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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.

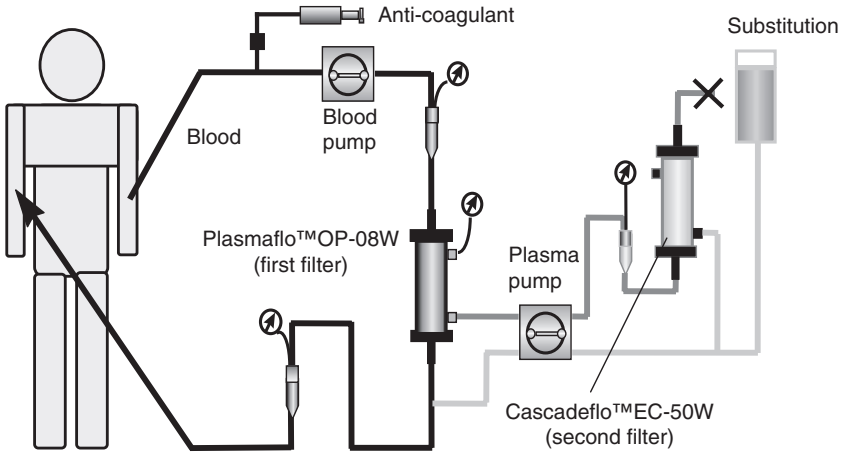

Figure 1 Schematic depiction of double filtration plasmapheresis (DFPP).

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.

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

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

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

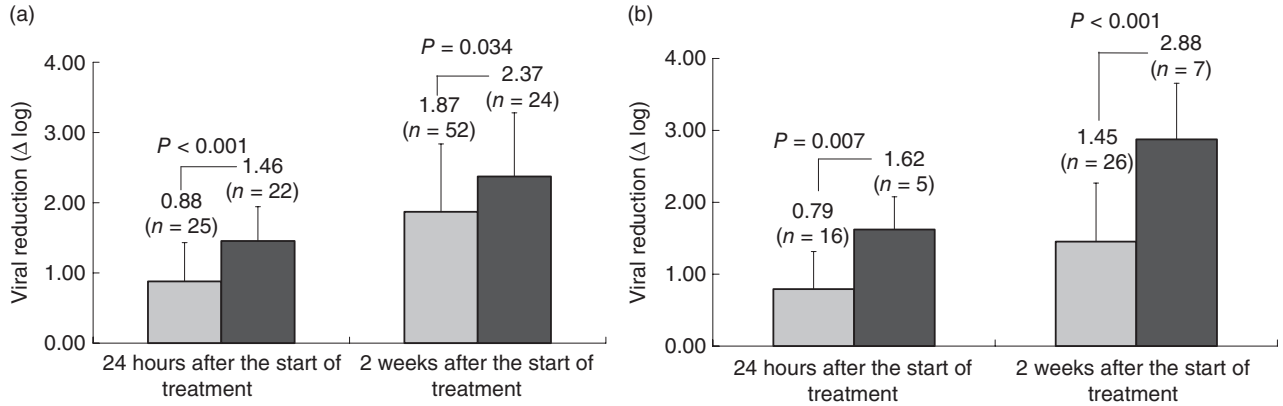

Figure 2 Viral reduction in groups with interferon alpha-2b and ribavirin, with and without double filtration plasmapheresis (DFPP). (a) Viral reduction in all patients. ■, Group 3 (all patients); ■, groups 2 and 4 (all patients). (b) Viral reduction in re-treated patients. ■, Group 3 (re-treated patients);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

