation of TVR occurred in 42.3% (11/26) of patients in the q8h group and 21.4% (6/28) of patients in the q12h group. Frequency of TVR discontinuation due to anaemia or renal damage was significantly higher in q12h than in q8h (6/26 [23%] versus 0/20 [0%], respectively; P=0.02; Additional file 1).

For anaemia, decreases of mean haemoglobin levels were similar during the initial 6 weeks. Although mean haemoglobin levels continued to decrease in the q8h group, haemoglobin levels stopped decreasing in the q12h group after week 6 (Figure 2). Low haemoglobin (<8.5 g/dl) occurred in 8 (30.8%) patients in the q8h group and 6 (23.1%) patients in the q12h group. The genotype of the ITPA single nucleotide polymorphism (SNP) had no significant effect on the frequency of anaemia. In terms of renal damage, during the 12 weeks of the triple therapy, estimated glomerular filtration rate decreased significantly more in the q8h group than in the q12h group (Figure 3).

Adherence to PEG-IFN and RBV treatment was higher in the q12h group, although the difference was not statistically significant (Additional file 1).

Table 2. Pharmacokinetic parameters of telaprevir at week 2

Pharmacokinetic parameter	TVR 750 mg q8h (n=10)	TVR 750 mg q12h (n=10)	P-value
C_{trough}, μg/ml	2.80 (1.33)	2.00 (0.59)	0.243
1 h, μg/ml	2.93 (1.35)	3.07 (0.81)	0.661
2.5 h, μg/ml	3.60 (1.66)	3.24 (1.22)	0.842
4 h, μg/ml	3.42 (1.40)	3.03 (1.02)	0.661
6 h, μg/ml	3.02 (1.41)	2.51 (0.97)	0.549
8 h, μg/ml	2.48 (1.37)	1.98 (0.77)	0.549
12 h, μg/ml	3.42 (1.47)	1.36 (0.70)	<0.001
C_{max}, μg/ml	3.90 (1.50)	3.74 (0.99)	0.720
$AUC_{24\ h}$, μg•h/ml[a]	74.91 (32.91)	57.16 (18.12)	0.243

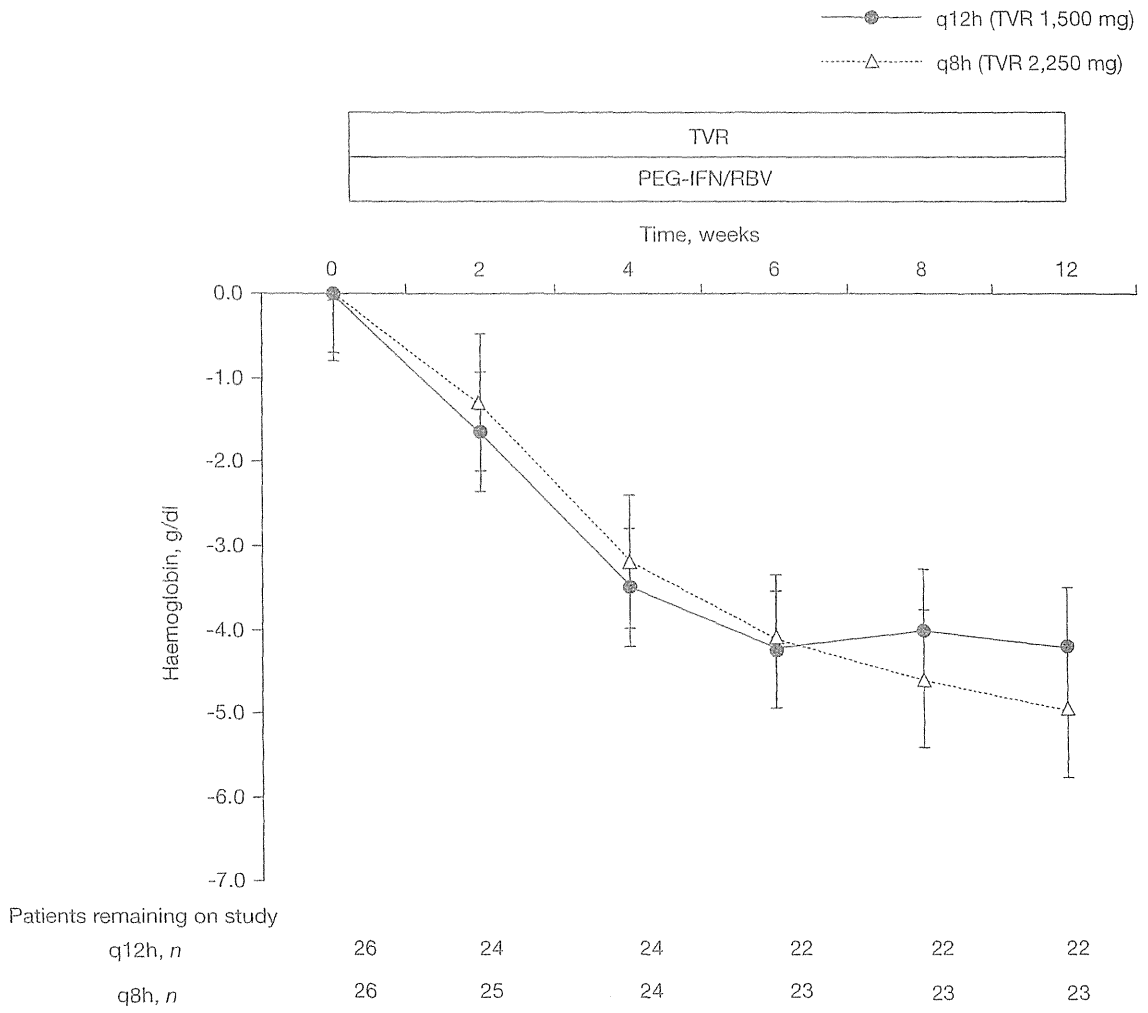
All values are expressed as mean (SD). [a]Area under the curve (AUC) at 24 h ($AUC_{24\ h}$) was calculated by multiplying $AUC_{8\ h}$ by 3 or $AUC_{12\ h}$ by 2. C_{max}, maximum plasma concentration; C_{trough}, trough plasma concentration; q8h, every 8 h; q12h, every 12 h; TVR, telaprevir.

