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Minimal Dose Interferon Suppository Treatment Suppresses Viral Replication with Platelet Counts and Serum Albumin Levels Increased in Chronically Hepatitis C Virus-Infected Patients: A Phase 1b, Placebo-Controlled, Randomized Study

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Animal studies have shown that rectally administrated interferon (IFN) is transferred into the lymphatic system via the rectal mucous membrane, suggesting that an IFN suppository could serve as another drug delivery method. We developed an IFN suppository and administered it to patients with chronic hepatitis C to evaluate its efficacy and safety. Twenty-eight patients with chronic hepatitis C participated in the study. The low-dose IFN suppository containing 1,000 international units (IU) of lymphoblastoid IFNα was administered to 14 patients daily for 24 weeks. Others had a placebo dosing. In 13 of the 14 IFN suppository-treated patients, viral load decreased at week 4. The serum hepatitis C virus (HCV) RNA levels (Log IU/mL, mean ± standard error) were 5.65 ± 0.18 before the treatment and 5.17 ± 0.27 at week 4 ($P = 0.01$). The 2′–5′ oligoadenylate synthetase activity increased, while the CD4/CD8 ratio decreased significantly. Interestingly, platelet counts and serum albumin levels were significantly increased during and after the treatment. No serious adverse events were observed. The low-dose IFN suppository treatment suppressed HCV replication, modifying host immunity, with increased platelet counts and serum albumin levels. The IFN suppository could be considered a new drug delivery method to preserve the quality of life of patients.

Introduction

More than 170 million people worldwide and approximately 2 million Japanese are infected with hepatitis C virus (HCV) (Nishioka and others 1991; The Global Burden of Hepatitis C Working Group 2004). In Japan, about 70% of hepatocellular carcinoma is caused by HCV infection (Nishioka and others 1991; Kaneko and others 1994). Controlling HCV infection in order to prevent disease progression and hepatocarcinogenesis is a crucial issue. The standard current treatment of chronically HCV-infected patients is a combination therapy of pegylated interferon (IFN) α and ribavirin. Unfortunately, this treatment can cause many adverse events; for example, fever, fatigue, arthralgia, anemia, bleeding tendency, retinal disorder, and depression, which frequently lead to deterioration of the "quality of life" of the patients during the long treatment period (24–72 weeks) (Manns and others 2001; Bruno and others 2004; Scotto and others 2005; Hiramatsu and others 2006). In addition, although rare, some suffer from serious side effects; for example, pneumonitis, cerebral hemorrhage, retinal bleeding, sepsis, and depression-induced suicide attempts. Furthermore, many cirrhotic patients are not considered eligible for this treatment because of the high risk of severe adverse events (Ghany and others 2011; Yee and others 2012). Therefore, treatment with a lesser possibility of such adverse effects is desired.

Previous animal experiments have shown that rectally administered IFN is easily transferred into the lymphatic system via the rectal mucous membrane (Yoshikawa and others 1984, 1985). Rectally dosed IFN is carried from the colorectal regional lymphatic system finally to the thoracic duct. This led us to speculate that the IFN might travel in portal blood and act in the liver. The local IFN action may even allow a lower dose to suppress HCV replication with minimal side effects. In this study, we developed a low-dose IFN suppository, which can preserve the bioactivity of the IFN contained within it for a long period. We then performed a Phase 1b, placebo-controlled, and randomized

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study for patients with chronic hepatitis C. The first aim of our study was to assess the antiviral effect and safety of the IFN suppository. Our second aim was to examine the pathobiological effects caused by the rectal IFN administration.

Patients and Methods

Participants and study design

Twenty-eight chronically HCV-infected patients participated in this study. All of them were seropositive for HCV RNA by a polymerase chain reaction (PCR) test (Cobas Amplicor HCV test v2.0; Roche Diagnostics, Basel, Switzerland). They had received a histological diagnosis of chronic hepatitis within the preceding 12 months. The exclusion criteria were the following: age less than 18 or more than 70, previous treatment of IFN, average daily intake exceeding 50 g of ethanol, choledocholithiasis, cirrhosis, autoimmune hepatitis, primary biliary cirrhosis, hepatitis B virus infection, human immunodeficiency virus infection, intravenous-drug use, drug-induced liver disease, and pregnancy. Fourteen patients were allocated to the IFN suppository group, and the others were allocated to the placebo one.

