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Complete Early Virological Response Was Highly Achieved by Double Filtration Plasmapheresis Plus IFN-Beta Induction Therapy for HCV-1b Patients With Relapse or No Response After Previous IFN Therapy

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Abstract: The efficacy of double filtration plasmapheresis (DFPP) plus interferon (IFN)-β induction therapy was preliminarily investigated in re-treated patients with chronic genotype 1b hepatitis C and high viral load (patients with relapse or non-response to previous IFN therapies). In eight patients with chronic hepatitis C, DFPP was performed five times over 2 weeks during IFN-β therapy, and 3 MU of IFN-β was administered twice a day for 2 weeks. Combination therapies with ribavirin and pegylated IFN-α2b (PEG-IFN-α2b) or pegylated IFN-α2a (PEG-IFN-α2a) were subsequently used. After 4 weeks, hepatitis C virus (HCV)-RNA tended to be more greatly decreased with DFPP combination therapy than with previous IFN therapy (4.5 ± 2.0 $\log_{10}$IU/mL vs. 2.9 ± 1.2 $\log_{10}$IU/mL). Rates of both rapid virological response and complete early virological response were significantly higher with DFPP and IFN-β induction therapy than with previous IFN therapy. DFPP plus IFN-β induction therapy produced a great reduction of viral load during the early stage of treatment and achieved a high early virological response, suggesting that this combination therapy may be useful as a new treatment modality for chronic hepatitis C patients in difficult-to-treat states. This combination may contribute to sustained virological response (SVR). The effects of DFPP on SVR and its significance remain to be clarified. **Key Words:** Complete early virological response, Double filtration plasmapheresis, IFN-β induction, Virus removal and eradication by DFPP.

The standard of care for chronic hepatitis C virus (HCV) has improved markedly since the approval of interferon (IFN) therapy more than a decade ago. Over the past 20 years, IFN therapy has improved to more effectively eliminate the virus, progressing from IFN-only therapy to combination therapy with ribavirin (RBV) and finally to pegylated IFN (PEG-IFN) therapy (1). However, even combined therapy with PEG-IFN and RBV for 48 weeks is unable to eliminate the virus in some 40% of hepatitis C cases, particularly those with genotype 1b and high viral load (2,3). Treatment options for patients who have relapsed or are refractory to treatment with PEG-IFN and RBV therefore need to be critically assessed.

The development of new methods in addition to the use of IFN has been anticipated, as well as drugs that can be used in combination with IFN. Sakai et al. investigated the possibility of enhancing the therapeutic effects of IFN by removing HCV from the blood using apheresis (4). Furthermore, Yamashita et al. reported the mechanism of clinical results by plasmapheresis, as HCV load in the blood was related to the treatment effects of IFN therapy, which could thus be enhanced by removing virus from the blood (5).

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The present study applied treatment with double filtration plasmapheresis (DFPP) plus IFN-β induction therapy to reduce blood levels of HCV-RNA in the early stage of IFN therapy for chronic hepatitis C patients for whom HCV was not eradicated by previous IFN therapy, and assessed the early virological response (EVR).

PATIENTS AND METHODS

Study protocols and therapeutic procedures

This was a retrospective, observational study. Subjects were patients who underwent DFPP therapy at the Saiseikai Niigata Daini Hospital, Niigata, Japan, between May 2008 and May 2010. All therapeutic procedures were carried out in accordance with the instructions from the manufacturers. We used Plasma flo OP-08W and Cascade flo EC-50W systems (Asahi Kasei Kuraray Medical, Tokyo, Japan) for plasma separation and the second filter, respectively. The volume of plasma processed during the course of each therapy session was 50 mL/kg. The course comprised five sessions over 2 weeks. During DFPP, patients were treated with IFN-β at 3 million units (MU) twice daily for 2 weeks as induction therapy. The 3 MU of IFN-β (Feron; Toray Industries, Tokyo, Japan) was dissolved in 100 mL of physiological saline or 5% glucose for injection, and the solution was administered twice daily by intravenous drip infusion over 30 min in the morning and evening. Subsequent to IFN-β, 1.5 μg/kg of PEG-IFN-α2b (Pegintron; Schering-Plough, Tokyo, Japan) was administered once a week. RBV (Rebetol; Schering-Plough) was administered at a dose of 600 mg/day (200 mg after breakfast, 400 mg after dinner) to patients weighing <60 kg, 800 mg/day (400 mg after breakfast, 400 mg after dinner) to those weighing 60–80 kg, and 1000 mg/day (400 mg after breakfast, 600 mg after dinner) to those weighing >80 kg. The daily dose of RBV was reduced by 200 mg when the hemoglobin level decreased to <10 g/dL, and RBV was discontinued when the hemoglobin level fell to <8.5 g/dL. When side effects made continued administration difficult, or when HCV-RNA levels did not decrease, IFN was discontinued at the discretion of the physician.