Table 3. Adverse events occurring in >5% of participants

Adverse event	TVR 750 mg q8h (n=26)	TVR 750 mg q12h (n=26)	P-value	All (n=52)
White blood cell count decreased	26 (100)	26 (100)	1.00	52 (100)
Platelet count decreased	26 (100)	26 (100)	1.00	52 (100)
Anaemia	26 (100)	26 (100)	1.00	52 (100)
Blood creatinine increased (eGFR decreased)	21 (80.8)	12 (46.2)	0.02	33 (63.5)
Skin rash	11 (42.3)	13 (50)	0.59	24 (46.2)
Blood uric acid increased	10 (38.5)	6 (23.1)	0.37	16 (30.1)
Anorexia	4 (15.4)	2 (7.7)	0.67	6 (11.5)
General fatigue	3 (11.5)	1 (3.8)	0.61	4 (7.7)

Data are n (%). eGFR, estimated glomerular filtration rate; q8h, every 8 h; q12h, every 12 h; TVR, telaprevir.

Figure 2. Time course of haemoglobin levels during the triple therapy

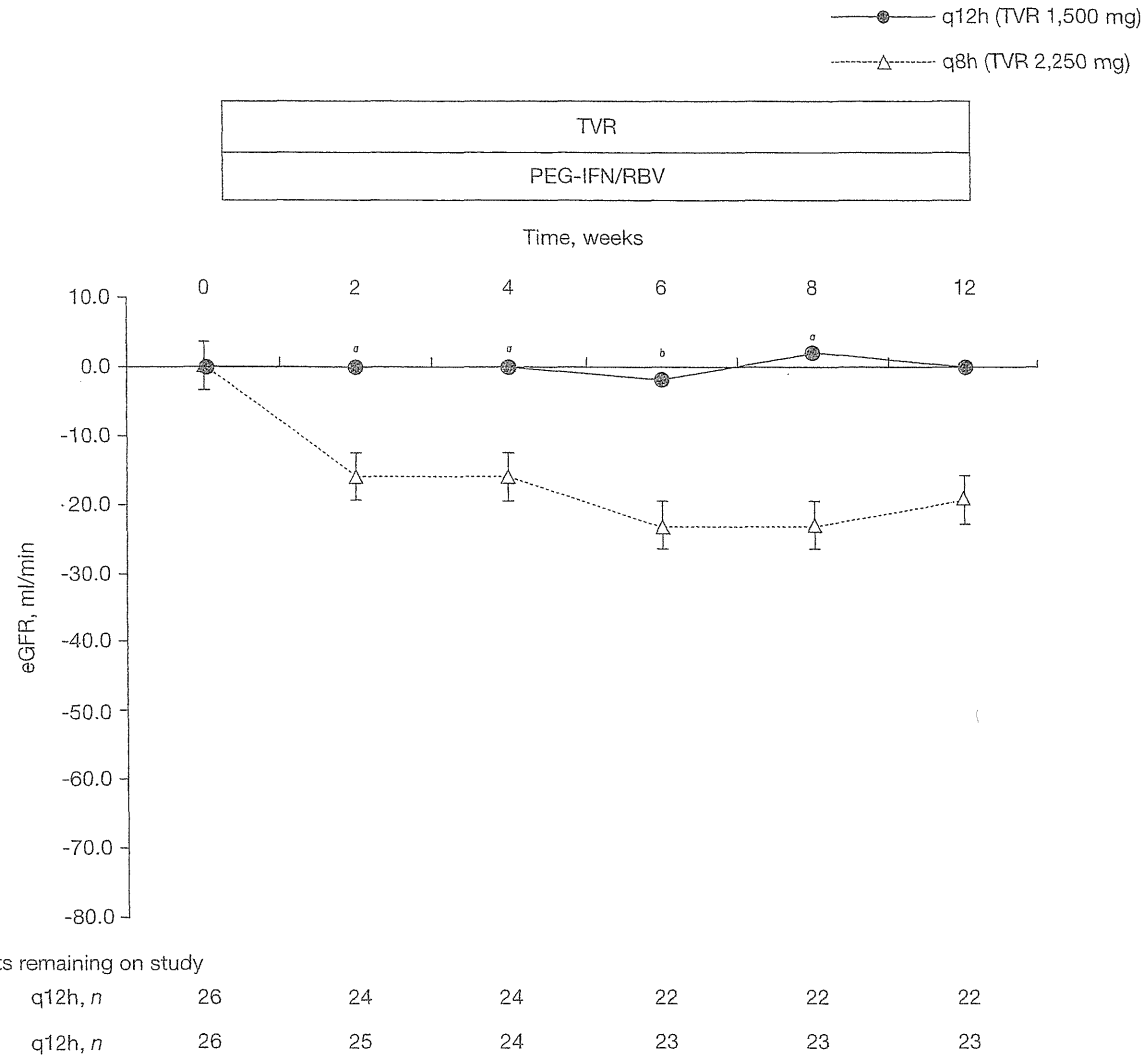


Change from baseline haemoglobin concentrations are noted as mean (SD). PEG-IFN, pegylated interferon; q8h, every 8 h; q12h, every 12 h; RBV, ribavirin; TVR, telaprevir.