This randomized, single-blind, and placebo-controlled study was conducted from October 1998 until October 2004 at the Department of Gastroenterology and Hepatology, Osaka General Medical Center (Osaka, Japan). Our primary objective was to evaluate the antiviral effect and safety of the IFN suppository. We also assessed the pathobiological effect of the IFN suppository.

The baseline characteristics of the patients are summarized in Table 1. There were no significant differences in the backgrounds of the IFN suppository and placebo groups. They were given the suppository containing 1,000 international units (IU) of IFN or placebo once every day for 24 weeks. Clinical and laboratory assessments were done every 4 weeks during treatment and until 24 weeks after the end of treatment. The long-term follow-up was also performed at 48 and 72 weeks after the end of the treatment.

Written consent to participate in this study was obtained from all the patients. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was also approved by the ethics committee of the Osaka General Medical Center.

Randomization and masking

The 28 patients were randomly assigned by a computer-generated randomization scheme to the IFN suppository group or the placebo one. The IFN suppository and placebo were prepacked into heat-sealed plastic molds and were identical in appearance. The patients were blinded to the allocation.

IFN suppository preparation

The IFN suppository contained a low dose (1,000 IU) of lymphoblastoid IFN α (BALL-1). In the Department of Pharmacy, the Osaka General Medical Center, it was prepared with IFN α (OIF; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) and hard fat (Pharmasol B-115; Nihon Yushi Co., Ltd., Tokyo, Japan), including an agent to prevent inactivation of the IFN due to the preparation process or long-time storage. The stable biological activity of IFN in the suppository was assessed using FL cells and Sindbis virus bioassay (Kono and Vilcek 1982; Fukuda and others 1988). The biological activity of the IFN in the suppository was well preserved (98% at 2 months after preparation, stored at 4°C).

Laboratory testing

The viral loads were measured by a quantitative PCR assay (Cobas Amplicor HCV Monitor Test v2.0; Roche Diagnostics; limit of quantization, 2.70–5.78 Log IU/mL). Serum samples showing high viral loads (>5.70 Log IU/mL) were 10-fold diluted and quantified again in order to precisely measure the high titer range. The HCV genotype was determined using PCR with a mixed primer set as previously reported (Chayama and others 1993). The CD4/CD8 ratio, 2′–5′ oligoadenylate synthetase (2–5 AS) activity, NK cell activity, interleukin (IL) 10, IFN α, and IFN γ in peripheral blood were tested before treatment and at week 8.

The CD4 or CD8-positive T-cell subset population in peripheral blood was measured by flowcytometry using monoclonal antibodies (Beckman Coulter, Inc., Brea, CA).

Table 1. Patient Characteristics at Baseline

Characteristics	*Interferon suppository* (n=*14*)	*Placebo* (n=*14*)	P *value*
Gender (male/female)	5/9	6/8	1.00[b]
Age (years)	59.3±11.2	54.4±8.9	0.22[c]
AST (IU/L)	46.5 (24–91)	37 (28–112)	0.43[d]
ALT (IU/L)	51 (17–101)	42.5 (16–159)	0.51[d]
Viral load (Log IU/mL)	5.65±0.68 (3.74–6.46)	5.67±0.56 (4.70–6.40)	0.96[c]
HCV genotype[a] (1b/2a+2b)	12/2	11/3	1.00[b]
Serum albumin (g/dL)	4.10±0.26	4.24±0.22	0.15[c]
Platelet count (10^4/mm^3)	16.1±4.07	16.5±5.23	0.83[c]
Histological diagnosis			
A score	1 (1–2)	1 (0–2)	0.82[d]
F score	1 (1–3)	1 (1–3)	0.86[d]

[a]Simmonds' classification, [b]Fisher's exact test, [c]Student's *t*-test, [d]Mann–Whitney *U* test.

A and F scores in histological diagnosis, AST and ALT levels are shown as medians (range). Other data are expressed as mean±standard deviation. The ranges of viral loads are shown as figures in the parentheses.

A score, necroinflammatory activity score; F score, fibrosis score; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.

