East Asia, including Japan, Korea, and Northern China, were determined by the PCR-RFLP method described previously.[12]

Quantification of HBV DNA and Sequencing. HBV DNA sequences spanning the S gene were determined by real-time detection PCR according to the method of Abe et al.,[25] with the detection limit of 100 copies/mL. HBV DNA sequences bearing core promoter, precore region, and the core gene were amplified by PCR with hemi-nested primers by the method described previously.[10] Negative samples were tested by another more sensitive second-round PCR with HB7F and HBV1917R (5′-CTC CAC AGT AGC TCC AAA TTC TTT A-3′). Thereafter, PCR products were directly sequenced with Prism Big Dye (Applied Biosystems, Foster City, CA) in the ABI 3100 DNA automated sequencer.

Construction of Plasmid and Site-Directed Mutagenesis of HBV DNA. Serum samples were obtained from two patients infected with HBV/Bj and a patient with Ce. HBV DNA was extracted from 100 μL serum using QIAamp DNA blood kit (QIAGEN, GmbH, Hilden, Germany). Four primer sets were designed to amplify two fragments covering the entire HBV genome. Amplified fragments were inserted into pGEM-T Easy Vector (Promega, Madison, WI) and cloned in DH5a competent cells (TOYOBO, Osaka, Japan). At least five clones of each fragment were sequenced and the consensus sequence determined. Among them, those containing the consensus sequence were identified and adopted as templates for further construction. Finally, 1.24-fold the HBV genome (nt 1413-3215/1-2185), just enough to transcribe oversized pregenome and precore mRNA, was constructed into pUC19 vector (Invitrogen Corp., Carlsbad, CA). For site-directed mutagenesis, the wild-type HBV was digested by *HindIII* and *EcoO65I* and ligated with the fragment carrying T1762/A1764 to produce 1.24-fold the genome carrying the core-promoter double mutation. Similarly, 1.24-fold the HBV genome with the precore stop-codon mutation (1896A) was generated. Further details are available online at: http://interscience.wiley.com/jpages/0270-9139/suppmat/index.html.

Cell Culture and DNA Transfection. For the standard replication assay, 10-cm-diameter dishes were seeded with 1×10^6 Huh7 cells each. After 16 hours of culture, cells were transfected with 5 μg DNA construct using the FuGENE 6 transfection reagent (Roche Diagnostics, Indianapolis, IN) and harvested 3 days later. Transfection efficiency was measured by cotransfection with 1 μg reporter plasmid expressing secreted alkaline phosphatase and estimating its enzymatic activity in the culture supernatant.

Southern Blot Hybridization. HBV DNA samples from cells at day 3 in culture were separated on 1.2% (wt/vol) agarose gel, transferred to a positive-charged nylon membrane (Roche Diagnostics), and hybridized with full-length HBV DNA labeled with alkaline phosphatase. Detection was performed with CDP-star (Amersham Biosciences, Piscataway, NJ), and signals were analyzed in the LAS-1000 image analyzer (Fuji Photo Film, Tokyo, Japan).

Statistical Analysis. Categorical variables were compared between groups by the chi-squared test and noncategorical variables by the Mann-Whitney *U*-test. A *P* value less than .05 was considered significant. Multivariate analyses with logistic regression were used to determine independent factors for fulminant hepatitis. STATA Software (StataCorp LP, College Station, TX) version 8.0 was employed for analyses.

Results

Demographic and Clinical Differences in Patients Infected With Various HBV Genotypes/Subgenotypes. Genotypes of HBV were not classifiable in 28 (8%), and sufficient clinical data were not available in 7 (2%) of the 336 patients with acute hepatitis B. Exclusive of these 35 patients, 301 (90%) were left for evaluation of HBV genotypes in reference to clinical outcome.

HBV genotypes/subgenotypes were Aa in 10 (3%), Ae in 33 (11%), Ba in 22 (7%), Bj in 22 (7%), Cs in 11 (4%), Ce in 192 (64%), D in 5 (2%), and G in 6 (2%); none of them were infected with F or H (Table 1). All six patients with HBV/G were co-infected with another genotype; Ae in two, Ba in two, and Ce in the remaining two. The mean age was lower in the patients with HBV/Ae than Ba ($P = .0001$), Aa ($P < .01$), Bj or Cs ($P < .05$ for each) and Ce than Ba ($P < .05$). Men predominated in HBV infections with foreign (Ae and Ba) compared with domestic genotypes (Bj and Ce) ($P < .05$).

HBeAg was detected in 79% of patients with HBV/Ae at a frequency much higher than that with Bj ($P < .005$), Ce ($P < .001$) or Ba ($P < .05$). HBeAg in four of the six (67%) patients with HBV/G was coded for by HBV of the other genotypes co-infecting them, because it has two stop codons and an insertion in the core gene that prohibit encoding HBeAg.[21] HBV DNA levels as well as HBeAg-positive rates at the presentation were higher in HBV/Ae than Ce ($P < .005$) or Bj ($P < .05$) infection.

The peak alanine aminotransferase (ALT) level was higher in HBV/Bj than Ae infection ($P < .05$). Fulminant hepatitis was significantly more frequent in patients infected with HBV/Bj (55%) than the other genotypes ($P < .05$); it occurred in two of the five (40%) patients with HBV/D, also. In reflection of severe clinical course,

Table 1. Clinical Characteristics of Patients Acutely Infected With HBV of Distinct Genotypes/Subgenotypes

Features	Genotypes/Subgenotypes							
	Aa (n = 10)	Ae (n = 33)	Ba (n = 22)	Bj (n = 22)	Cs (n = 11)	Ce (n = 192)	D[a] (n = 5)	G[a,b] (n = 6)
Age (years)	42.2 ± 13.1	31.2 ± 10.3[d]	41.5 ± 10.7[e]	43.5 ± 19.1	38.5 ± 11.1	36.3 ± 15.0	38.6 ± 20.8	42.7 ± 17.5
Men	8 (80%)	30 (91%)[f]	19 (86%)[g]	9 (41%)	7 (64%)	122 (64%)	2 (40%)	6 (100%)
HBeAg positive	7 (70%)	26 (79%)[h]	11 (50%)	8 (36%)	8 (73%)	101 (53%)	1 (20%)	4 (67%)
ALT (IU/L)	1875 ± 759	2070 ± 1113[i]	2523 ± 1185	3472 ± 2720	2269 ± 995	2610 ± 1719	2559 ± 1672	2142 ± 722
Duration of elevated ALT (weeks)[c]	7.9 ± 5.8	9.5 ± 6.2	8.8 ± 3.7[j]	6.0 ± 2.5	10.1 ± 7.5	7.7 ± 5.1	5.7 ± 2.1	9.8 ± 1.5
Total bilirubin (mg/dL)	14.1 ± 10.3	9.0 ± 7.2	9.3 ± 5.9	10.9 ± 9.0	11.0 ± 13.8	9.8 ± 10.7	8.2 ± 2.2	13.0 ± 7.8
HBV DNA (log copies/mL)								
Median	4.76	6.08[k]	5.15	4.93	5.61	4.94	5.91	5.97
(range)	(2.90-8.08)	(2.00-8.46)	(2.00-8.19)	(2.00-8.44)	(2.00-8.50)	(2.00-9.06)	(2.00-8.37)	(3.35-7.11)
<2.00 (undetectable)	0 (0%)	1 (3%)	2 (9%)	3 (14%)	2 (18%)	28 (15%)	1 (20%)	0 (0%)
Medication with								
Lamivudine	1 (10%)	9 (27%)	2 (9%)	5 (23%)	2 (18%)	28 (15%)	4 (80%)	2 (33%)
Steroid	0	3 (9%)	0	5 (23%)	1 (9%)	16 (8%)	0	0

[a]Patients with HBV genotype D or G were not included in the analysis.
[b]All patients with HBV genotype G were co-infected with HBV of another genotype; Ae in two, Ba in two, and Ce in two.
[c]Exclusive of the 16 patients who died of fulminant hepatitis, 3 receiving liver transplantation and 10 without clinical data available.
[d]$P = .0001$, Ae vs. Ba. $P < .01$, Ae vs. Aa. $P < .05$, Ae vs. Bj or Cs.
[e]$P < .05$, Ba vs. Ce.
[f]$P = .0001$, Ae vs. Bj. $P < .005$, Ae vs. Ce.
[g]$P < .005$, Ba vs. Bj. $P < .05$, Ba vs. Ce.
[h]$P < .005$, Ae vs. Bj. $P < .01$, Ae vs. Ce. $P < .05$, Ae vs. Ba.
[i]$P < .05$, Ae vs. Bj.
[j]$P < .01$, Ba vs. Bj. $P < .05$, Ba vs. Ce.
[k]$P < .005$, Ae vs. Ce. $P < .05$, Ae vs. Bj.

the peak ALT level tended to be high in patients with HBV/Bj.