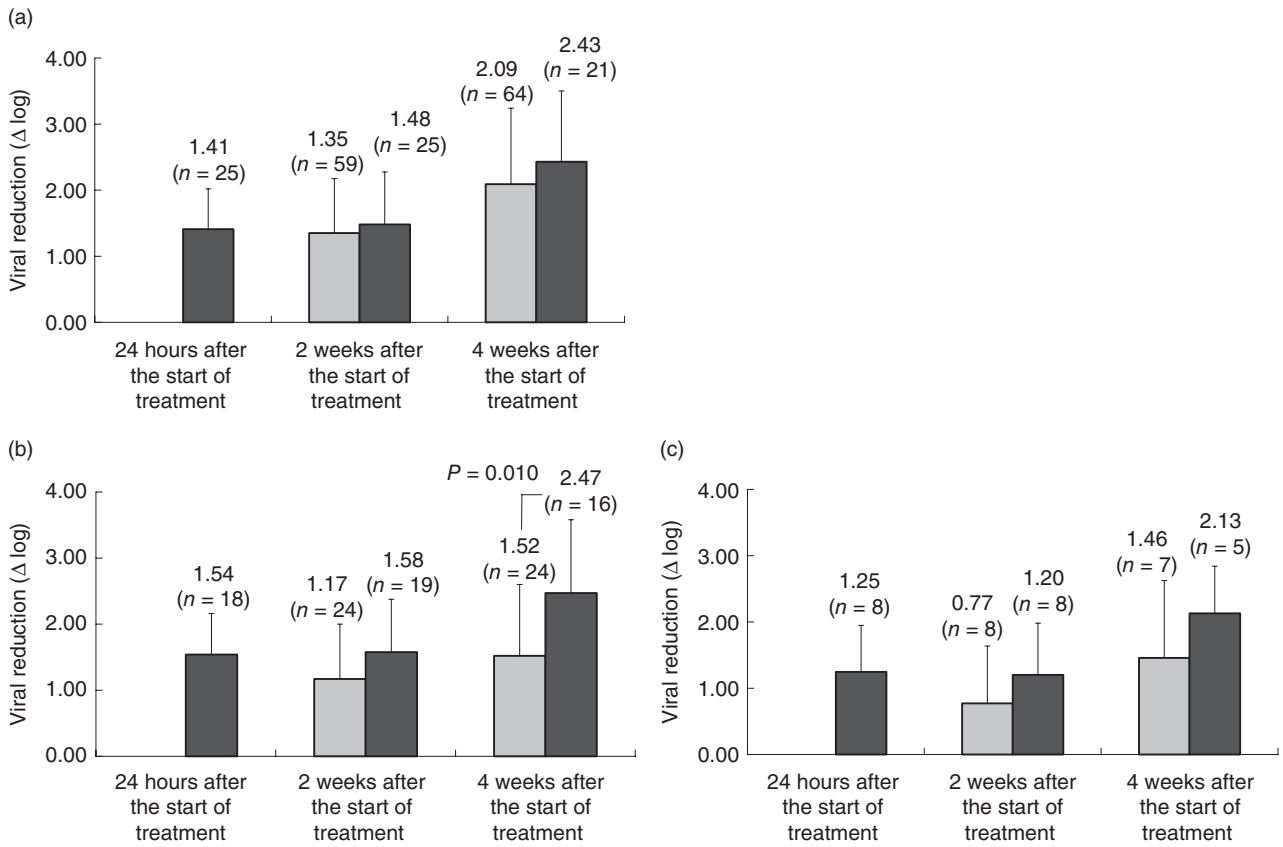

Figure 3 Viral reduction in groups with pegylated interferon alpha-2b and ribavirin, with and without double filtration plasmapheresis (DFPP). (a) Viral reduction in all patients. ■, Group 6 (all patients); ■, group 5 (all patients). (b) Viral reduction in re-treated patients. ■, Group 6 (re-treated 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.

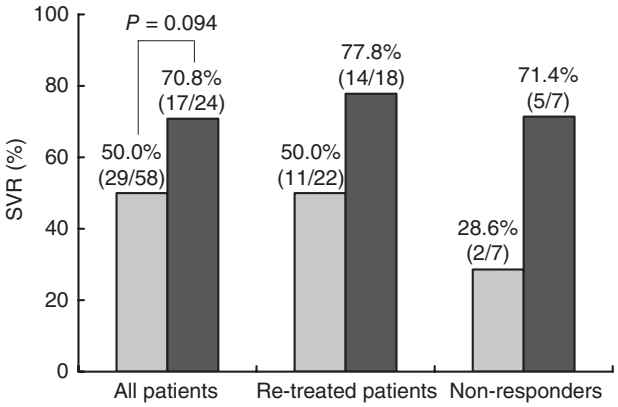

Figure 4 Sustained virus response rates (SVR) in patients treated with pegylated interferon alpha-2b and ribavirin, with and without double filtration plasmapheresis (DFPP). ■, Group 6; ■, group 5.

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]

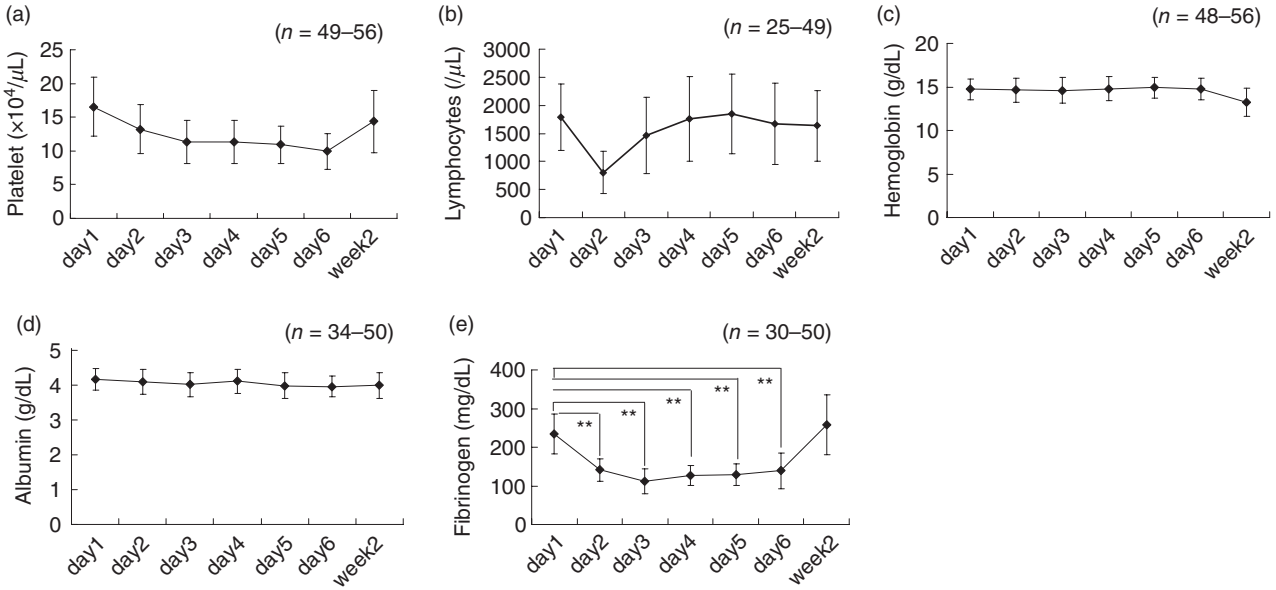

Figure 5 Changes in laboratory data during interferon treatment with double filtration plasmapheresis (DFPP). Data are expressed as mean ± SD. $^{**}P < 0.001$.

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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