Table 2. Results of Interferon Treatment

	Early-Intervention Group SR/N (%)*	Late-Intervention Group SR/N (%)*	Total SR/N (%)*	P Value†
After short-term IFN therapy				
$< 1 \times 10^5$ copies/mL‡				
1b	6/6 (100)	4/7 (57)	10/13 (77)	>.1
others	2/2 (100)	2/2 (100)	4/4 (100)	
total	8/8 (100)	6/9 (67)	14/17 (82)	>.1
$\geq 1 \times 10^5$ copies/mL‡				
1b	4/6 (67)	0/6 (0)	4/12 (33)	.061
others	1/1 (100)	0/0 (0)	1/1 (100)	
total	5/7 (71)	0/6 (0)	5/13 (38)	.021
Total	13/15 (87)	6/15 (40)	19/30 (63)	.021
After follow-up IFN therapy				
$< 1 \times 10^5$ copies/mL‡				
1b	6/6 (100)	5/7 (71)	11/13 (85)	>.1
others	2/2 (100)	2/2 (100)	4/4 (100)	
total	8/8 (100)	7/9 (78)	15/17 (88)	>.1
$\geq 1 \times 10^5$ copies/mL‡				
1b	6/6 (100)	1/6 (17)	7/12 (58)	.015
others	1/1 (100)	0/0 (0)	1/1 (100)	
total	7/7 (100)	1/6 (17)	8/13 (62)	.005
Total	15/15 (100)	8/15 (53)	23/30 (77)	.006

Abbreviation: SR: sustained virological response.

*(%): Rate of sustained virological response.

†P value for the comparison of early-intervention group to late-intervention group by Fisher exact test (2-tailed).

‡Copies/mL refers to HCV-RNA.

late-intervention group, after both initial and follow-up therapy ($P = .021$ and $P = .006$, respectively). All patients who were considered to have a sustained virological response were monitored for at least 2 years after treatment; during this period, serum ALT levels did not exceed the ULN in any of these patients. In addition, in these patients HCV-RNA remained negative at the end of the 2-year period. All patients seroconverted and became anti-HCV antibody–positive.

Results of short-term IFN administration, stratified by viral load and genotype, were as follows. A low viral load in the early-intervention group was associated with a sustained virological response in all patients, regardless of genotype. In the late-intervention group, the sustained virological response rate was 57% (4/7) for patients with genotype 1b. In the early-intervention group, among patients with a high viral load, a sustained virological response occurred in 5 of 7 (71%) patients and in 4 of 6 (67%) of those with genotype 1b. In the late-intervention group, sustained virological responses did not occur in any patient with a high viral load. Thus, the sustained virological response rate was significantly higher in the early-intervention group than in the late-intervention group ($P = .021$).

After follow-up IFN therapy, sustained virological responses occurred in the 2 patients with a high viral load in the early-intervention group, so that sustained virological responses eventually occurred in all patients in this group. In the late-intervention group, a sustained virological response occurred after follow-up therapy in 1 patient with a low viral load and 1 patient with a high viral load. Results after follow-up indicated that the sustained virological response rate for patients with a high viral load in the early-intervention group was significantly higher than that in the late-intervention group ($P = .005$).

The presence or absence of symptoms at the onset of hepatitis and the efficacy of IFN therapy were investigated. In the early-intervention group, 8 patients were symptomatic and 7 patients were asymptomatic. There was no difference in the sustained virological response rate between symptomatic patients (88%, 7 out of 8 patients) and asymptomatic patients (86%, 6 out of 7 patients) after short-term therapy. In the late-intervention group, 9 patients were symptomatic and 6 patients were asymptomatic. After short-term therapy, the sustained virological response rate was higher in the symptomatic patients (56 %, 5 out of 9 patients) than in the asymptomatic patients (17%, 1 out of 6 patients), but the difference was not significant. After additional therapy, the sustained virological response rate was higher in the symptomatic patients (67%, 6 out of 9 patients) than in the asymptomatic patients (33%, 2 out of 6 patients), but the difference was not significant. There was, therefore, no relationship between the presence or absence of symptoms at the onset of hepatitis and the efficacy of IFN therapy.