Presumed infection routes of 301 patients were sexual transmission in 172 (57%), blood transfusion in 4 (1%), medical accidents in 17 (6%), and unknown in the remaining 108 (36%).

Clinical Outcome of Patients With Acute Hepatitis B. Fulminant hepatitis developed in 40 (13%) patients. To cope with severe acute liver disease, lamivudine and steroid were administered to 53 (18%) and 25 (8%) patients, respectively. Fulminant hepatitis led to death in 16 (5%) patients, and three (1%) received liver transplantation. Exclusive of the 40 patients with fulminant hepatitis who received various treatments and five without clinical data, 256 (85%) were followed for the chronic outcome (Fig. 1). Serum ALT levels stayed elevated for longer than 24 weeks for the diagnosis of chronic hepatitis in eight (3%) of them. Among them, five had cleared HBsAg from serum until then, and therefore, their liver function abnormality was not attributed to persistent HBV infection. Table 2 summarizes persistence of HBV infection in the 256 patients with acute hepatitis; 253 (99%) lost serum HBsAg by 6 months. Hence, HBV infection evolved into chronicity in only 3 of the 256 (1%) patients, representing 2 of the 32 (6%) infected with HBV/Ae and 1 of the 21 (5%) with Ba. All of the three with chronic outcome had low-titered IgG anti-HBc at the presentation, and two of them had been negative for HBsAg before the presentation. None of them had received lamivudine or steroid treatment during their acute phase of illness. Of the patients without antiviral therapy, chronic outcome was significantly more frequent in those infected with HBV/Ae than non-Ae genotypes (9% 2/23 vs. 0.5% 1/187, $P = .032$).

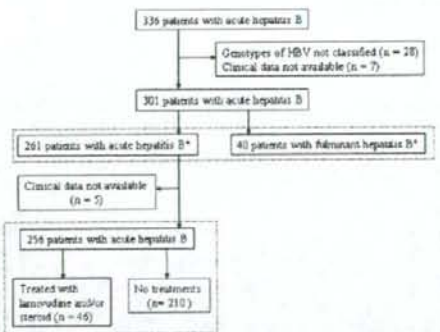

Fig. 1. A flow diagram of 336 patients studied. Comparison was made between patients with fulminant and acute self-limited hepatitis (upper dotted area), and the chronicity was compared between patients with and without treatments (lower dotted area). *Of 301 patients, 37 were negative for HBV DNA, including 27 with acute and 10 with fulminant hepatitis.

Table 2. Persistence of HBV Infection in the Patients With Acute Hepatitis Who Did or Did Not Receive Lamivudine or Steroid

Treatment	Total	Genotypes/Subgenotypes							
		Aa (n = 8)[a]	Ae (n = 32)[a]	Ba (n = 21)[a]	Bj (n = 10)[a]	Cs (n = 10)[a]	Ce (n = 167)[a]	D (n = 3)[a]	G (n = 5)[a]
Total (n = 256)	3/256 (1.2%)	0	2/32 (6%)c	1/21 (5%)	0	0	0	0	0
Lamivudine (n = 36)[b]	0/36 (0%)	0/1 (0%)	0/9 (0%)	0/2 (0%)	0	0/1 (0%)	0/19 (0%)	0/2 (0%)	0/2 (0%)
Steroid (n = 16)[b]	0/16 (0%)	0	0/3 (0%)	0	0	0/1 (0%)	0/12 (0%)	0	0
Neither	3/210 (1.4%)	0/7 (0%)	2/23 (9%)c	1/19 (5%)	0/10 (0%)	0/8 (0%)	0/139 (0%)	0/1 (0%)	0/3 (0%)

[a]Exclusive of 40 patients with fulminant hepatitis and 5 without clinical data available.
[b]Six patients received steroid along with lamivudine.
[c]$P < .05$, Ae vs. non-Ae.

Comparison Between Patients With Fulminant and Acute Self-Limited Hepatitis. Table 3 compares demographic, clinical, and virological characteristics between the 40 patients with fulminant and the 261 with acute self-limited hepatitis for whom analysis was feasible. Patients with fulminant hepatitis were significantly older (44.7 ± 16.3 vs. 36.0 ± 14.3 years, $P = .0017$), less predominantly male (43% vs. 71%, $P = .0005$) and less often positive for HBeAg (23% vs. 60%, $P < .0001$) than those with acute hepatitis. Peak ALT and total bilirubin levels were higher for fulminant than acute hepatitis ($P < .0001$), reflecting severe hepatic lesions. Notably, the median HBV DNA level was lower in patients with fulminant than acute hepatitis (4.89 vs. 5.19 log copies/mL, $P = .0178$); the frequency of undetectable HBV DNA at the presentation was higher in fulminant hepatitis (25% vs. 10%, $P = .0086$). Lamivudine or steroid was given significantly more often to patients with fulminant hepatitis.

There were marked differences in the distribution of genotypes between patients with fulminant and acute hepatitis. HBV/Ae was less frequent (0% vs. 13%, $P = .0121$), whereas Bj was more often (30% vs. 4%, $P < .0001$) in patients with fulminant than acute hepatitis. Although HBV/Ce tended to be less frequent in patients with fulminant than acute hepatitis (55% vs. 65%), the difference fell short of being significant.

Precore stop-codon mutation (G1896A) and core-promoter double mutation (A1762T/G1764A) were more

Table 3. Comparison Between Patients With Fulminant and Acute Self-Limited Hepatitis Who Were Infected With HBV

Features	Fulminant (n = 40)	Acute (n = 261)	P Value
Age (years)	44.7 ± 16.3	36.0 ± 14.3	.0017
Men	17 (43%)	186 (71%)	.0005
HBeAg positive	9 (23%)	157 (60%)	<.0001
ALT (IU/L)	4315 ± 2889	2284 ± 1221	<.0001
Total bilirubin (mg/dL)	20.5 ± 16.4	8.3 ± 7.3	<.0001
HBV DNA (log copies/mL)			
Median	4.89	5.19	.0178
(range)	(2.00-8.44)	(2.00-9.06)	
<2.00 (undetectable)	10 (25%)	27 (10%)	.0086
Treatment			
Lamivudine	16 (40%)	37 (14%)	.0003
Steroid	9 (23%)	16 (6%)	.0022
Genotypes/subgenotypes			
Aa	1 (2.5%)	9 (3%)	NS
Ae	0 (0%)	33 (13%)	.0121
Ba	1 (2.5%)	21 (8%)	NS
Bj	12 (30%)	10 (4%)	<.0001
Cs	1 (2.5%)	10 (4%)	NS
Ce	22 (55%)	170 (65%)	NS
D	2 (5%)	3 (1%)	NS
G	1 (2.5%)	5 (2%)	NS
Mutations[a]			
nt 1753 and/or nt1754[b]	11/30 (37%)	28/234 (12%)	.0003
A1762T/G1764A	15/30 (50%)	39/234 (17%)	<.0001
G1896A	16/30 (53%)	21/234 (9%)	<.0001
G1899A	7/30 (23%)	8/234 (3%)	<.0001

[a]Exclusive of 37 patients in whom precore region and core-promoter could not be amplified by PCR.
[b]T1753C/A/G and/or T1754C/A/G.