Measurement of HCV-RNA and virological response

The quantity of HCV-RNA was measured using the COBAS TaqMan HCV test (detection limit, 1.2 log IU/mL; Roche Diagnosis, Tokyo, Japan) and the high-range Amplicore HCV monitor method (detection limit, 5 kIU/mL; Roche Diagnosis).

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 Δlog was then calculated as $\Delta\log = \log A - \log B = \log(A/B)$.

HCV-RNA levels were investigated before the start of treatment and at 4 and 12 weeks after the start of treatment. A HCV-RNA level <1.2 log IU/mL after 4 weeks of treatment was regarded as a rapid virological response (RVR). Early virological response can be broken down into two subgroups. The first are those patients who have at least a 2-$\log_{10}$ drop in HCV-RNA after 12 weeks of treatment but still have detectable viremia. These patients achieve what is called partial EVR. The second subgroup comprises those patients who have achieved complete EVR (cEVR), with completely undetectable HCV-RNA levels after 12 weeks of treatment.

RESULTS

We measured the viral mutation of six patients. Virus mutation in the core region was as fol1ows: wild type (3 patients) and mutant type (3 patients) at aa 70; and wild type (3 patients) and mutant type (3 patients) at aa 91. Interferon sensitivity-determining regions demonstrated mutation 0 (1 patient), mutation 1 (4 patients) and mutation 2 (1 patient) (Table 1).

Changes from baseline in HCV-RNA at week 4 were 4.5 ± 2.0 $\log_{10}$IU/mL for DFPP plus IFN-β induction therapy and 2.9 ± 1.2 $\log_{10}$IU/mL for previous IFN therapy (Fig. 1). Changes from baseline in HCV-RNA at week 12 were 5.3 ± 2.0 $\log_{10}$IU/mL for DFPP plus IFN-β induction therapy and 4.6 ± 2.2 $\log_{10}$IU/mL for previous IFN therapy (Fig. 1).

Overall viral dynamics of DFPP plus IFN-β induction therapy for 4 weeks and PEG-IFN/RBV showed a reduction in viral load of 2 log in 87.5% (7/8) and 87.5% (7/8) at 4 and 12 weeks after the start of treatment, respectively (Table 1).

The RVR rate was significantly higher with DFPP plus IFN-β induction therapy than with previous IFN therapy. Furthermore, cEVR rate was significantly higher with DFPP plus IFN-β induction therapy than with previous IFN therapy, even in cases of re-treated patients with chronic genotype 1b hepatitis C and high viral load (relapsers or non-responders to previous IFN therapies) (Fig. 2).

A typical case of DFPP plus IFN-β induction therapy is presented here (Fig. 3).