Discussion

With the introduction of TVR, the eradication rate of HCV has improved significantly [5–7]. However, severe adverse effects associated with TVR have also been reported, some or which occur more frequently in Japanese patients [8,9]. The dose of TVR for use in triple therapy was determined based on a dose-finding study conducted in the US and Europe [15], which found that the q8h dosage regimen achieved the greatest reduction of HCV RNA. However, body weights of Japanese patients who were treated with TVR, PEG-IFN-α2b and RBV [9] were 61–63 kg compared to 79–91 kg among American and European patients who were treated with boceprevir, PEG-IFN-α2b and RBV combination therapy [16]. As the dose of TVR is the same among countries where triple therapy is approved, we considered the possibility that the dose of TVR might be too high for smaller Japanese patients and could be reduced. Suzuki *et al.* [17] previously reported that the antiviral effect of triple therapy was similar when patients were given TVR at 1,500 mg/day (q8h at 500 mg) compared with those given at 2,250 mg/day (q8h at 750 mg) in the Japanese patients, suggesting that reduction of TVR might be possible. However, the treatment period of their study was only 12 weeks, and the study was a non-randomized controlled study with a small number of patients. Therefore, we conducted a randomized controlled trial to confirm that the dose reduction is as effective as the approved regimen. Therefore, we also attempted to test if TVR is as effective when administered at 12 h intervals instead of 8 h intervals, based on a pharmacokinetics study in which Marcellin *et al.* [18] found no difference in viral response and safety profiles

Figure 3. Time course of eGFR levels from baseline during triple therapy



Statistically significant differences between patients treated with 1,500 mg versus 2,250 mg telprevir (TVR): $^{a}P<0.01$, $^{b}P<0.05$. eGFR, estimated glomerular filtration rate; PEG-IFN, pegylated interferon; q8h, every 8 h; q12h, every 12 h; RBV, ribavirin.

between patients treated with the triple therapy with TVR 2,250 mg (q12h) and TVR 2,250 mg (q8h). Furthermore, Buti *et al.* [19] reported that the effectiveness and safety were similar between patients treated with triple therapy with 2,250 mg TVR (q12h) and 2,250 mg TVR (q8h) in the OPTIMIZE trial (Phase IIIb).

We showed in this study that the effect of TVR given q12h at 750 mg with PEG-IFN-α2b and RBV is the same as TVR given q8h among Japanese chronic hepatitis C patients. However, four patients failed to achieve SVR_{12}, and all treatment was discontinued within 4 weeks in these patients. Safety profiles were similar except for differences in the frequency of anaemia and renal damage. Haemoglobin levels continued to decline only in patients who received the larger 2,250 mg dose, whereas haemoglobin levels plateaued by week 6 in patients who received the 1,500 mg dose. We also found that the 1,500 mg dosage was also accompanied with a lower frequency of renal damage (Figure 3). Incidence of TVR discontinuation was significantly less frequent in patients treated with the 1,500 mg regimen. These results suggest that reduction of TVR to 1,500 mg and administration of the drug q12h is as effective as the approved 2,250 mg dose and is less likely to result in premature termination of TVR therapy (Additional file 1).

We assessed the effect of reduced TVR only in patients who relapsed under previous PEG-IFN/RBV therapy or had the IL28B SNP rs8099917 TT genotype that is associated with a good response to IFN therapy. Patients who had relapsed during previous

PEG-IFN/RBV therapy have been reported to respond well to triple therapy [9]. The majority of patients with the rs8099917 TT genotype have also been reported to successfully eradicate the virus with triple therapy [20,21]. The effect of TVR reduction on patients who are expected to be difficult to treat should be further explored in a different trial.

Until recently it was unknown why SNPs near the IL28B locus, such as rs8099917 and rs12979860, are associated with the outcome of IFN therapy. However, the recent characterization of IFNL4 and its association with polymorphism ss469415590 (TT or ΔG) has shed light on this issue [22]. Genotype ss469415590 TT, which fails to express functional IFNL4, is associated with both eradication of HCV by PEG-IFN plus RBV combination therapy as well as spontaneous clearance of the virus [22]. As this polymorphism is in strong linkage disequilibrium with rs8099917 and rs12979860 in Asian populations [22], it is assumed that in the majority of patients the IL28B and IFNL4 ss469415590 genotypes are in complete linkage disequilibrium, and in fact, there was only one patient who had a discrepancy between ss469415590 and rs8099917 genotypes (Additional file 1). Taken together, patients with ss469415590 genotype TT/TT are expected to be successfully treated with the 1,500 mg regimen.

Our results were obtained from Japanese patients with body weights between 61 and 63 kg in each group of patients (Table 1). Results obtained here should be confirmed in patients with a larger body weight. Alternatively, administration of TVR based on body weight should be considered in order to maintain high eradication rates while reducing the risk of adverse effects. However, it should be noted that the limitations of the study are the relatively small patient numbers and enrolling two main groups including prior relapsers and treatment-naive patients with favourable INFL4 genotypes. A more comprehensive study is essential in the future.

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Disclosure statement

The authors declare no competing interests.