Table 4. Multivariate Analysis for Factors Independently Associated With Fulminant Hepatitis

Factors	Odds Ratio	95% Confidence Interval	P Value
Age (yr)			
<34[a]	1		
≥34	3.472	1.094-11.023	.0347
Sex			
Male	1		
Female	2.272	0.780-6.613	.1323
HBeAg			
Positive	1		
Negative	3.344	1.065-10.506	.0387
ALT (IU/L)			
<2200[a]	1		
≥2200	2.094	0.683-6.414	.1957
Total bilirubin (mg/dL)			
<10.0[a]	1		
≥10.0	18.818	4.320-81.980	<.0001
HBVDNA (log copies/mL)			
<5.00[a]	1		
≥5.00	1.042	0.367-2.961	.9383
Treatment			
Lamivudine (−)	1		
Lamivudine (+)	2.650	0.814-8.625	.1056
Steroid (−)	1		
Steroid (+)	2.515	0.668-9.472	.1728
Genotypes/Subgenotypes			
Non-Bj	1		
Bj	7.001	1.737-28.228	.0062
Mutations			
nt 1753 and/or 1754[b]			
Absent	1		
Present	2.316	0.698-7.683	.1700
A1762T/G1764A			
Absent	1		
Present	1..013	0.295-3.478	.9841
G1896A			
Absent	1		
Present	4.157	1.265-13.657	.0189
G1899A			
Absent	1		
Present	2.525	0.534-11.949	.2427

[a]Median values.
[b]T1753C/A/G or T1754C/A/G.

frequent in patients with fulminant than acute hepatitis (53% vs. 9% and 50% vs. 17%, respectively, $P < .0001$ for each). Likewise, mutations in core-promoter at nt 1753 or nt 1754, and G1899A mutation were more frequent in patients with fulminant than acute hepatitis ($P = .0003$ and $P < .0001$, respectively).

Factors Independently Associated With the Development of Fulminant Hepatitis. Various factors found in association with fulminant hepatitis were evaluated for the independence in multivariate analysis (Table 4). Age 34 years or older (odds ratio 3.47 [95% confidence interval 1.09-11.02], $P = .035$), HBV/Bj (7.00 [1.74-28.23], $P = .006$), HBeAg-negative (3.34 [1.07-10.51], $P = .039$), total bilirubin ≥10.0 mg/dL (18.82 [4.32-81.98], $P < .0001$) and G1896A (4.16 [1.27-13.66], $P = .019$) were independent risk factors for the development of fulminant hepatitis.

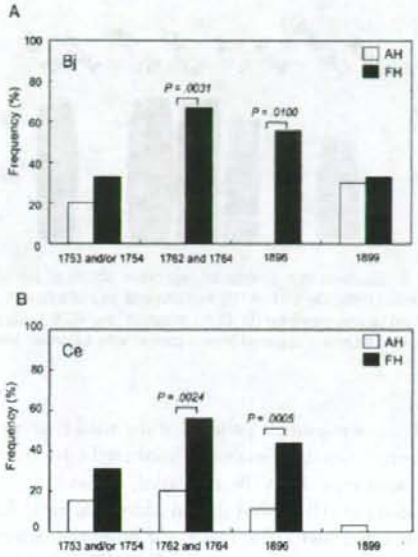

Fig. 2. Frequencies of precore and core-promoter mutations compared between patients with fulminant and acute self-limited hepatitis who were infected with HBV/Bj (A) or Ce (B).

In view of the majority of Japanese patients who were infected with Bj or Ce, mutations in the precore region and core-promoter were compared between those with fulminant and acute self-limited hepatitis for each subgenotype (Fig. 2). G1896A and A1762T/G1764A were significantly more frequent in patients with fulminant than acute hepatitis infected with either HBV/Bj or Ce (56% vs. 0% and 67% vs. 0% for Bj or 44% vs. 11% and 56% vs. 22% for Ce, respectively, $P \leq .01$ for all). For the patients infected with HBV/Bj, in particular, precore and core-promoter mutations were highly frequent in those with fulminant hepatitis (56% and 67%, respectively), whereas they occurred in none of those with acute hepatitis. G1899A was equally frequent in both patients with fulminant and acute hepatitis infected with HBV/Bj; it was rarely seen in those with Ce. Mutations involving nt 1753 or nt 1754 tended to be more frequent in patients with fulminant than acute hepatitis.

***Replication of the Wild-Type HBV as Well as Precore and Core-Promoter Mutants* In Vitro.** Full-length HBV DNA of the wild-type HBV/Bj from a patient with chronic hepatitis B was incorporated with G1896A or A1762T/G1764A mutation *in vitro*. Another plasmid was constructed with HBV/Bj_58 carrying G1896A from a fulminant patient. Figure 3 compares

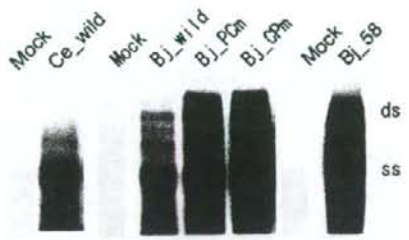

Fig. 3. Southern blot analysis for replicative activity of the wild-type HBV clones (HBV/Ce_wild and Bj_wild), as well as mutants with precore (Bj_PCm) or core-promoter (Bj_CPm) mutation, and Bj_58 with precore stop-codon mutation obtained from a patient with fulminant hepatitis.

densities of migration patterns of the wild-type, precore, and core-promoter mutants in Southern blotting analysis. The wild-type HBV/Bj displayed a band for single-stranded (ss) HBV DNA and an additional band for double-stranded (ds) HBV DNA. Of note, the densities of these bands were far greater for HBV/Bj mutants incorporated with precore or core-promoter mutation, as well as Bj_58 with the precore mutation, thereby indicating much enhanced replicative activity of precore or core-promoter mutant *in vitro*. Although the intracellular HBV DNA level for the wild-type HBV/Bj was comparable with that for the wild-type Ce (Fig. 3), the extracellular HBV DNA level in culture media was approximately threefold higher for Bj than Ce ($P < .01$) (Sugiyama M et al., manuscript in submission).

Discussion

A nationwide survey of genotypes/subgenotypes in patients with acute HBV infection from Japan during the past 2 decades has examined their influence on fulminant and chronic outcomes. The study was feasible in a country where mass vaccination has not been performed because of an extremely high efficacy of immunoprophylaxis on babies born to carrier mothers; it has decreased the persistent HBV carrier rate from 1.4% to 0.3%.[26] Acute HBV infection keeps increasing, however, predominantly through promiscuous sexual contacts in Japan.

Fulminant hepatitis developed rather frequently in 40 of the 301 (13%) patients. This is likely due to selection bias because the study included only patients who were hospitalized for acute hepatitis B. Exclusion of subclinical cases of acute HBV infection would have overestimated the incidence of fulminant hepatitis. Regardless of such a selection bias, influence of HBV genotypes/subgenotypes was evident in comparison with the 40 patients with fulminant and the 261 with acute self-limited hepatitis. Remarkably, none of the 33 patients infected with HBV/Ae developed fulminant hepatitis. In sharp contrast, 12 of the 22 (55%) patients infected with HBV/Bj developed it. Furthermore, both precore (G1896A) and core-promoter (A1762T/G1764A) mutations were detected significantly more frequently in patients with fulminant than acute self-limited hepatitis. In infection with HBV/Bj, in particular, the frequency of core-promoter mutation was much higher in the patients with fulminant (67%) than that reported in those with chronic hepatitis (16%).[27] Precore and core-promoter mutations are very frequent in patients with fulminant hepatitis from Asia[28-30] and the Middle East.[31] The failure in detecting these mutations in Western countries[32-35] could be attributed to frequent HBV/Ae and rare Bj there. In multivariate analysis, HBeAg-negative, HBV/Bj, and the precore stop-codon mutation for G1896A were independent risk factors for the development of fulminant hepatitis (Table 4). Various mutations at nt 1753 for enhanced HBV replication,[36] as well as those adjacent at nt 1754 prevailing in patients with fulminant hepatitis,[37] occurred more frequently in patients with fulminant than acute self-limited hepatitis. Host factors, such as age and total bilirubin, contributed to the development of fulminant hepatitis as well (Table 4).

In vitro replication analysis demonstrated the intracellular HBV DNA level of the wild-type HBV/Bj comparable with that of the wild-type Ce (Fig. 3). The extracellular HBV DNA level of HBV/Bj-clone, however, was much higher than those of the other genotypes, indicating its strong inclination to be secreted from cells (Sugiyama et al., manuscript in submission). Such a high concentration of HBV/Bj in the circulation of patients would rapidly and extensively promote infection of hepatocytes.