The 2–5 AS activity was evaluated by radioimmunoassay (Eiken Kagaku Co., Ltd., Tokyo, Japan). NK cell activity was tested by ^{51}Cr-release cytotoxic assay (Perkin Elmer, Inc., Waltham, MA). Serum IL10 level was measured by enzyme immunoassay (BioSource International, Inc., Camarillo, CA). Serum IFN α and IFN γ were tested by radioimmunoassay (Dainabot Co., Ltd., Tokyo, Japan) and enzyme immunoassay (JIMRO, Gunma, Japan), respectively. Histological evaluation was performed by the histological scoring system of Desmet and others (1994).

Statistical analysis

We made no prospective calculations of statistical power. We selected the sample size to provide information on the effect and safety of the IFN suppository in the phase Ib trial. Differences between the IFN suppository and placebo groups before the treatment were compared by means of Student's *t*-test, Fisher's exact test, and the Mann–Whitney *U* test. To evaluate the statistical significance of the change in virus-related and immunological markers, platelet counts, and albumin levels, we used the paired *t*-test and Wilcoxon-signed rank test. The changes of viral loads, platelet counts, and serum albumin levels during and after the treatment were also assessed by one-way repeated measures ANOVA. The significance level used was 0.05 for all the tests. The statistical analysis was done with Stat View (version 5.0.1). All analyses were done on an intention-to-treat basis.

Results

Eligible patients were recruited from October 1998 to December 2002. All 14 patients treated with the IFN suppository completed the dosing. During long-term follow up, 2 patients were lost at 48 and 72 weeks after the end of treatment. One sought and received the standard IFN therapy at 72 weeks. Eleven patients completed the follow-up study. In the placebo group, 1 patient discontinued the placebo administration at week 16 but participated in the follow-up study. One patient was lost at 48 weeks after the end of the treatment. One sought and received the standard IFN therapy at 72 weeks. The follow up was completed with 12 control patients (Fig. 1).

Change of HCV viral load

In 13 of the 14 IFN suppository-treated patients, viral loads were decreased at week 4. One patient showed a more than 2 log drop of the viral load. However, breakthrough of viral replication (more than 0.3 Log elevation from the viral load nadir) was seen in 5 patients at week 24. After the end of the treatment, 2 patients had marked viral reactivation. One showed 2 log viral load elevation at 4 weeks after the end of the treatment, and the other showed 0.7 log elevation at 8 weeks after the treatment (Fig. 2).

The viral loads [Log IU/mL, mean ± standard error (S.E.) and ranges] of the IFN suppository cohort were 5.65 ± 0.18 (3.74–6.46) before the treatment, 5.17 ± 0.27 (2.78–6.36) at week 4, 5.21 ± 0.23 (2.95–6.32) at week 8, 5.26 ± 0.20 (3.77–6.42) at week 12, 5.23 ± 0.22 (3.48–6.49) at week 16, 5.23 ± 0.23 (3.15–6.45) at week 20, and 5.39 ± 0.22 (3.30–6.61) at week 24. Significant viral load suppression compared with pretreatment was seen during dosing ($P = 0.01$ at week 4, 0.002 at week 8, 0.005 at week 12, 0.002 at week 16, 0.002 at week 20, and 0.04 at week 24) (Fig. 3). One-way repeated measures ANOVA confirmed the significant suppression of the viral loads during the dosing ($P < 0.001$) (Fig. 3). In the placebo cohort, the one-way repeated measures ANOVA showed no statistically significant change of the viral loads while they fluctuated.

Changes of 2–5 AS activity, CD4/CD8 ratio, NK cell activity, IL10, IFN α, and IFN γ levels in peripheral blood

In the IFN suppository-treated cohort, 2–5 AS activity was significantly increased by the treatment. The activity (pmol/dL) was 87.9 ± 12.7 (mean ± S.E.) before the treatment and 120.8 ± 18.2 at week 8 ($P = 0.008$). On the other hand, the CD4/CD8 ratio was decreased significantly by the treatment.