Sustained Virological Response and Eradication of HCV-RNA During Initial Therapy. Table 3 shows the virological state of patients who became negative for HCV-RNA during initial IFN therapy. HCV-RNA became negative at Week 1 after the start of treatment in 13

Table 3. Patients Becoming HCV-RNA Negative During Short-Term Therapy and Sustained Virological Response

	Early-Intervention Group N = 15	Late-Intervention Group N = 15	Total N = 30
HCV-RNA negative at Week 1 after starting treatment	13*	5*	18
HCV-RNA negative at Week 2 after starting treatment	15**	8**	23
HCV-RNA negative at the completion of 4-week treatment	15**	8**	23
SR (%)† after short-term therapy	13 (87%)	6 (40%)	19 (63%)
SR (%)† after follow-up therapy	15 (100%)	8 (53%)	23 (77%)

Abbreviation: SR, sustained virological response.

*P = .008 for the comparison of early-intervention group to late-intervention group by the Fisher exact test (2-tailed)

**P = .006 for the comparison of early-intervention group to late-intervention group by the Fisher exact test (2-tailed).

†(%): Rate of sustained virological response.

Table 4. Efficacy of Initial Interferon Treatment Classified According to Factor

	Sustained Virological Response N = 19	No Response N = 11	P Value*
Age (y, mean ± SD)	40 ± 12	37 ± 8	.467
Gender (male/female)	14/5	5/6	.238
Baseline test values (before IFN therapy)			
Serum ALT (IU/L, mean ± SD)	493 ± 201	406 ± 143	.218
HCV load (low/high)†	14/5	3/8	.023
(10^5 copies/mL, mean ± SD)‡	1.78 ± 2.24	4.40 ± 2.81	.009
Genotype (1b/others)	14/5	11/0	.129
Time of treatment initiation (early-intervention group/late-intervention group)	13/6	2/9	.021
HCV-RNA at Week 1 (negative/positive)	18/1	0/11	<.001

Abbreviation: y, years.

*P value for comparison with sustained virological response to nonresponse by the Fisher exact test (2-tailed) or the unpaired Student *t* test.

†HCV load: low (< 1×10^5 copies/mL), high (≥ 1×10^5 copies/mL).

‡Copies/mL refers to HCV-RNA.

patients in the early-intervention group and in 5 patients in the late-intervention group; the higher conversion rate for the early-intervention group was significant (P = .008). A sustained virological response occurred after initial treatment in all 18 patients who tested negative for HCV-RNA at Week 1. An additional 5 patients were negative for HCV-RNA at Week 2. Of these, 1 patient from the late-intervention group underwent a sustained virological response at the completion of the initial therapy; a recrudescence of disease occurred in the other 4 patients. After follow-up therapy, all 4 of these patients also underwent a sustained virological response. All 7 patients who continued to be positive for HCV-RNA at Week 2 were in the late-intervention group. These 7 patients continued to be HCV-RNA positive after completion of the initial treatment and continued to be virological nonresponders after follow-up therapy.

Factors Influencing the Efficacy of Initial IFN Treatment. Table 4 shows the results of initial IFN treatment classified according to various factors. Univariable analysis of sustained virological response after initial therapy yielded the following results: age (P = .467), gender (P = .238), pretreatment serum ALT levels (P = .218), HCV load (P = .009), genotype (P = .129), timing of initial treatment (P = .021), and loss of HCV-RNA by Week 1 (P < .001). Significant factors were viral load (low viral load group), the timing of initial treatment (early-intervention group), and loss of HCV-RNA by Week 1.

Discussion

Our randomized controlled trial in patients with acute hepatitis C demonstrates that short-term (4 weeks) IFN therapy is effective when the treatment is initiated 8 weeks after the onset of acute hepatitis. In this study, to demonstrate the efficacy of short-term IFN therapy for acute hepatitis C, we applied strict criteria for the diagnosis; patients with posttransfusion hepatitis were excluded. As substantiated by our study, acute hepatitis C is associated with a few subjective symptoms that are easily overlooked by many patients. Diagnosis requires finding elevated serum ALT levels, and conversion from HCV-RNA negative to HCV-RNA positive.