Enhanced replication capacities of precore (G1896A) and core-promoter (A1762T/G1764A) mutants for HBeAg-minus and -reduced phenotypes, respectively, were demonstrated in a replication model *in vitro* (Fig. 3). These observations were concordant with those in previous reports[38,39]; however no data are available on the replication of HBV/Bj *in vitro*, either of the wild-type or variants with these mutations. Extremely high intracellular and extracellular expressions of viral DNA were observed for the HBV/Bj clone with precore stop-codon mutation from a patient with fulminant hepatitis. These results might implicate high replication due to mutations of precore region and core-promoter in the induction of fulminant hepatitis. In support of this view, Bocharov et al.[40] have proposed that enhanced HBV replication would efficiently stimulate immune reactions, represented by the cytotoxic T lymphocyte response, suggesting that enhanced replication by HBV/Bj or precore/

core-promoter mutation might lead to fulminant hepatitis.

That HBV DNA levels were lower in patients with fulminant than acute hepatitis, despite a high replication capacity of HBV/Bj incriminated in the development of fulminant hepatic failure, may seem surprising. Because destruction of hepatocytes proceeds swiftly in patients with fulminant hepatitis, hepatic mass for HBV to thrive would have been extremely reduced in them at presentation. As a consequence, some patients with fulminant hepatitis B are without serum HBsAg; they are diagnosed by high-titered IgM anti-HBc.[41] On the contrary, HBV DNA levels were higher in the patients with HBV/Ae than Bj (Table 1); those with Ae tend to delay reducing HBV DNA, some of whom have chronic outcome. Combined, correlating HBV DNA levels with the clinical outcome in acute HBV infection would be difficult.

A wide variation has been seen in the rate of persistence after acute HBV infection in adulthood. No chronic outcomes of acute hepatitis B were seen in female recipients of red blood cells contaminated with HBV (0/28)[42] or patients in an acupuncture-associated outbreak (0/35).[43] In marked contrast, they ranged from 0.2% (14/715) in Greece[44] through 2.7% (1/37) in university students in Taiwan[45] to 10.4% (5/8) in Alaskan Eskimos[46] and 12.1% (7/58) in Germany.[47] HBV genotypes are implicated in a high rate of persistence in European countries where HBV/A is predominant.[48] In Japan, also, adulthood infection tends to persist longer with HBV/A than B or C (23% 3/13 vs. 13% 1/8 or 12% 3/25).[49] In the current series on 256 patients with acute hepatitis B in Japan who were followed rigorously, HBV infection persisted in only three (1%), representing 2 of the 32 (6%) with HBV/Ae and 1 of the 21 (5%) with Ba. Hence, 99% of patients lost their HBsAg by 6 months. Persistence of HBV observed in the patients with HBV/Ae (6%) is less frequent than that in 4 of the 31 (13%) patients with Ae from a hospital in metropolitan Tokyo.[49] The difference would be ascribable, at least in part, to lamivudine given to some patients in this study (18%). All patients treated with lamivudine recovered from acute hepatitis, whereas none of the three patients with chronic outcome had received antiviral treatment during their acute phase of illness, indicating that lamivudine might be able to prevent the chronic outcome. Likewise, some patients from metropolitan Tokyo, in whom HBV persisted,[49,50] had received immunosuppressants in the acute phase of infection before referral to their hospital.

Using cell culture and chimeric mice models for the replication system of different genotype/subgenotype clones, we have observed that the replication of HBV is the highest for HBV/Bj or C and the lowest for Aa/Ae (Sugiyama M et al., manuscript in submission). It is probable that the propensity of HBV/A infection to chronicity would be due to less intensive immune response against its slow viral dynamics. Taken together, the infection with HBV/A appears to persist longer than those with the other genotypes; this needs to be confirmed by further investigation in patients from various countries.

In conclusion, persistence of HBV after acute infection is rare and occurs more often in patients infected with HBV/Ae than others. Fulminant outcome is frequent in hospitalized patients and associated with HBV/Bj accompanied by the lack of serum HBeAg as well as high replication due to precore stop-codon mutation (G1896A), a finding supported by an *in vitro* replication model.

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Original Article

Double filtration plasmapheresis and interferon combination therapy for chronic hepatitis C patients with genotype 1 and high viral load

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Aim: The efficacy and safety of double filtration plasmapheresis (DFPP) plus interferon (IFN) combination therapy were compared with those of IFN therapy alone in 193 chronic hepatitis C patients having a high hepatitis C virus ribonucleic acid load of difficult-to-treat genotype 1b.

Methods: All patients received either interferon alpha-2b (IFN-α-2b) monotherapy or combination therapies with ribavirin and IFN-α-2b or pegylated interferon alpha-2b (PEG-IFN-α-2b). Each patient individually decided whether to receive concomitant DFPP. DFPP was immediately followed by IFN treatment, and up to five sessions were given during the first week.

Results: Sixty patients decided to receive DFPP. In the DFPP plus PEG-IFN-α-2b therapy group ($n = 30$), viral load reduction at 4 weeks after the start of treatment was greater than in non-DFPP ($n = 74$) (2.47 *vs* 1.52, log, $P = 0.010$), and the sustained virus response was also higher (77.8% *vs* 50.0%), even in cases of re-treated patients (relapsers or non-responders to previous IFN therapies). Adverse events, mild and transient, were observed in 38.3% of all DFPP-treated patients.

Conclusion: DFPP plus IFN combination therapy produced a great reduction of viral load during the early stage of treatment and achieved a high sustained virus response, suggesting that this combination therapy may be a new modality for chronic hepatitis C patients at difficult-to-treat states.

Key words: combination therapy, double filtration plasmapheresis, early viral reduction, non-responder, relapser, sustained virus response

INTRODUCTION

IT IS WELL known that some cases of chronic hepatitis C ultimately progress to hepatic cirrhosis and hepatocellular carcinoma.[1,2] Over the past 20 years, interferon (IFN) therapy has improved to more effectively eliminate the virus, from IFN-only therapy, to its combination therapy with ribavirin, and to pegylated interferon (PEG-IFN) therapy.[3] Nevertheless, even combined therapy with PEG-IFN and ribavirin for 48 weeks is unable to eliminate the virus in some 40% of hepatitis C cases.[4,5]

Researchers are therefore actively developing new drugs to replace IFN, as well as drugs that can be used in

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combination with IFN. Also, attempts are being made to physically remove hepatitis C virus (HCV) particles from the blood. Granulocyte apheresis, plasma exchange and hemofiltration have been applied to HCV-infected patients for treatment of cryoglobulinemia and vasculitis, and these modalities are shown to reduce HCV ribonucleic acid (RNA) in the blood during the treatment.[6–10] Marson *et al.* reported that low-density lipoprotein-cholesterol apheresis and plasma exchange in hypercholesteremia patients with HCV infection reduced the quantity of HCV-RNA in the blood of some cases.[11] Ishida *et al.* found that hemodialysis, hemofiltration and peritoneal dialysis in chronic dialysis patients infected with HCV produced significantly lower HCV-RNA levels in the blood.[12] There are reports of combined granulocyte apheresis with IFN therapy for chronic hepatitis C,[13–15] and also reports claiming that early reduction of the virus is important in the treatment of chronic hepatitis C.[16,17] Thus, the potential effectiveness of IFN therapy combined with early virus removal by a physical method is of particular interest. Moreover, Sakai *et al.* reported the mechanism of clinical results by plasmapheresis whereby HCV in the blood was related to the treatment effects of IFN therapy, which could be enhanced by removing the virus from the blood.[18–20]

In the present study, we treated chronic hepatitis C patients with double filtration plasmapheresis (DFPP) in order to reduce the blood levels of HCV-RNA at the early stage of IFN therapy.