TABLE 1. *Early viral dynamics with DFPP plus IFN treatment*

Case	Age/sex	Liver biopsy	Viral mutation			Previous IFN therapy		DFPP plus IFNβ induction therapy	Combined medicine	Viral dynamics of previous treatment			Viral dynamics after DFPP + IFN		
		Grade/Stage	ISDR	aa70	aa91		Outcome			Before treatment	log drop 4 weeks	log drop 12 weeks	Before treatment	log drop 4 weeks	log drop 12 weeks
1	40/M	A1/F1	1	wild	mutant	PEG–IFN	Relapser	IFNβ (3MU 2/day) 2 weeks → PEG–IFNα–2b + RBV	Fluvastatin	6.4	2.7	6.4	7.1	3.7	5.9
2	62/F	A1/F0	ND	ND	ND	IFNβ + RBV	NR	IFNβ (3MU 2/day) 2 weeks → PEG–IFNα–2a + RBV	Fluvastatin	5.4	0.8	0.3	7.1	4.9	7.1
3	54/F	A2/F2	1	mutant	wild	IFNβ + RBV	Relapser	IFNβ (3MU 2/day) + RBV 2 weeks → PEG–IFNα–2b + RBV	Teprenon	6.9	4.0	5.7	7.1	5.9	7.1
4	45/F	A2/F2	0	mutant	mutant	IFNβ + RBV	NR	IFNβ (3MU 2/day) + RBV 2 weeks → PEG–IFNα–2b + RBV	Fluvastatin Teprenon	7.3	1.8	1.9	6.6	0.5	0.7
5	57/M	A1/F1	ND	ND	ND	PEG–IFNα–2b + RBV	Relapser	IFNβ (3MU 2/day) + RBV 2 weeks → PEG–IFNα–2b + RBV	Fluvastatin Teprenon	6.0	3.2	6.0	6.2	6.2	6.2
6	64/M	A2/F2	2	wild	wild	IFNα	Relapser	IFNβ (3MU 2/day) + RBV 2 weeks → PEG–IFNα–2b + RBV	Fluvastatin Teprenon	6.0	4.3	6.0	4.2	4.2	4.2
7	63/M	A2/F2	1	wild	wild	PEG–IFNα–2b + RBV	Relapser	IFNβ (3MU 2/day) + RBV 2 weeks → PEG–IFNα–2b + RBV	Fluvastatin Teprenon	6.0	2.3	6.0	6.7	6.7	6.7
8	73/M	A2/F4	1	mutant	mutant	PEG–IFNα–2a + RBV	Relapser	IFNβ (3MU 2/day) + RBV 2 weeks → PEG–IFNα–2b + RBV	Fluvastatin Teprenon	6.8	3.9	4.5	5.5	3.7	5.5

aa, amino acid; ISDR, interferon sensitivity region; DFPP, double filtration plasmapheresis; IFN, interferon; ND, not done; NR, non-responder; PEG-IFN, pegylated interferon; RBV, ribavirin.

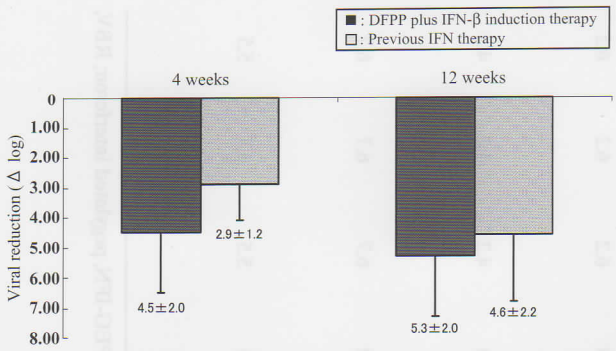
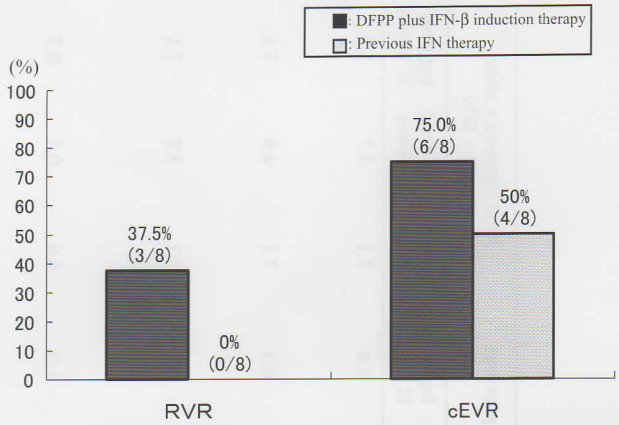


FIG. 1. Log changes in viral reduction from baseline during the initial 12 weeks of treatment for chronic hepatitis C virus infection, genotype 1b and high viral load.



RVR, rapid virological response; cEVR, complete early virological response

FIG. 2. Rapid virological response (RVR) and complete early virological response (cEVR) rate in double filtration plasmapheresis (DFPP) plus interferon (IFN)-β induction therapy and previous IFN therapy.

CASE 3

Patient: A 54-year-old female.

Object: IFN therapy.

Medical history: Fractured pelvis in a traffic accident in 1990; history of blood transfusion at that time.

Smoking history: None.