There are no standard methods for treatment of acute hepatitis C. Both IFN-alfa[3–5,13,15–17] and IFN-beta[12,14] have been used. Previous studies have involved predominantly asymptomatic patients with posttransfusion hepatitis.[13–15] Recent studies by European researchers[4,5] have shown that 75% or 68% of patients have jaundice at the onset of acute hepatitis C, but Japanese studies have found that few patients have jaundice at the onset.[12,19] The latter finding is consistent with the results of this study, in which only 3 of 34 patients had jaundice. The reason for this difference in the incidence of jaundice at the onset of acute hepatitis C between Europeans and Japanese is unclear. A recent study of the natural history of acute hepatitis C found spontaneous viral clearance within 5 weeks of the onset of symptoms in a high proportion of patients with symptomatic hepatitis.[4] As a 24-week course of IFN therapy starting immediately after the onset of acute hepatitis C was very effective (sustained response of 95%),[3] deciding when to start IFN therapy is important; spontaneous clearance of HCV may occur within 5 weeks of the onset of acute hepatitis C. In addition, asymptomatic acute hepatitis C is common in Japan. Therefore, in this study we monitored serum ALT levels and HCV-RNA for 8 weeks before initiating of IFN therapy. Clearance of HCV occurred within this 8-week period in 4 of 34 patients. All patients in the late-intervention group underwent some changes in serum ALT levels during the 1-year period of observation, but none of these patients became HCV-RNA negative after 1 year.

Recent reports of European studies[4,5] indicated a high rate (67%-68%) of spontaneous HCV clearance within 12 weeks of the onset of acute hepatitis C, but the corresponding rate was low in our study (12%). The reason for this difference is unclear, but the fact that we observed our patients for only an 8-week period may have been a contributing factor. In Japan, patients are often referred to a specialist hospital by the doctor who made the initial di-

agnosis of acute hepatitis, if treatment is considered to be necessary. Since most patients referred do require treatment, it is possible that patients in whom hepatitis resolves spontaneously would have been excluded. Spontaneous clearance of HCV should be investigated in a large group of patients who have been followed from the time of infection to the onset of acute hepatitis C. In our study, spontaneous clearance of HCV was not observed in any patients who had a needle-stick injury and who had been followed from the time of the infection to the onset of acute hepatitis C. The natural history of acute hepatitis C in the Japanese seems to be different from that in Europeans, but there are no data available to indicate whether this is the case.

We found no relationship between the presence or absence of symptoms at the onset of hepatitis and the efficacy of IFN therapy, although spontaneous clearance of HCV occurred in many patients with symptoms at the onset.[4,5] In this study, short-term daily IFN therapy was associated with a high (87%) sustained virological response rate. When a recrudescence of disease occurred in the early-intervention group, patients received an additional 20-week course of follow-up therapy; sustained virological responses occurred in all patients. In this respect our results are similar to those reported by Jaeckel et al.[3] It appears that IFN therapy is more effective in acute hepatitis C than in chronic hepatitis C. Due to IFN treatment's high cost and frequency of adverse events, it is desirable to shorten the duration of treatment with IFN as much as is practicable.

When we studied the efficacy of IFN therapy in patients in whom HCV-RNA became negative during short-term (4 weeks) treatment, we found that it was necessary for patients to become HCV-RNA negative within the first week of treatment for a sustained virological response to occur. A sustained virological response occurred in all 18 patients, in whom this early event occurred; monitoring for at least the following 2 years indicated that no recurrence of HCV-RNA positivity and no relapse of elevated serum ALT levels occurred in any of these patients. In IFN therapy for chronic hepatitis C, the sustained virological response rate is low if the HCV load does not decrease early after the start of therapy.[20] These findings suggest that short-term IFN therapy may be associated with satisfactory results in acute hepatitis C, if patients become HCV-RNA negative within the first week of treatment. Conversion from HCV-RNA positive to HCV-RNA negative within 2 weeks of the start of IFN therapy is one indicator that treatment of chronic hepatitis C may be efficacious.[21,22] We also found that a 20-week course of follow-up IFN therapy may be associated with a sustained virological response in patients who are HCV-RNA negative within 2 weeks of starting treatment.