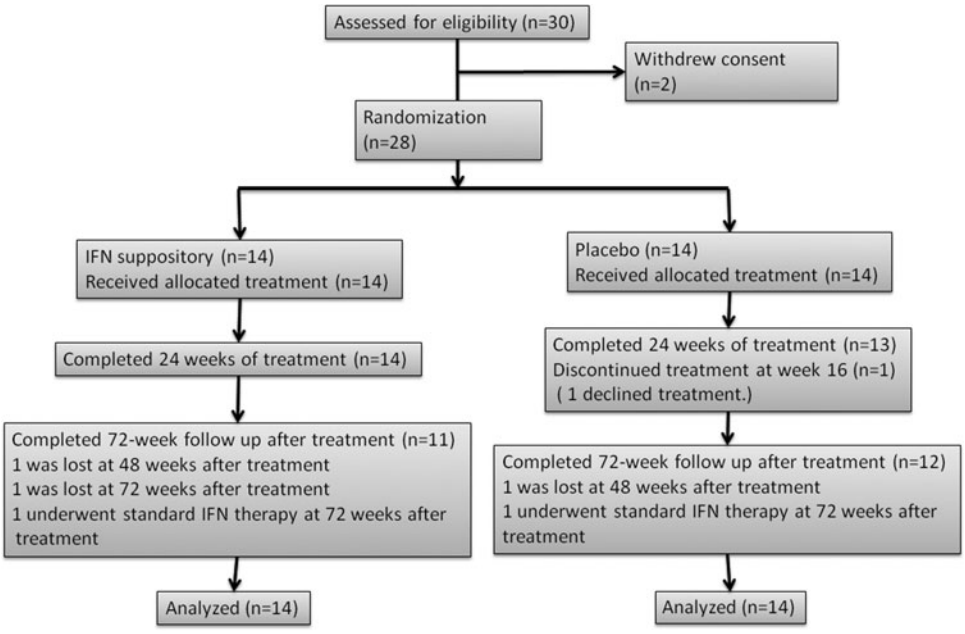

FIG. 1. Study flow diagram. We randomized patients to receive IFN suppository or placebo for 24 weeks. In the IFN suppository cohort, 2 patients were lost at 48 and at 72 weeks after treatment. One underwent standard IFN therapy at 72 weeks after treatment. In the placebo cohort, 1 patient declined continued dosing of the placebo at week 16. One patient was lost at 48 weeks after treatment. One underwent standard IFN therapy at 72 weeks after treatment. IFN, interferon.

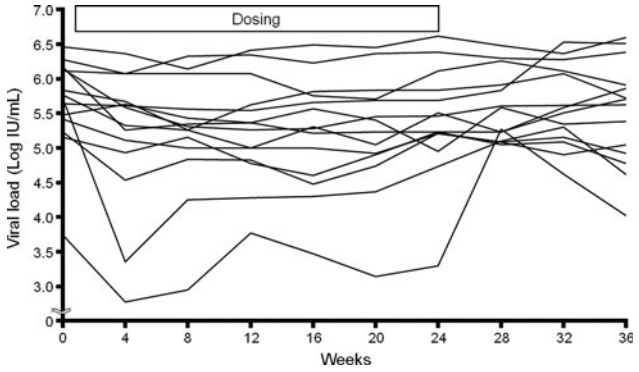

FIG. 2. Changes of viral loads for individual patients during and after the IFN suppository treatment. The viral loads were measured every 4 weeks.

The ratio was 2.67 ± 0.35 (mean ± S.E.) before and 2.07 ± 0.26 at week 8 ($P = 0.01$). In contrast, NK cell activity, IL10, and IFN γ levels were not significantly changed. IFN α was not detected either before or at week 8 in the serum of any patient. No significant change of these markers was observed in the placebo patients.

Changes of platelet and leukocyte counts and hemoglobin concentration

Platelet counts increased during the IFN suppository treatment, and maximum counts were observed at 4 weeks after the end of the treatment. The platelet counts gradually decreased thereafter, whereas significant elevation was sustained till 72 weeks after the end of the treatment. The platelet counts ($\times 10^4/mm^3$, mean ± S.E.) were 16.1 ± 1.1 before the treatment, 18.8 ± 1.4 at 4 weeks after the end of the treatment ($P = 0.002$), and 18.6 ± 1.8 at 72 weeks after the treatment ($P = 0.02$). One-way repeated measures ANOVA confirmed the significant increase of platelet counts during and after the treatment ($P = 0.006$) (Fig. 4).