History of current medical condition: The patient was diagnosed with chronic hepatitis C in 2006. Thereafter, she was monitored by a local doctor. In February 2008, treatment with IFN therapy was started, so liver function tests yielded abnormal results: alanine aminotransferase (ALT), 62 IU/L; and aspartate aminotransferase (AST), 47 IU/L. The patient showed infection with genotype 1b HCV with high viral load (6.9 $\log_{10}$IU/ml). Liver biopsy showed mild necroinflammation with portoportal fibrosis (grade A2, stage F2).

Course: Anti-viral treatment (IFN-β and RBV) was initiated in February 2008. As a result of therapy, liver function test results normalized. HCV-RNA levels decreased, but were not completely eliminated. Combination therapy with IFN-β and RBV was stopped after finishing the 48-week protocol.

After the end of IFN treatment, serum levels of AST (24 IU/L) and ALT (11 IU/L) increased. Like-

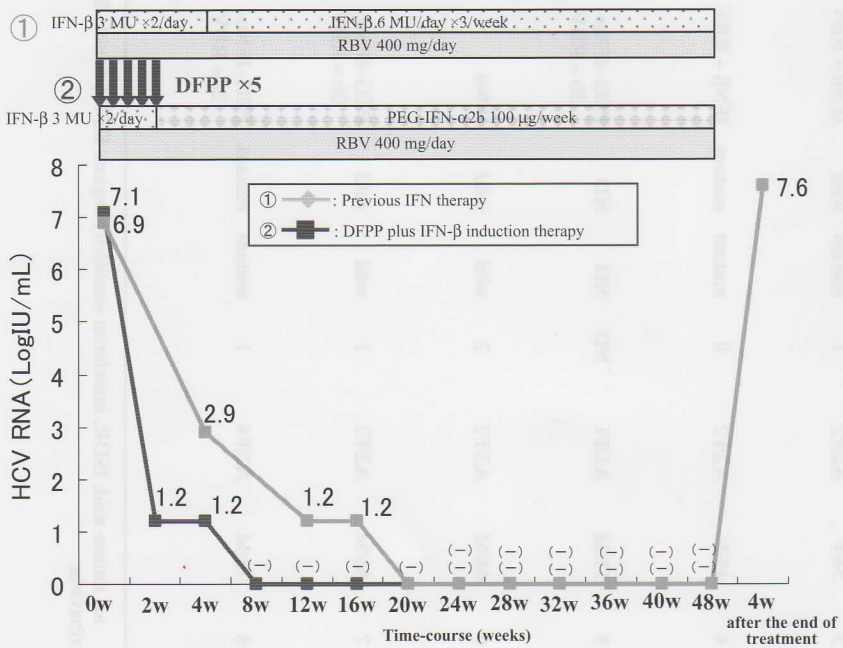


FIG. 3. Clinical course. Double filtration plasmapheresis (DFPP) combined with interferon (IFN)-β and ribavirin (RBV) therapy was done. The amount of hepatitis C virus (HCV)-RNA decreased rapidly with DFPP. The patient was diagnosed with complete early virological response (cEVR).

wise, HCV-RNA levels increased again. The patient was therefore diagnosed as a relapser.

From July 2010, combination therapy with IFN-β and RBV during DFPP was administered for a second time. During DFPP treatment, levels of HCV-RNA and ALT decreased dramatically. After DFPP plus IFN-β at 3 MU twice daily and RBV for 2 weeks, the patient was scheduled to receive PEG-IFN-α2b and RBV combination therapy. HCV-RNA was not detectable during the 8-week treatment. She was diagnosed as showing cEVR.

DISCUSSION

Interferon therapy has been developed to more effectively eliminate HCV. Nevertheless, even the most popular combined therapy with PEG-IFN and RBV for 48 weeks is unable to remove HCV in some 49% of difficult-to-treat hepatitis C patients with genotype 1b having a high viral load of HCV-RNA. Currently, the overall sustained virological response (SVR) rates associated with either PEG-IFN-α2a or PEG-IFN-α2b and RBV are approximately 55–65% across all genotypes (3).

The importance of a successful re-treatment strategy for refractory or relapsed patients with HCV cannot be overemphasized. DFPP is a type of plasmapheresis therapy, representing an apheretic technique to remove high molecular weight substances including HCV from the plasma by a second filter. Use of DFPP was approved in Japan in April 2008 for the retreatment of chronic hepatitis C patients with genotype lb and high viral load, in whom HCV was not eradicated by previous IFN therapy.

After DFPP, HCV-RNA levels have been shown to decrease transiently by approximately 50–90% immediately after plasmapheresis and to return to either pre-treatment or higher levels within approximately 4–6 h (6–8).