Additional files

Additional file 1: Supplementary tables illustrating a comparison between IFNL3 (rs8099917) and IFNL4 (ss469415590) genotypes; SVR_{12} rates stratified by response to previous therapy, age, gender and platelet count; adverse events leading to discontinuation of all treatment or TVR only; and the rate of treatment completion without reduction or discontinuation can be found at http://www.intmedpress.com/uploads/documents/3050_Kawakami_Additional_File_1.pdf

Additional file 2: Supplementary figures displaying the study design; enrolment and outcomes; and the cumulative rate of undetectable HCV RNA in serum during treatment can be found at http://www.intmedpress.com/uploads/documents/3050_Kawakami_Additional_File_2.pdf

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Short Communication

Potential of a no-touch pincer ablation procedure for small hepatocellular carcinoma that uses a multipolar radiofrequency ablation system: An experimental animal study

Yusuke Kawamura,[1,2] Kenji Ikeda,[1,2] Taito Fukushima,[1,2] Tasuku Hara,[1,2] Tetsuya Hosaka,[1,2] Masahiro Kobayashi,[1,2] Satoshi Saitoh,[1,2] Hitomi Sezaki,[1,2] Norio Akuta,[1,2] Fumitaka Suzuki,[1,2] Yoshiyuki Suzuki,[1,2] Yasuji Arase[1,2] and Hiromitsu Kumada[1,2]

[1]*Department of Hepatology,* [2]*Okinaka Memorial Institute for Medical Research, Toranomon Hospital, Tokyo, Japan*

Aim: Treatment of hepatocellular carcinoma located on the liver surface is frequently difficult because direct puncture of the tumor must be avoided during needle insertion. The aim of this study was to investigate the utility of a no-touch pincer ablation procedure that uses a multipolar radiofrequency ablation (RFA) system for a tumor located on the liver surface.

Methods: The experimental animals were three pigs, and RFA was performed with two internally cooled bipolar electrodes. Three ablative procedures were compared: linear insertion at regular 13-mm intervals (pattern 1; virtual target tumor size, <10 mm); fan-shape insertion, maximum interval 20 mm (pattern 2; virtual target tumor size, <15 mm); and 25 mm (pattern 3; virtual target tumor size, <20 mm). All electrodes were inserted at a 30-mm depth. For patterns 1 and 2, ablation was performed on three other parts of the liver, and for pattern 3, ablation was performed on two other parts.

Results: For the median transverse and longitudinal diameter to the shaft, with the pattern 1 procedure, the ablative areas were 32 mm × 30 mm, and with the pattern 2 procedure, the ablative areas were 27 mm × 30 mm with carbonization of the liver surface. In contrast, with the pattern 3 procedure, the ablative areas were 45 mm × 26 mm; however, the ablative margin did not reach the surface, and carbonization was not apparent.

Conclusion: The no-touch pincer ablation procedure (with an electrode interval of ≤20 mm) may be useful when performed with two internally cooled bipolar electrodes for small nodules that protrude from the liver surface.

Key words: bipolar, hepatocellular carcinoma, multipolar, no-touch ablation, radiofrequency ablation

INTRODUCTION

AMONG THE AVAILABLE treatment options for hepatocellular carcinoma (HCC), surgical resection is generally considered to be a local eradication method that can provide a satisfactory long-term outcome.[1–8] Recent advances in imaging procedures have led to increased detection of early-stage HCC and to improved survival due to the increased identification of patients in whom hepatic resection is possible.[9,10]

For patients who are not eligible for surgery for various reasons (e.g. lack of sufficient liver function for surgical resection), percutaneous local therapy is a viable therapeutic option. Several local ablation therapies are available, including percutaneous ethanol injection, percutaneous acetic acid injection, cryotherapy, percutaneous microwave coagulation therapy and radiofrequency ablation (RFA). In addition to surgical resection, local ablation therapies, particularly RFA, are considered to be local eradication methods for HCC that can provide good long-term outcomes.[11] Therefore, in recent years, RFA has become a widely used option for the primary treatment of small-size HCC. However, we often

Correspondence: Dr Yusuke Kawamura, Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan. Email: k-yusuke@toranomon.gr.jp

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encounter cases of HCC that are difficult to treat with RFA as a result of tumor location, especially nodules that protrude from the liver surface. In addition, a relationship between percutaneous local approaches to HCC (including tumor biopsy) and tumor seeding has been reported previously,[12,13] and with regard to the risk of treatment-related tumor seeding, the following risk factors have been reported: tumor size, tumor location (subcapsular portion), α-fetoprotein level, tumor stage and histopathological grade.[14,15] Therefore, a no-touch approach to local therapy may be considered an ideal treatment method for HCC.

Recently, a multipolar ablation system became available. Until now, in Japan, monopolar electrodes have typically been used, and the present cases are usually treated with some technical arrangement. For example, in the case of using a multi-tined expandable electrode, after obliquely inserting the electrode to avoid direct puncture of the target tumor, the multi needles are expanded toward the target tumor via non-tumor tissue, or in the case of using an internally cooled electrode, multiple insertions are made to avoid direct puncture of the target tumor, and RFA is performed after each insertion. However, these methods do not always provide enough of a treatment effect due to the influence of uncertain treatment procedures and natural, direct puncture to a tumor is indispensable. In contrast, a multipolar ablation system that uses an internally cooled bipolar electrode can combine the use of one to three electrodes at the same treatment session. When three electrodes are used, this system can treat large tumors; however, in the case of small tumors, it is not really necessary to use three electrodes to treat the target tumor. In addition, when we used this multipolar ablation system, usually electrodes were inserted into HCC, but in theory, this system can use no-touch ablation. However, to our knowledge, there are no technical reports that describe a non-direct punctual RFA method that uses a bipolar ablation system for HCC located on the liver surface. In this experimental animal study, we assumed that a small (<20 mm) HCC nodule protruded from the liver surface, and examined proper pincer ablation methods using two internally cooled bipolar electrodes.