METHODS

Patients in the study

THE PATIENTS INCLUDED 89 cases treated with interferon alpha-2b (IFN-α-2b) therapy at 15 facilities in Japan between 2002 and 2004, and 104 cases treated with pegylated interferon alpha-2b (PEG-IFN-α-2b) therapy in 2004 (Table 1). A total of 182 patients underwent liver biopsy in order to clarify the staging and grading of chronic hepatitis C. All patients were confirmed to be HCV-RNA positive with high transaminase levels persisting for 6 months or longer, and their HCV-RNA genotype was 1b, the blood levels of which exceeded 100 KIU/mL, as determined by the Amplicore HCV monitor method (Roche, Tokyo, Japan), prior to the start of corresponding therapies. All patients were negative for hepatitis B surface antigen. Patients' age ranged from 20 and 69 years. Patients with platelet counts $\leq 10 \times 10^4/\mu L$, leukocyte counts $\leq 3000/\mu L$, or hemoglobin levels ≤12 g/dL were excluded from the study. Patients were divided into six groups according to the respective methods of treatment as follows: Group 1; five cases treated with DFPP plus IFN-α-2b for 24 weeks. Group 2; 10 cases treated with DFPP plus IFN-α-2b and ribavirin for 24 weeks. Group 3; 59 cases given IFN-α-2b and ribavirin for 24 weeks. Group 4; 15 cases treated with DFPP plus IFN-α-2b for 48 weeks and ribavirin for the first 24 weeks. Group 5; 30 cases treated with DFPP plus PEG-IFN-α-2b and ribavirin for 48 weeks. Group 6; 74 cases given PEG-IFN-α-2b and ribavirin for 48 weeks. The total dose of IFN-α-2b for 24 weeks was 432×10^6 units or more, and more than 864×10^6 units for 48 weeks. The dose of PEG-IFN-α-2b was 1.5 μg/kg per week, and the dose of ribavirin was either 600 mg/day or 800 mg/day.

Each patient individually decided whether to receive concomitant DFPP. There were no significant differences in patient backgrounds among the six groups. The study was conducted with the written informed consent of individual patients and with the approval of the review boards of the respective medical facilities.

Double filtration plasmapheresis and blood collection

Blood was collected from the peripheral vein for DFPP, and a Plasmaflo™ OP-08W (Asahi Kasei Medical, Tokyo, Japan) was used to separate the blood into plasma and cell components. The virus was then removed from the separated plasma by a second filter (Cascadeflo™ EC-50W; Asahi Kasei Medical) with an average pore size of 30 nm (Fig. 1). For each session, the final volume of treated plasma was 50 mL/kg. The number of sessions and the days when DFPP was given were decided by the physicians, based on the reduced plasma fibrinogen levels during DFPP and patient wishes.

HCV-RNA measurement

The quantity of HCV-RNA was measured by the original Amplicore HCV monitor method (detection limit: 0.5 KIU/mL) for groups 1–4, and by the high-range Amplicore HCV monitor method (detection limit: 5 KIU/mL) for groups 5 and 6. The quantity determined by the original method was converted into a high-range value using a regression formula ($y = 1.3983x - 1.8285$).[21] The quantity for viral response rate was measured by the qualitative Amplicore HCV monitor (detection limit: 0.05 KIU/mL), and any quantity below the detection limit was taken to be negative.

Table 1 Background characteristics of patients with chronic hepatitis C

	Group 1 ($n = 5$) 24 weeks of IFN + DFPP	Group 2 ($n = 10$) 24 weeks of IFN + 24 weeks of Rib + DFPP	Group 3 ($n = 59$) 24 weeks of IFN + 24 weeks of Rib	Group 4 ($n = 15$) 48 weeks of IFN + 24 weeks of Rib + DFPP	Group 5 ($n = 30$) 48 weeks of PEG-IFN + 48 weeks of Rib + DFPP	Group 6 ($n = 74$) 48 weeks of PEG-IFN + 48 weeks of Rib	Statistical analysis
Sex (male/female)	4/1	8/2	33/26	11/4	21/9	46/28	NS, χ^2 (Yates' correction)
Age (years)	53 ± 13	53 ± 4	56 ± 9	50 ± 14	54 ± 8	55 ± 10	NS, ANOVA
Weight (kg)	59.0 ± 6.1	64.5 ± 7.3	62.1 ± 10.4	61.1 ± 10.4	68.9 ± 10.4	64.6 ± 10.6	NS, ANOVA
Liver biopsy							
Grading (0/1/2/3)	0/1/2/0	0/4/5/1	1/22/32/3	0/9/6/0	1/14/11/1	0/28/40/1	NS, χ^2 (Yates' correction)
Staging (0/1/2/3/4)	0/1/1/1/0	0/3/7/0/0	1/19/16/17/6	2/5/8/0/0	2/11/11/3/0	0/26/21/14/8	NS, χ^2 (Yates' correction)
Not done	2	0	1	0	3	5	
HCV-RNA (KIU/mL), n (%)							NS, χ^2 (Yates' correction)
100–500	1 (20)	2 (20)	22 (37)	5 (33)	10 (33)	24 (32)	
500 or above	4 (80)	8 (80)	37 (63)	10 (67)	20 (67)	50 (68)	
ALT (IU/L)	105.0 ± 67.9	85.7 ± 34.8	106.8 ± 72.2	105.1 ± 73.9	73.2 ± 46.7	87.5 ± 63.8	NS, ANOVA
Past IFN treatment, n (%)							NS, χ^2 (Yates' correction)
Naive	0 (0)	7 (70)	29 (50)	10 (66)	8 (27)	45 (61)	
Relapser or non-responder	5 (100)	3 (30)	28 (47)	4 (27)	22 (73)	29 (39)	
Unknown (relapser or non-responder)	0 (0)	0 (0)	2 (3)	1 (7)	0 (0)	0 (0)	

Data are presented as mean ± SD.
ALT, alanine aminotransferase; DFPP, double filtration plasmapheresis; IFN, interferon alpha-2b; NS, not significant; PEG-IFN, pegylated interferon alpha-2b; Rib, ribavirin.

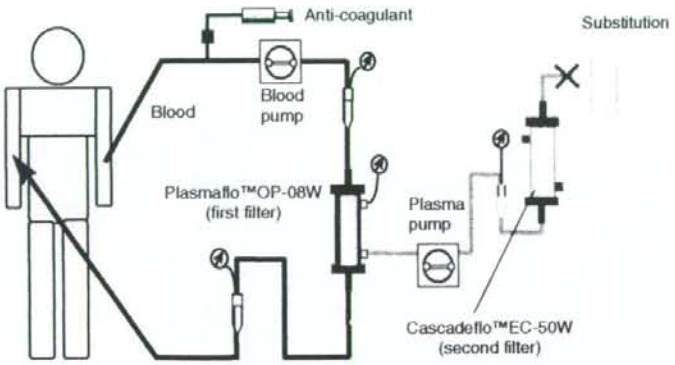

Figure 1 Schematic depiction of double filtration plasmapheresis (DFPP).

Performance of virus removal at second filter inlet and outlet

Plasma was collected from the inlet and outlet of the second filter during a session of DFPP, when the treated plasma volume reached half of the target quantity, and also when DFPP was completed. The changes in quantities of HCV-RNA were evaluated with these collected plasma samples.

Quantity of virus removed by a single session of DFPP

The quantity of virus removed by a single session of DFPP was computed based on two different hypotheses.

In the first hypothesis, in which the liver is assumed not to produce HCV, a one-compartment model was used to calculate the quantity of virus removed in a single session of DFPP.

HCV RR = $1 - \exp(-SC \times V_{pt}/V_p)$

HCV RV = HCV RR $\times C_{pre} \times V_p$

HCV RR: HCV removal ratio

HCV RV: HCV removed volume

V_p: total plasma volume (bodyweight $\times$ 1/13) $\times$ (1 – hematocrit value/100)

V_{pt}: plasma volume treated by DFPP

C_{pre}: HCV-RNA quantity before DFPP

SC: sieving coefficient (set at 1)

In the second hypothesis, in which the liver is assumed to produce HCV, the quantity of virus removed in a single session of DFPP was calculated from the quantities of HCV-RNA in the serum collected before and after the first session of DFPP.

Viral reduction and viral response rate

In order to determine the viral reduction (Δlog), the quantity of HCV-RNA was determined and converted into a log of virus quantity at the beginning of treatment (A), as well as the virus quantity at each of the virus measurement points (B). The following was then calculated: $\Delta\log = \log A - \log B = \log (A/B)$. In groups 2, 3 and 4, viral reduction was determined by collecting blood, before, at 24 h, and at 2 weeks after the start of DFPP or IFN-α-2b therapy. In groups 5 and 6, blood was collected, before, at 24 h, and at 2 and 4 weeks after the start of DFPP or PEG-IFN-α-2b therapy.