In this study, significant predictive factors were viral load (low viral load), timing of initial treatment (early-intervention group), and conversion to HCV-RNA negative at Week 1. Although viral load is the best predictor of the efficacy of IFN therapy for chronic hepatitis C,[10,11] it was also found to be a significant predictor of a sustained virological response in patients with acute hepatitis C. Our results suggest that a short course (4 weeks) of IFN therapy for acute hepatitis C may be associated with satisfactory results in patients who have low viral loads, by whom IFN therapy is started early, or who become HCV-RNA negative within one week of the initiation of treatment.

In chronic cases that had been monitored for 1 year, the viral elimination rate was also high for those with a low viral load. As fulminant hepatic failure is rare in acute hepatitis C, it was previously believed that spontaneous clearance of HCV would occur after the hepatitis had spontaneously subsided. Accordingly, patients were followed for 1 year, rather than having costly IFN treatment at an early stage of the disease. However, in this study we found that IFN therapy was more effective when the treatment was initiated at an early stage after the onset of acute hepatitis. We studied 2 groups; treatment was initiated after 8 weeks of monitoring in the early-intervention group and after observation for a year or more in the late-intervention group. However, we were unable to investigate the timing of initiation of IFN therapy after the onset of acute hepatitis C. Hofer et al.[4] reported that patients with acute hepatitis C had a high rate of spontaneous viral clearance within one month of onset of symptoms, and that IFN therapy was indicated in patients who failed to clear the virus within 35 days of the onset of symptoms. Gerlach et al.[5] observed that spontaneous clearance of HCV usually occurs within 12 weeks of the onset of symptoms; no spontaneous clearance of HCV occurred after 16 weeks. The timing of IFN therapy for acute hepatitis C should, therefore, be investigated in a larger study.

In conclusion, short-term (4 weeks) IFN therapy at an early stage of acute hepatitis C may be associated with satisfactory results, especially when HCV-RNA becomes negative within the first week of treatment. If the patient remains HCV-RNA positive after Week 1 of IFN treatment, a 24-week course of IFN therapy is recommended to try to achieve a satisfactory result.

References

1. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 1997;22:825–832.