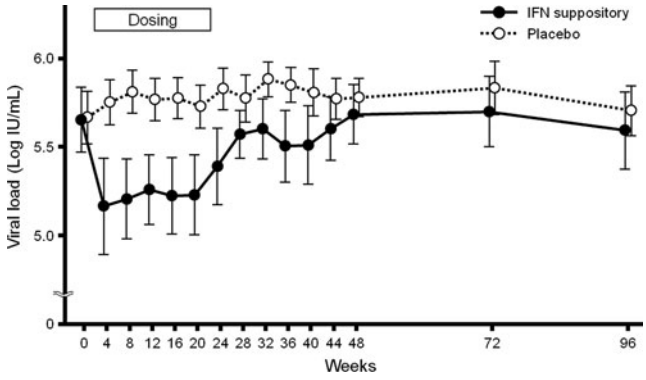

FIG. 3. Changes of viral loads during and after dosing. The results are expressed as means ± S.E. *Closed circles* and *solid lines* represent the IFN suppository-treated patient data, while *open circles* and *dotted lines* are for the controls. The viral loads were suppressed significantly during IFN suppository treatment (The P values were 0.01 at week 4, 0.002 at week 8, 0.005 at week 12, 0.002 at week 16, 0.002 at week 20, and 0.04 at week 24). Statistical significance during dosing was confirmed by one-way repeated measures ANOVA ($P < 0.001$). No significant change of viral loads was observed in the placebo patients. S.E., standard error.

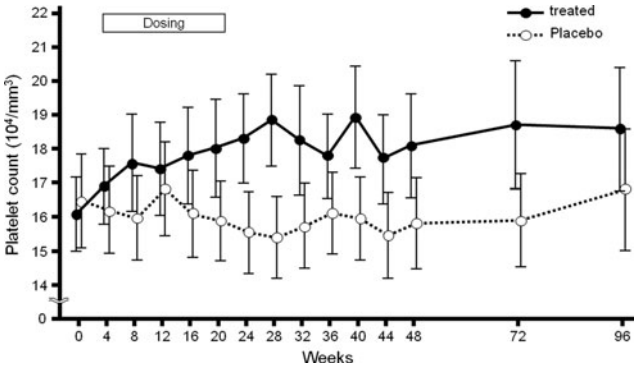

FIG. 4. Changes of platelet counts during and after dosing. The results are expressed as means ± S.E. *Closed circles* and *solid lines* represent the IFN suppository-treated patient data, while *open circles* and *dotted lines* are those for the controls. The IFN suppository treatment led to a significant increase in platelet counts compared with before dosing ($P < 0.01$ except $P = 0.06$ at week 4, $P = 0.01$ at 8 and 48 weeks after the end of treatment, and $P = 0.02$ at 72 weeks after treatment). No significant change of platelet counts was seen in the placebo group.

Leukocyte counts ($/mm^3$) showed a significant increase at week 8 and week 16 ($P = 0.01$ and $P = 0.04$, respectively, compared with pretreatment). No significant change was seen in the hemoglobin concentration.

No significant change of platelet or leukocyte counts or hemoglobin concentration was seen in the placebo group.

Changes of hepatic transaminases and serum albumin levels

No significant change was seen in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), or γ-glutamyl tarnspeptidase (γ-GTP) levels in the IFN suppository-treated cohort. On the other hand, serum albumin showed a significant increase during and after the treatment. Compared with pretreatment levels, a statistically significant difference was seen at 4 and 16 weeks after the end of the treatment ($P = 0.02$ and $P = 0.04$, respectively). The serum albumin levels (g/dL, mean ± S.E.) were 4.10 ± 0.07 before, 4.27 ± 0.09 at 4 weeks after the end of the treatment, and 4.26 ± 0.08 at 16 weeks after the end of the treatment. One-way repeated measures ANOVA showed a significant increase of the albumin levels until 16 weeks after the end of the treatment ($P = 0.02$).

No significant change was observed in the placebo cohort.

Safety

There were no serious adverse events that led to dosing interruption or discontinuation. Adverse events occurred in 2 out of 14 (14.3%) patients in the IFN suppository group and in 2 out of 14 (14.3%) in the placebo group. In the IFN group, 1 patient had nausea and mild colic pain in the lower abdomen at week 8. The symptoms were resolved in 2 days without treatment. The other patient had epigastralgia at week 20. It was cured with 5-day medication treatment using H_2 blocker. In the placebo group, 2 patients had nausea at week 2 and 18, which was resolved in a few days without any medication. No adverse laboratory findings in

hematology or clinical chemistry testing were seen in either the IFN suppository or placebo group.