Patients whose HCV-RNA became negative on the Amplicore HCV monitor qualitative method and whose transaminases were within the normal range at 24 weeks after the completion of IFN therapy were considered to exhibit a sustained viral response (SVR).

Evaluation of DFPP safety

The subjective and objective adverse events of DFPP were observed, and five clinical items were also measured; platelet count, lymphocyte count, hemoglobin levels, albumin levels, and fibrinogen levels. These were determined before the first session of DFPP, and before the successive DFPP on the second, third, fourth, fifth and sixth days, and at 2 weeks after the last session of DFPP.

Statistical analysis

Statistical analysis consisted of an analysis of variance for patient background factors, and a paired *t*-test for quantities of HCV-RNA at the second filter inlet during DFPP. The *t*-test was used for viral load reductions and the Fisher's exact test for viral response rates among the groups. The *t*-test was two-tailed, and differences of $P < 0.05$ were considered significant.

RESULTS

Combination therapy of IFN and DFPP

OF THE 193 cases examined, 133 received IFN therapies alone, while the remaining 60 underwent DFPP. SVR was not evaluated in the following patients. One patient in group 1 withdrew her consent before receiving DFPP. One patient in each of groups 2, 4, and 5 failed to come to the facility due to personal reasons. There were seven patients in group 3, three in group 4, one in group 5, and 10 in group 6 who terminated IFN therapies before the scheduled treatment was completed. Also, there was one patient in group 1, one in group 4, three in group 5, and six in group 6 who continued IFN therapies after the scheduled treatment was completed.

The number of DFPP sessions performed was five in six patients, four in 10, three in 42, two in one, and one in one. The time spent for DFPP treatment was 100–480 min (average, 194 ± 105 min).

Virus removal performance at second filter inlet and outlet in DFPP

The quantity at the second filter inlet was 1720 ± 1481 KIU/mL when the treated plasma volume reached half the target quantity, and 1520 ± 1057 KIU/mL when DFPP was completed. At the outlet, the quantity of HCV-RNA was below the detection limit in all but two cases in which the removal rate was 99.98% or higher.

Quantity of virus removed by a single session of DFPP

The total plasma volume of patients undergoing DFPP ranged from 1200 mL to 4168 mL (average, 2945 ± 544 mL). The average plasma volume by a single session of DFPP was 46.7 ± 8.9 mL/kg bodyweight. The total treated plasma volume by DFPP ranged from 2030 mL to 4650 mL (average, 3161 ± 420 mL).

From the standpoint of the first hypothesis, in which the liver is assumed not to produce HCV $(4.69 \pm 4.50) \times 10^9$ IU HCV was removed in a single session of DFPP (HCV RV) and the HCV removal ratio (HCV RR) was 66.3 ± 7.1%. For the second hypothesis, which assumes that the liver produces HCV, the quantities of HCV-RNA in sera were compared before and after a single session of DFPP. The quantity of HCV-RNA ranged from 130 KIU/mL to 13 853 KIU/mL (average, 2392 ± 2139 KIU/mL) before DFPP, but after DFPP the quantity fell significantly to 27–4699 KIU/mL (average, 1494 ± 969 KIU/mL) ($P < 0.001$), showing that a single session of DFPP removed $(3.08 \pm 5.81) \times 10^9$ IU HCV and that the removal ratio was 26.1 ± 36.4%

Viral reduction effects in combined treatment with DFPP

All cases in groups 2 and 4 receiving DFPP plus IFN-α-2b and ribavirin showed viral load reduction significantly larger at 24 h after the start of treatment than in group 3 receiving non-DFPP ($P < 0.001$). At 2 weeks after the start of treatment, the viral load reduction in the groups undergoing DFPP exceeded 2 log units and was significantly larger than the reduction in the groups undergoing non-DFPP ($P = 0.034$). The viral load reduction for patients re-treated with DFPP who were either relapsers or non-responders following previous IFN therapy was (1.62 ± 0.46) log at 24 h after the start of treatment, and (2.88 ± 0.78) log at 2 weeks. These values were significantly larger than (0.79 ± 0.52) log at 24 h and (1.45 ± 0.81) log at 2 weeks in Group 3 without DFPP ($P = 0.007$ and $P < 0.001$, respectively) (Fig. 2).

In Group 5 treated with DFPP plus PEG-IFN-α-2b and ribavirin, the viral load reduction 2 weeks after the start of treatment was (1.48 ± 0.80) log in all cases (1.58 ± 0.80) log in the re-treated patients, and (1.20 ± 0.78) log in the former non-responders. All of these values were larger than the corresponding values in group 6 without DFPP. In addition, the reduction in group 5 at 4 weeks after the start of treatment in these cases was (2.43 ± 1.07) log (2.47 ± 1.11) log and (2.13 ± 0.71) log, respectively, and exceeded 2 logs in group 6, in which the reduction was (1.52 ± 1.08) log in the re-treated patients ($P = 0.010$) and (1.46 ± 1.17) log in the-non-responders (Fig. 3).

Sustained virus response rate

In patients treated with IFN-α-2b, SVR was seen in one of three cases in group 1, three of nine cases in group 2, nine of 52 cases in group 3, and all of 10 cases in group 4. In patients treated with PEG-IFN-α-2b, SVR was seen in 17 of 24 (70.8%) in group 5, and in 29 of 58 (50.0%) in group 6 ($P = 0.094$). In the re-treated patients, SVR was seen in 14 of 18 (77.8%) in group 5, and in 11 of 22 (50.0%) in group 6, and in non-responders, in five of seven (71.4%) in group 5, and in two of seven (28.6%) in group 6 (Fig. 4).

Safety of DFPP treatment

When DFPP treatment was conducted, 23 of 60 cases (38.3%) experienced some adverse events with 32 reported incidents in total (Table 2). A drop in blood

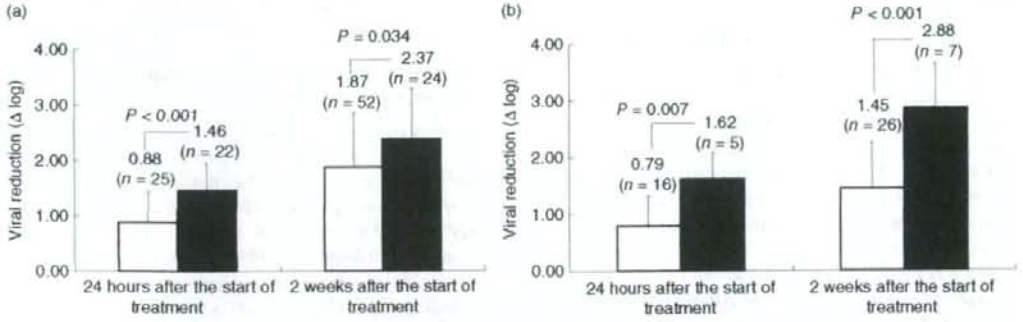

Figure 2 Viral reduction in groups with interferon alpha-2b and ribavirin, with and without double filtration plasmapheresis (DFPP). (a) Viral reduction in all patients. ▒, Group 3 (all patients); ■, groups 2 and 4 (all patients). (b) Viral reduction in re-treated patients. ▒, Group 3 (re-treated patients); ■, groups 2 and 4 (re-treated patients). Data are expressed as mean ± SD.