METHODS

Summary of experimental procedures

WE USED A bipolar RFA device (CelonPOWER System; OLYMPUS Winter & Ibe GmbH [Telto, Germany]) and two internally cooled bipolar electrodes (30-mm, 15-G, CelonProSurge; OLYMPUS Winter & Ibe GmbH). RFA was applied in the livers of three normal female domestic pigs (each pig's weight was 60 kg) under general anesthesia maintained until killing. The abdomen was opened so that the needle could be inserted under an ultrasonography (US) guide directly into the upper region of the liver where the thickness was larger than 3.5 cm. As a pig liver consists of five thin lobes, RFA sessions were performed two to three times in each liver for evaluation of the "no-touch pincer ablation procedure". After the experiments were completed, the animal was killed, and the ablated liver lobes were excised immediately. The specimen was cut in the plane of the needle tract and photographed to evaluate the shape and size of the ablated zone (white zone). The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Toranomon Hospital.

Protocol of the no-touch pincer ablation procedure

We used a bipolar RFA device (CelonPOWER System; OLYMPUS Winter & Ibe GmbH), and all ablation procedures were performed with two internally cooled bipolar electrodes (30-mm, 15-G, CelonProSurge; OLYMPUS Winter & Ibe GmbH). Internal liquid circulation of the applicator enables the efficiency of coagulation to be increased. The delivery rate was set to 30 mL/min of saline solution at room temperature. The liquid flow was provided by a triple peristaltic pump, which is part of the system. The electrodes were operated by a power control unit working at 470 kHz and providing a maximum output power of 250 W (OLYMPUS Winter & Ibe GmbH). In this study, output power and total energy in each session were fixed at 60 W and 25 kJ, respectively, according to the dosimetry table for the bipolar RFA system (CelonPOWER System; OLYMPUS Winter & Ibe GmbH).

With regard to the ablation protocol, we performed the following three types of ablation procedure: linear insertion, at regular 13-mm intervals (pattern 1); fan-shape insertion, maximum interval of 20 mm (pattern 2); and 25 mm (pattern 3). All electrodes were inserted at a 30-mm depth from the liver surface under a US guide (Fig. 1). Each ablation procedure was performed for the following number of times: pattern 1, three sessions; pattern 2, three sessions; and pattern 3, two sessions. In this study, we assumed that the size of the virtual target tumor was less than 10 mm in pattern 1,

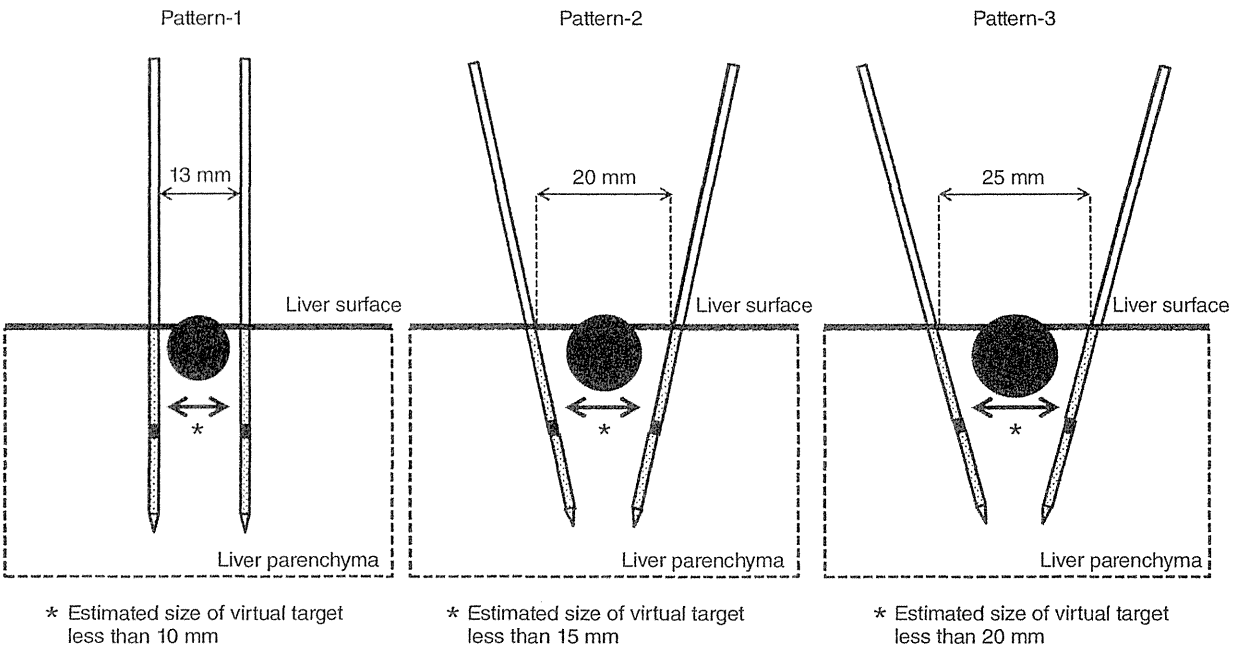


Figure 1 Protocol for a pincer ablation procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface.

less than 15 mm in pattern 2 and less than 20 mm in pattern 3.

Measurement procedure of the ablative margin

After completion of the experiments, the animal was killed and the ablated liver lobes were excised immediately. The specimen was cut in the plane of the needle tract and photographed to evaluate the shape and size of the ablated zone (white zone).

Statistical analysis

The size of the ablated zone and the duration of ablation were compared among the three groups with the Kruskal–Wallis test. All values are expressed as medians. A *P*-value of less than 0.05 denoted the presence of a statistically significant difference.

RESULTS

Features of the no-touch pincer ablation procedure

THE THREE TYPES of pincer ablation procedure applied to the pig liver were performed in the area shown in Figure 2(a).