A few reports have described the efficacy of DFPP combined with IFN against chronic hepatitis C. Fujiwara et al. reported that DFPP together with IFN administration produced a substantial reduction in viral load during the early stages of treatment and achieved a high rate of SVR (9), suggesting this treatment as a new modality for chronic hepatitis C patients in difficult-to-treat states, whereas Yamashita et al. found only a transient effect of DFPP.

IFN-β is thus approved for the treatment of chronic hepatitis C in Japan (10). Okushin et al. reported on the early and high rate of disappearance of plasma HCV-RNA with administration of IFN-β twice a day, i.e. drip infusion of 3 MU of IFN-β in the morning and evening. Twice-daily administration of IFN-β for the first 4 weeks as induction therapy followed by IFN-α2b monotherapy showed a higher SVR rate than once-daily administration of IFN-β or IFN-α monotherapy (11). Furthermore, studies have reported that administration of IFN-β at 3 MU in the morning and evening for 2 weeks reduces HCV-RNA levels by 3 logIU/mL in many cases, whereas co-administration of PEG-IFN and RBV decreases levels by 1–2 logIU/mL (12,13), clearly indicating that the former regimen results in a higher rate of HCV-RNA decrease in the early phase of treatment.

In this study, we used DFPP plus IFN-β induction therapy to enhance the efficacy of treatment for chronic hepatitis C patients in whom HCV was not eradicated by earlier PEG-IFN/RBV combination therapy. We then assessed early viral dynamics associated with SVR to determine the efficacy of DFPP and IFN-β in patients with chronic hepatitis C who had not responded to previous IFN therapy.

Both EVR and RVR, which are indicated by loss of serum HCV RNA at weeks 12 and 4, respectively, are closely related to the SVR rate (14,15).

The EVR milestone also carries a strong predictive value for SVR. EVR can be broken down into two subgroups. The first comprises patients who have at least a 2-$\log_{10}$ drop in HCV-RNA after 12 weeks of treatment, but still show detectable viremia. These patients have achieved partial EVR. The second subgroup comprises patients who have completely undetectable HCV RNA levels after 12 weeks of treatment; these patients have achieved cEVR.

The first study to document this was by Davis et al. in 2003, who found that patients who experienced cEVR with co-administration of PEG-IFN-α2b and RBV went on to show an SVR rate of 84%, compared with only 22% for patients who achieved partial EVR (14,15).

Virus removal and eradication (VRAD) by DFFP is utilized to eliminate HCV by means of a modified DFPP that uses a second filter with the largest pore size.

To be able to predict SVR with PEG-IFN/RBV treatment, reduction of the HCV-RNA viral load by week 4 is considered essential. A 2 log reduction in HCV-RNA viral load by week 4 is prerequisite to achieving SVR with PEG-IFN/RBV treatment (16).

In our study of DFPP plus consecutive intravenous IFN-β treatment for 2 weeks, a reduction in viral load of 2 log was achieved in 50% of six patients at 12 weeks after the start of treatment.

In the treatment of genotype 1 chronic hepatitis C with PEG-IFN and RBV, relapse occurs in about

30% of patients after the end of treatment (2,3). Particularly in late viral responders, the rate of relapse is high (59%) after 48 weeks of treatment (17). Reducing the relapse rate and raising the SVR rate is vital. This requires (i) a dose increase; and (ii) prolongation of treatment.

More effective methods of therapy need to be developed to treat genotype 1b HCV infection, and final virological and biochemical outcomes for more patients treated with twice daily administration of IFN-β should be examined.

A combination of DFPP plus IFN-β induction produced a great reduction in the viral load during the early stage of combination therapy and may help achieve a high rate of SVR in such patients.

From the above considerations, DFPP plus consecutive intravenous IFN-β treatment for 2 weeks is a promising regimen for non-SVR patients with genotype lb and high viral loads who have previously been treated with PEG-IFN/RBV therapy.

Further study is needed to elucidate the SVR rate in a larger number of patients given DFPP plus IFN treatment, particularly with consecutive intravenous IFN-β.

CONCLUSION

Double filtration plasmapheresis was effective in non-responders who had previously received IFN therapy. IFN-β induction with DFPP followed by PEG-IFN/RBV may be promising as a therapy for patients with chronic HCV infection. 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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