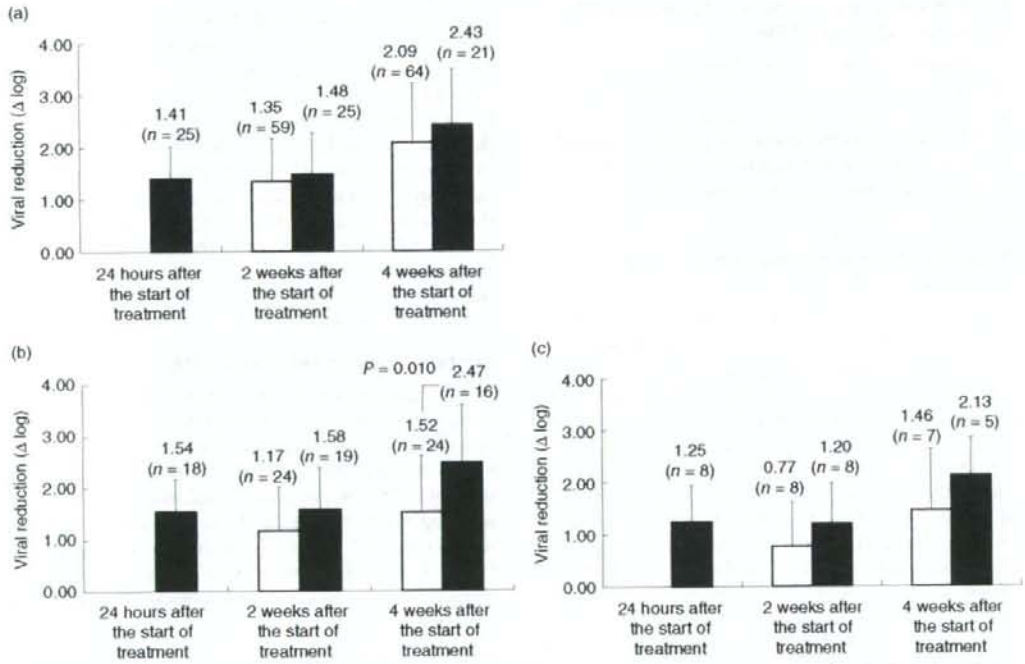

Figure 3 Viral reduction in groups with pegylated interferon alpha-2b and ribavirin, with and without double filtration plasmapheresis (DFPP). (a) Viral reduction in all patients. ▒, Group 6 (all patients); ■, group 5 (all patients). (b) Viral reduction in re-treated patients. ▒, Group 6 (re-treated patients); ■, group 5 (re-treated patients). (c) Viral reduction in non-responders. ▒, Group 6 (non-responders); ■, group 5 (non-responders). Data are expressed as mean ± SD.

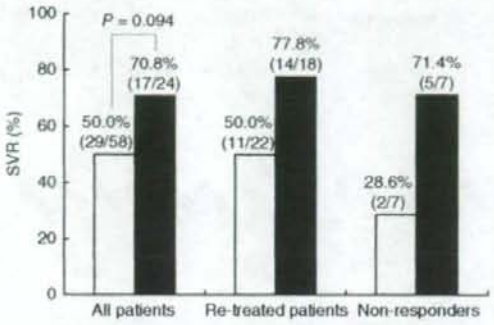

Figure 4 Sustained virus response rates (SVR) in patients treated with pegylated interferon alpha-2b and ribavirin, with and without double filtration plasmapheresis (DFPP). ▒, Group 6; ■, group 5.

pressure was observed in four cases, but recovered after giving intravenous 100–200 mL normal saline solution. One case lost consciousness, but recovered after 2–3 min by Ambu pressure and oxygen inhalation and giving atropine sulfate and intravenous metoclopramide. Because the drop in blood pressure and loss of consciousness were temporary, all four patients continued to receive DFPP onwards. Minor disorder was found in 10 cases, but was temporary and recovered without any treatment. All other adverse events were also temporary.

Figure 5 demonstrates changes in the platelet count, lymphocyte count, hemoglobin levels, albumin levels, and fibrinogen levels. The platelet count and lymphocyte count fell temporarily during DFPP, but recovered to initial levels within 2 weeks in all cases. There were no changes in hemoglobin and albumin levels. The fibrinogen levels fell significantly from 234 ± 52 mg/dL to 142 ± 29 mg/dL ($P < 0.001$) on the day after DFPP (removal rate = 37.8%). This reduction continued during the period of DFPP, but recovered to initial levels within 1 week after the completion of DFPP. There were no bleeding or other adverse events sometimes accompanying a decline in fibrinogen levels.

Table 2 Adverse events during double filtration plasmapheresis treatment

Symptom	No. cases	No. incidents
Minor disorder	10	11
Decline of blood pressure	4	5
Loss of consciousness	1	1
Fever	2	2
Chills	2	2
Vomiting	2	2
Nausea	5	6
Pain in right brachial	1	1
Vagal reaction	2	2

DISCUSSION

DOUBLE FILTRATION PLASMAPHERESIS has been applied to many diseases and its safety has been established.[22,23] In the present study, DFPP was applied to chronic hepatitis C patients in combination with IFN therapy, and the adverse events were all those characteristic of DFPP, such as minor disorder, reduced blood pressure and nausea. It is reported that chronic hepatitis C patients experience a decline in the number of platelets or lymphocyte count, and that giving IFN can induce further reductions.[24] With DFPP, only a temporary decline in these two levels was noted. Because fibrinogen has a molecular weight of 340 000 and some of this can be removed (removal rate: 37.8%), there was a case in the present study in which fibrinogen levels fell below 100 mg/dL but recovered to the initial levels within 1 week after completion of DFPP. However, none of the cases in this study experienced bleeding or other serious adverse events. This demonstrates that DFPP can be used safely in combination with IFN therapy to treat chronic hepatitis C.

In order to assess the efficacy of virus removal, HCV-RNA load was recorded at the inlet and outlet of the second filter, when treated plasma volume reached half the targeted volume, and when DFPP was completed. As a result, the filter removed at least 99.98% of the virus. This would indicate that almost all of the HCV with an average particle diameter of 55–65 nm[25] was trapped by the second filter with an average pore size of 30 nm[26] Moreover, because the virus quantity fell below the detection limit in almost all cases, it is certain that the second filter efficiently removed the virus throughout DFPP.

The quantity of virus removed by a single session of DFPP was assayed in consideration of two different hypotheses. In the first hypothesis that the liver does not produce HCV, a computation based on a one-compartment model yielded $(4.69 \pm 4.50) \times 10^9$ IU as the quantity of virus removed by DFPP and 66.3 ± 7.1% as its removal ratio. Under these circumstances, new viruses are produced in the liver even after viral removal by DFPP, because the liver produces 10^{12} HCV per day.[27]

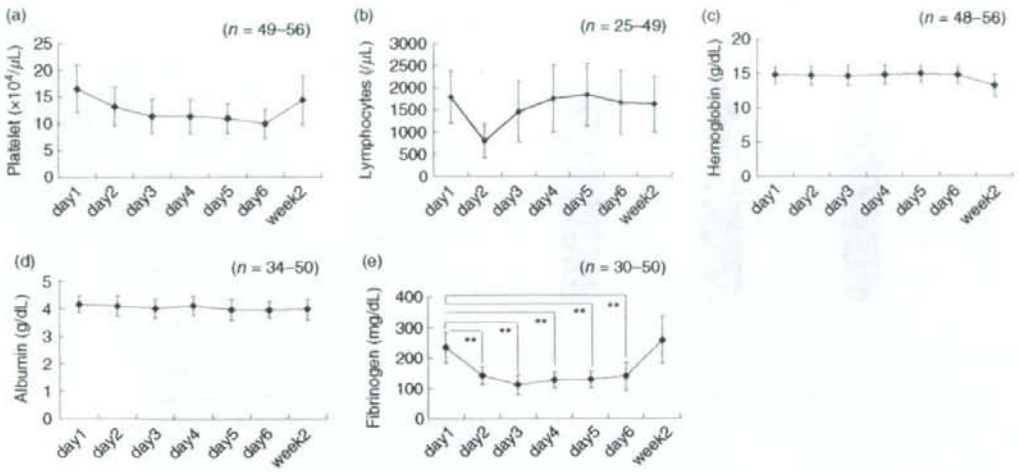

Figure 5 Changes in laboratory data during interferon treatment with double filtration plasmapheresis (DFPP). Data are expressed as mean ± SD. $^{**}P < 0.001$.

In the second hypothesis that the liver can produce HCV, the quantity of virus removed by DFPP was calculated from actual clinical results. The quantity of HCV-RNA in the serum significantly decreased from an average of 2392 ± 2139 KIU/mL before DFPP to 1494 ± 969 KIU/mL after DFPP ($P < 0.001$). DFPP removed $(3.08 \pm 5.81) \times 10^9$ IU of the virus and the removal ratio was 26.1 ± 36.4%, despite the virus being newly produced by the liver.