Discussion

With consideration of maximal safety and effective biological activity, we prepared a low-dose IFN suppository. The dose was determined as follows. It is reported that 0.1 IU/mL of IFN α is enough to induce 2–5AS activity in cultured cells (Uno and others 1998). According to a previous report on rectal administration of IFN to rats (Yoshikawa and others 1985), a 1,000-unit IFN suppository is estimated to introduce approximately 0.01 IU/mL of IFN concentration into the lymph of the thoracic duct in a human being. Therefore, the IFN concentration in upstream lymph nodes in the mesenteric or hepatic hilar regions is expected to be more than 0.01 IU/mL. For these reasons, we set 1,000 U as the minimal effective dose for the clinical trial.

The IFN suppository treatment suppressed viral replication, although the dose of IFN was very low ($1/10^4$ to $1/10^3$ compared with standard injection dose), and most of the patients were difficult to treat because of their being genotype 1b and having high viral loads. With regard to reproducibility and accuracy of the Cobas Amplicor HCV test v2.0, they reported that the coefficients of variation were 1.0% to 1.5% for log10-transformed HCV RNA levels (Tawara and others 2000). Therefore, about 0.3 to 0.5 log drops of the mean viral loads seen during the IFN suppository dosing are considered significant antiviral effects. Elevated 2–5 AS activity and decreased CD4/CD8 ratio, which was considered to be associated with immune pressure to eliminate HCV (Pham and others 1995; Kiefersauer and others 1997; Urbani and others 2008), confirmed the antiviral action and immune modulation. However, 5 patients showed viral breakthrough at week 24. After the end of the treatment, 2 patients experienced marked viral reactivation on release from the antiviral pressure of the treatment.

Interestingly, in addition to the antiviral effect, platelet counts and serum albumin levels were elevated during and after the treatment in contrast to standard IFN treatment. Unexpectedly, platelet count elevation remained till 72 weeks after the end of the treatment. It is uncertain whether the IFN suppository treatment could improve liver function to increase platelet counts, because transaminase levels did not decrease significantly. Since some cytokines enhance megakaryocyte proliferation (Tsuji-Takayama and others 1996; Muraoka and others 1997; Huang and others 2007), rectal IFN administration might augment thrombocytosis by modifying the immune system. On the other hand, Kodama and others (2010) showed that platelets have an antifibrotic role in suppressing type 1 collagen expression via the hepatocyte growth factor (HGF)-Met signaling pathway. According to their findings, the increase of platelet counts induced by the IFN suppository itself could be beneficial to liver function.

With regard to safety, the IFN suppository treatment showed a few minimal adverse events and no significant difference from the placebo cohort. The influenza-like symptoms usually seen in standard IFN treatment were not observed, and the patients' quality of life was well preserved. Neither hematological disorder nor depression was observed in this study.

Although the pegylated IFN and ribavirin combination therapy is the standard of care against HCV infection, many patients are not indicated for it because of advanced liver disease, old age, thrombocytopenia, leukocytopenia, or anemia. Hopefully, IFN-free antiviral therapy for HCV can become available in the future. Recently, Lok and others (2012) reported that 4 out of 11 patients treated with an NS5A replication complex inhibitor and an NS3 protease inhibitor reached sustained virologic response. In contrast, they also showed that 9 out of 10 patients achieved sustained virologic response when treated with these direct-acting antiviral agents added to the pegylated IFN and ribavirin combination. This could imply that only suppression of viral replication by direct-acting antiviral agents without immune activation could jeopardize complete eradication of HCV. When we finished this study in October 2004, the pegylated IFN and ribavirin combination therapy had just become available and was shown to improve antiviral outcome. Since we did not have a specific strategy for the IFN suppository as distinguished from the pegylated IFN and ribavirin therapy at that time, we hesitated about reporting our findings. However, combination therapy of direct-acting antiviral agents has recently become a possible option due to its antiviral significance and less toxic features. We consider that the IFN suppository might have the add-on benefit of being an IFN injection-free therapy which can help preserve patients' quality of life.

To the best of our knowledge, this is the first report showing the efficacy of a low-dose IFN suppository against HCV infection. A further study of dose escalation is needed in order to determine the optimal dose for efficacy and safety. However, in this study, we have shown that rectal administration of IFN led to biological activity in the hosts as well as antiviral activity against HCV and may serve as a new drug delivery method.

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Author Disclosure Statement

No competing financial interests exist.

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