Table 1 summarizes the features of each pincer ablation procedure for the treatment of the virtual target located on the liver surface.

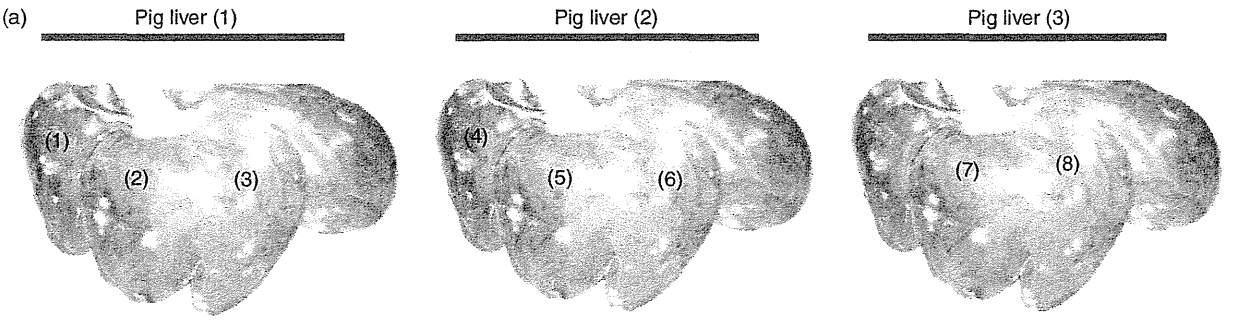
In the median (range) transverse and longitudinal diameter to the shaft, ablative areas were: pattern 1, 32 (27–35) mm × 30 (30–35) mm; pattern 2, 27 (25–35) mm × 30 (30–32) mm; and pattern 3, 45 (40–50) × 26 (25–27) mm. There were no significant differences in the size of each ablative area among the three ablation procedures. However, with the pattern 3 procedure, the transverse diameter to the shaft was larger than with the other procedures, and as a result, the ablative form was flatter. On the other hand, patterns 1 and 2 acquired sufficient ablative areas that covered the liver surface with carbonization of the surface; however, with pattern 3, the ablative areas did not reach the liver surface, and carbonization of the liver surface was not apparent (Fig. 2b–d).

In addition, there were no significant differences among ablation procedures in the duration of ablative time.

DISCUSSION

WE OFTEN ENCOUNTER cases of HCC that are difficult to treat with RFA as a result of tumor location, especially nodules that protrude from the liver

(a)



Ablative pattern	*Location*
Pattern-1: linear insertion, at regular 13-mm intervals	(1)(2)(5)
Pattern-2: fan-shape insertion, maximum interval, 20 mm	(3)(4)(7)
Pattern-3: fan-shape insertion, maximum interval, 25 mm	(6)(8)

(b) Representative ablative images: pattern-1

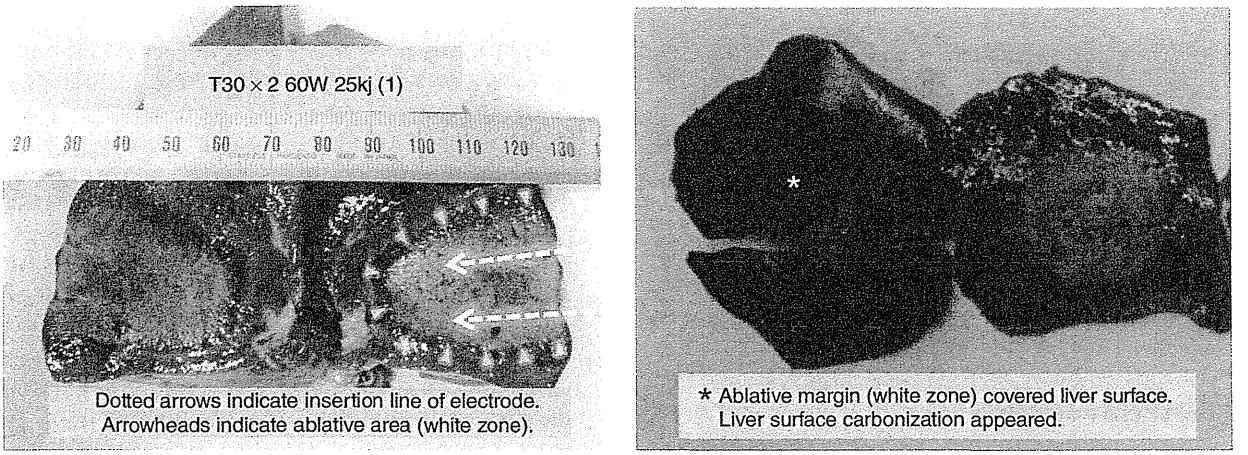
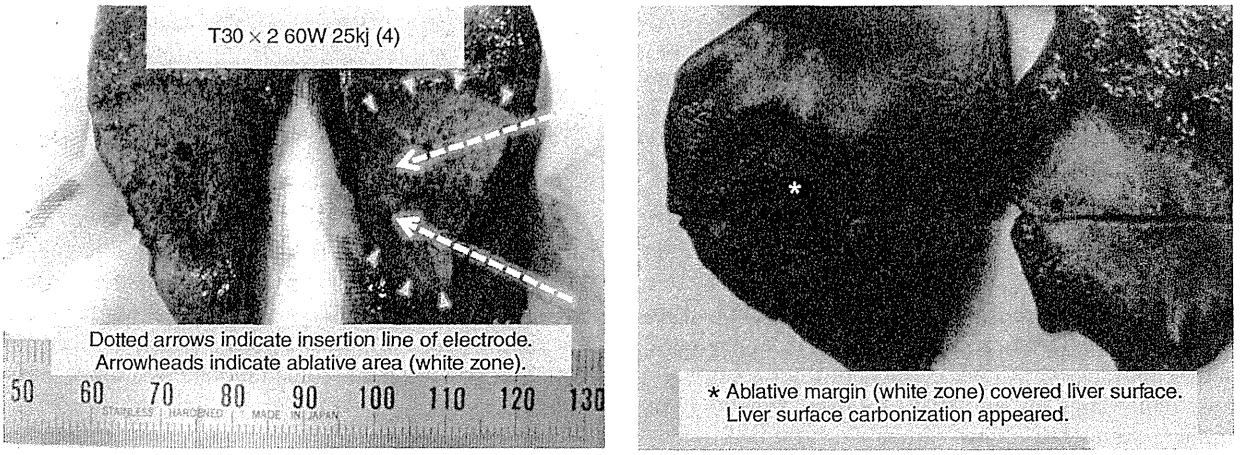