Moreover, the present study demonstrated that the viral load reduction at 24 h after the start of IFN-α-2b therapy was significantly greater in the groups undergoing DFPP combined with IFN therapy than in non-DFPP groups ($P < 0.001$, Fig. 2a; $P = 0.007$, Fig. 2b). The underlying mechanism is not known; however, there may be several explanations for the significant reduction with the combination DFPP treatment. One explanation is that the reduced viral return to the infected liver by combination DFPP treatment may have a favorable effect on the IFN efficacy. Another possibility is that DFPP treatment changes the viral nature in the blood, and affects the efficacy. In chronic hepatitis C patients, there are two fractions of HCV particles in the blood according to its buoyant density which relate viral titer or disease states.[28–30] One is a high-density fraction in which HCV particles form an immunocomplex with immunoglobulin G (IgG), the other is a low-density fraction where HCV particles bind to low-density lipoprotein (LDL). Removal of circulating HCV by DFPP treatment decreases the amount of the high-density fraction of HCV particles, and may be associated with the IFN response, as we reported that the lower ratio of the high-density fraction of HCV was associated with the response to interferon treatment.[18–20]

The quantity of HCV-RNA showed that DFPP combined with IFN therapy reduced viral numbers at all samplings (i.e. at 24 h, 2 weeks, and 4 weeks after the start of IFN therapy). In particular, the groups receiving DFPP combined with IFN-α-2b and ribavirin, or PEG-IFN-α-2b and ribavirin showed a 2 log or greater viral load reduction at 2 weeks or 4 weeks after the start of IFN therapy, respectively. Davis reported that an early viral reduction of 2 logs or more is important in removing the virus.[31] The use of DFPP at the start of IFN therapy may constitute a crucial treatment for viral removal.

DFPP was effective in non-responders who had previously received IFN therapy. Moreover, in the groups treated with PEG-IFN-α-2b therapy alone, non-responders showed a viral load reduction of (1.46 ± 1.17) log at 4 weeks after the start of treatment, whereas the reduction was (2.13 ± 0.71) log in the groups with DFPP. This difference was reflected in the SVR, which was two of seven cases (28.6%) in patients without DFPP and five of seven cases (71.4%), suggesting that forcible removal of the virus by DFPP can make

non-responders responsive to IFN therapy in a short period of time.

The groups of treatment in the present study were selected by patients' wishes, and thereby the patients were not randomly assigned to the groups. Nevertheless, there was little difference in patient clinical background factors (Table 1). Significant difference of rate of SVR between IFN with and without DFPP was not statistically obtained in this study using a limited number of patients. However, DFPP is assumed to provide effective treatment even for chronic hepatitis C patients resistant to IFN therapy. Further study is clearly necessary to determine the effectiveness of this combination therapy, and to understand the mechanisms of virus production and elimination by DFPP.

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CASE REPORT

Reactivation of hepatitis in a bladder cancer patient receiving chemotherapy

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Abstract Reactivation of hepatitis is a serious complication of chemotherapy in hepatitis B virus (HBV) carriers. There are many reports of this in lymphoma patients but few in urological cancer patients. A 59-year-old woman with bladder cancer who was an HBV carrier developed severe liver dysfunction after 2 cycles of chemotherapy. The diagnosis was reactivation of hepatitis. She improved with administration of lamivudine with a steroid and is currently well without disease. Care should be taken about the risk of reactivation when performing chemotherapy in HBV carriers and prophylaxis by lamivudine should be considered.

Keywords Hepatitis B virus · Chemotherapy · Reactivation · Lamivudine

Introduction

Reactivation of hepatitis is a serious complication of chemotherapy in hepatitis B virus (HBV) carriers. There have been a number of cases reported during treatment of lymphoma; however, only one case has so far been reported for urological cancer treatment [1]. We herein report a case of chemotherapy-induced hepatitis flare in a patient with bladder cancer.

A 59-year-old woman was diagnosed as having infiltrating bladder carcinoma with metastasis to local lymph nodes. She was an HBV carrier but had no active hepatitis and a normal alanine aminotransferase level (ALT, normal < 40 IU/l), positive HBs Ag, positive anti-HBe Ab, positive serum HBV-DNA (4.9×10^6 copies/ml, normal $< 4 \times 10^2$ copies/ml). Following cystectomy she received chemotherapy consisting of methotrexate, epiadriamycin and cisplatin. At the end of the second cycle of chemotherapy, just after the withdrawal of 4 mg/day dexamethazone for antiemesis, she presented with general fatigue and appetite loss. Laboratory tests showed ALT elevated to 2,000 IU/l (Fig. 1). Although her serum HBV-DNA level decreased to 4.7×10^2 copies/ml, she was diagnosed as having reactivation hepatitis because a thorough evaluation did not indicate any other cause for liver dysfunction. She was started on lamivudine at 100 mg/day with 500 mg of steroid therapy for 3 days. One week later she developed reversible posterior leukoencephalopathy syndrome, and required an anticonvulsion agent and respiratory care for 1 month. She improved 3 months later; ALT

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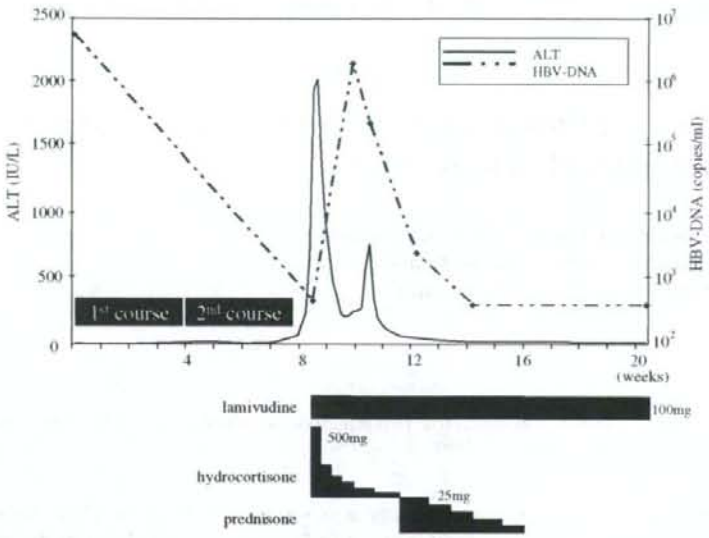

Fig. 1 The time courses of ALT and HBV-DNA

normalized and serum HBV-DNA was undetectable when lamivudine was stopped. After the discontinuance of chemotherapy she has been well without evidence of cancer recurrence or hepatitis for 2 years of follow-up.

Discussion

Reactivation of HBV is well known in lymphoma patients undergoing cytotoxic chemotherapy. The mechanism of flare in HBV carriers has not been clearly elucidated, though a possible mechanism is that increased HBV-infected hepatocytes due to immunosuppressive agents, which are disintegrated by the attack of restored activated T-cells after the withdrawal of the agents [2].

The frequency of HBV reactivation in HBs Ag-positive lymphoma patients receiving chemotherapy was reported to range from 15% to 20%. The HBV-DNA usually rises and drops rapidly soon after ALT elevation, so the true incidence of HBV reactivation might be underestimated in retrospective studies. In fact, we did not detect a rise of HBV-DNA during the clinical course. Patients with positive HBV-DNA have a risk for flare-up. In addition, the use of steroids was reported to be a risk for reactivation. Upon withdrawal of steroids, there is an intense rebound in cytotoxic T-cell function that coincides with a surge in serum ALT and decreases in the levels of HBs Ag and HBV-DNA [3].

Lamivudine inhibits reverse transcription activity and DNA synthesis, is well tolerated and the adverse effects are mild. However, long-term lamivudine use is associated with the development of lamivudine-resistant mutant strains of HBV. Despite this risk, prophylaxis against chemotherapy-induced reactivation is recommended [4].

There has hitherto been only one report of HBV reactivation in urological cancer chemotherapy. However, as our case demonstrates, this remains a possibility and care should be taken about reactivation when performing chemotherapy in HBV carriers; monitoring HBV-DNA is mandatory and prophylaxis by lamivudine should be considered.

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