Figure 2 (a) Schema of the ablative areas of each pincer ablation procedure in the three pig livers. (b) One of the ablative shapes and the margin achieved with the pattern 1 procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface. With this pattern, we inserted the electrodes linearly (maximum interval for each electrode was 13 mm). The ablative margin covered the liver surface with carbonization of the liver surface. (c) One of the ablative shapes and the margin achieved with the pattern 2 procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface. With this pattern, we used a fan-shape insertion method (maximum interval for each electrode was 20 mm). The ablative margin covered the liver surface with carbonization of the liver surface. (d) Ablative shape and margin achieved with the Pattern 3 procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface. With this pattern, we used a fan-shape insertion method (maximum interval for each electrode was 25 mm). The ablative area close to the liver surface was larger than with the other procedures. However, the ablative margin did not cover the liver surface, and carbonization of the liver surface was not apparent.

(c) Representative ablative images: pattern-2



(d) Representative ablative images: pattern-3

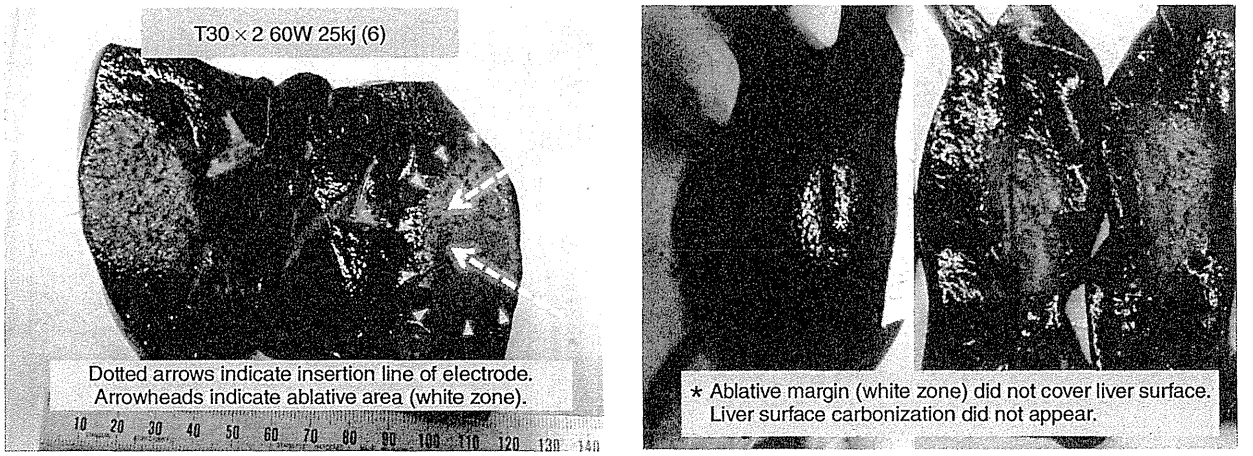


Figure 2 *Continued*

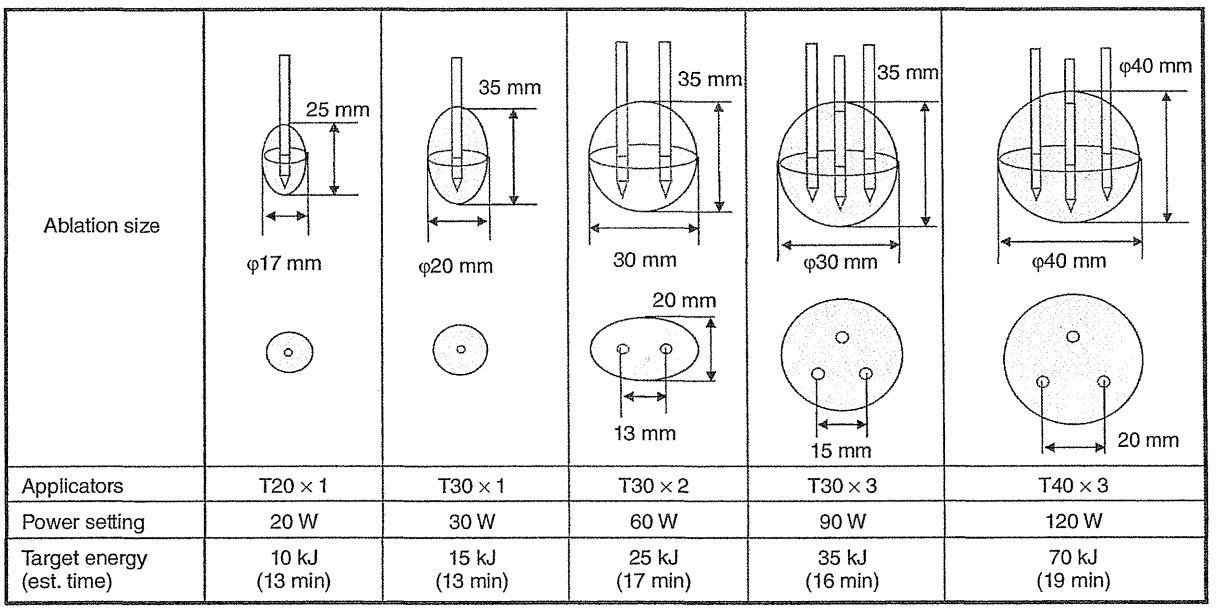
surface. In these situations, a multipolar ablation system that uses internally cooled bipolar electrodes may be suitable for treatment. With a multipolar ablation system, we can combine the use of one to three electrodes at the same treatment session, and when three electrodes are used, this system can treat a large tumor. However, in the case of small tumors (<20 mm), it is not really necessary to use three electrodes for treatment of the target tumor. However, in the dosimetry table of this bipolar system in Figure 3, which was made from previously reported early clinical data[16] and basic analysis, when two internally cooled bipolar electrodes are used (30 mm, 15-G, CelonProSurge; OLYMPUS Winter & Ibe GmbH), the recommended interval of each electrode in this system was 13 mm. With this regulation, we can treat only small tumors (<13 mm) when we perfume no-touch pincer ablation using two electrodes. Therefore, in this study we assumed a virtual target tumor with a tumor diameter less than 20 mm, and investigated the efficacy of a no-touch pincer ablation procedure and the maximum size of the tumor using two internally cooled bipolar electrodes for nodules that

Table 1 Features of each pincer ablation procedure for the treatment of the virtual target located on the liver surface

	Pattern 1			Pattern 2			Pattern 3		*P*
	1	2	3	1	2	3	1	2	
Duration	13′46″	13′16″	12′58″	14′38″	13′50″	13′30″	13′05″	12′40″	$P = 0.151$
Ablated area									
Transverse diameter, mm	27	35	32	25	27	35	45	40	$P = 0.113$
Longitudinal length, mm	35	30	30	32	30	30	27	25	$P = 0.102$
Ablated area covered liver surface	Yes	Yes	Yes	Yes	Yes	Yes	No	No	
Liver surface carbonization appeared	Yes	Yes	Yes	Yes	Yes	Yes	No	No	

protrude from the liver surface. In addition, we investigated only the fan-shape insertion method at a maximum interval of 20–25 mm. The reason for this is that in an actual RFA procedure, it is occasionally difficult to insert two electrodes in the same intercostal space for slightly large nodules that protrude from the liver surface; therefore, in this study, we examined a fan-shape ablation method that assumed two different intercostal approaches. Our results showed that with the pattern 3 treatment procedure, we could not acquire a sufficient ablative margin to the side of the liver surface. From these results, tumors of 20 mm or more may not be suitable for a no-touch pincer ablation procedure that uses two internally cooled bipolar electrodes in this bipolar system.

In contrast, with the pattern 1 and 2 treatment procedures, we acquired a sufficient ablative margin to the side of the liver surface with carbonization of the liver



- The data are based on Frericks et al., Radiology (2005) 237: 1056–1062. The reported average efficacy was ~0.5 millitre ablation volume per kilojoule.
  From these data, the required energy for an ablation sphere or ellipsoid of given diameter was calculated.
- The application of blood flow interruption (e.g. Pringle's manoeuvre, embolization) allows for a significant reduction of the target energy.

Disclaimer: this dosimetry table does not replace the monitoring of actual ablation sizes. The ablation diameters are approximations based on statistical data; they are not guaranteed for individual clinical cases. Ablation size and shape as well as the procedure time may significantly vary due to tumor physiology and vascular structure. A deviation from the recommended applicator distances may also have an impact on the ablation dimensions.

Figure 3 Dosimetry table for the CelonPOWER system (in Japan).

surface. These results may indicate that tumors of less than 15 mm are candidates for the no-touch pincer ablation procedure that uses two internally cooled bipolar electrodes in this bipolar system.

Finally, this experimental animal study had some limitations. First, the number of animals was very small, and the target tumor was a virtual tumor. Second, an additional examination regarding a no-touch linear insertion procedure for maximum intervals of 20 mm and 25 mm for each electrode was not enforced. Third, we could not investigate the same fan-shape ablation procedure using monopolar RFA in this study, because we assumed it would be too difficult to carry out a two-step insertion method using a monopolar electrode under the influence of a first ablation for nodules that protrude from the liver surface. Fourth, we could not investigate the pathological changes in the ablative area in this study. Therefore, with only these study results, it may not be possible to draw conclusions regarding the utility of the fan-shape insertion method using a bipolar RFA device. To solve these problems, we must carry out an additional large-scale study that includes pathological examination in the near future.

Finally, to summarize the points to be noted at the time of performing the pincer ablation procedure, first, we should insert the needle carefully under US guidance, because in this procedure, measuring the distance of the needle tip from the liver surface and the two needle intervals on the liver surface correctly is the most important point.

Second, with this procedure, we should pay attention to the risk of thermal damage to the visceral peritoneum. Therefore, if possible, thermal protection using measures such as artificial ascites should be considered.

Third, in this study, we did not observe a portal or hepatic vein thrombus in the ablative area. However, this study was performed mainly in the vicinity of the liver surface, and usually this area does not include large vessels. Therefore, we need to use caution as with monopolar ablation when we ablate near large vessels.

In conclusion, the no-touch pincer ablation procedure (with an electrode interval of ≤20 mm) may be useful when performed with two internally cooled bipolar electrodes for small HCC tumors that protrude from the liver surface.

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