2. Takano S, Yokosuka O, Imazeki F, Tagawa M, Omata M. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. HEPATOLOGY 1995;21:650–655.
3. Jaeckel E, Cornberg M, Wedemeyer H, Santantonio T, Mayer J, Zankel M, et al. German Acute Hepatitis C Therapy Group. Treatment of acute hepatitis C with interferon alfa-2b. N Engl J Med 2001;15:1452–1457.
4. Hofer H, Watkins-Riedel T, Janata O, Penner E, Holzmann H, Steindl-Munda P, et al. Spontaneous viral clearance in patients with acute hepatitis C can be predicted by repeated measurements of serum viral load. HEPATOLOGY 2003;37:60–64.
5. Gerlach JT, Diepolder HM, Zachoval R, Gruener NH, Jung MC, Ulsenheimer A, et al. Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance. Gastroenterology 2003;125:80–88.
6. Ikeda K, Saitoh S, Arase Y, Chayama K, Suzuki Y, Kobayashi M, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. HEPATOLOGY 1999;29:1124–1130.
7. Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, et al. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. HEPATOLOGY 1998;27:1394–1402.
8. Imazeki F, Yokosuka O, Fukai K, Saisho H. Favorable prognosis of chronic hepatitis C after interferon therapy by long-term cohort study. HEPATOLOGY 2003;38:493–502.
9. Arif A, Levine RA, Sanderson SO, Bank L, Velu RP, Shah A, et al. Regression of fibrosis in chronic hepatitis C after therapy with interferon and ribavirin. Dig Dis Sci 2003;48:1425–1430.
10. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferoh alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;26:975–982.
11. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958–965.
12. Omata M, Yokosuka O, Takano S, Kato N, Hosoda K, Imazeki F, et al. Resolution of acute hepatitis C after therapy with natural beta interferon. Lancet 1991;338:914–915.
13. Viladomiu L, Genesca J, Esteban JI, Allende H, Gonzalez A, Lopez-Talavera JC, et al. Interferon-alpha in acute posttransfusion hepatitis C: a randomized, controlled trial. HEPATOLOGY 1992;15:767–769.
14. Takano S, Satomura Y, Omata M. Effects of interferon beta on non-A, non-B acute hepatitis: a prospective randomized, controlled-dose study. Gastroenterology 1994;107:805–811.
15. Lampertico P, Rumi M, Romeo R, Craxi A, Soffredini R, Biassoni D, et al. A multicenter randomized controlled trial of recombinant interferon-alpha 2b in patients with acute transfusion-associated hepatitis C. HEPATOLOGY 1994;19:19–22.
16. Hwang SJ, Lee SD, Chan CY, Lu RH, Lo KJ. A randomized controlled trial of recombinant interferon alpha-2b in the treatment of Chinese patients with acute post-transfusion hepatitis C. J Hepatol 1994;21:831–836.
17. Ohnishi K, Nomura F, Nakano M. Interferon therapy for acute post-transfusion non-A, non-B hepatitis: response with respect to anti-hepatitis C virus antibody status. Am J Gastroenterol 1991;86:1041–1049.
18. Simmonds P, Alberti A, Alter HJ, Bonino F, Bradley DW, Brechot C, et al. A proposed system for the nomenclature of hepatitis C virus genotypes. HEPATOLOGY 1994;19:1321–1324.
19. Oketani M, Higashi T, Yamasaki N, Shinmyozu K, Osame M, Arima T. Complete response to twice-a-day interferon-beta with standard interferon-alpha therapy in acute hepatitis C after a needle-stick. J Clin Gastroenterol 1999;28:49–51.
20. Jessner W, Gschwantler M, Steindl-Munda P, Hofer H, Watkins-Riedel T, Wrba F, et al. Primary interferon resistance and treatment response in chronic hepatitis C infection: a pilot study. Lancet 2001;358:1241–1242.
21. Nomura H, Kimura Y, Morita C, Tada H, Okamoto O, Shiraishi G, et al. Usefulness of HCV-RNA assays in efficacy evaluation of interferon treatment for chronic hepatitis C: Amplicor HCV assay and branched DNA probe assay. J Infect 1997;34:349–355.
22. Yamaji K, Hayashi J, Kawakami Y, Furusyo N, Sawayama Y, Kishihara Y, et al. Hepatitis C viral RNA status at two weeks of therapy predicts the eventual response. J Clin Gastroenterol 1998;26:193–199.

ISSN: 1545-4452 *www.ccp-journal.com*

Timeframe of Acceptance of Dying in End-Stage AIDS Patients During Their Final Week

Toshio Makie*, Muneaki Harada, Yoshiaki Nose, Chiharu Kubo
Kyushu University

Tsuyoshi Nakamura
Nagasaki University

Jun Hayashi
Kyushu University Hospital

Seizaburo Kashiwagi
National Kyushu Medical Center Hospital

ABSTRACT - This study investigates the demographics that are predictive of acceptance of dying and the timeframe of acceptance of dying in a group of end-stage AIDS patients. Subjects were thirty-five end-stage AIDS patients (20 hemophiliacs, 15 non-hemophiliacs). Two physicians evaluated each patient's acceptance of dying by reviewing nursing records of their final week of life. A piecewise linear logistic regression analysis was applied to the demographic and timeframe factors. Based on the patients' behavior recorded by nurses, age at death and number of days before death were significantly related to the acceptance of dying. Over time, the patients' acceptance of dying increased to 40% between the sixth and fourth days before death, but then drastically decreased to 15%, regardless of age, in the last four days. Knowledge of this timeframe may be useful for psychological strategies during a patient's final week.

Key Words: Dying, AIDS, Acceptance, Terminal Illness

A patient at the end of life requires careful, active attention to his or her psychological status for the purposes of dying with dignity, being as comfortable and calm as possible, and accepting death. During this critical time, the psychological status of patients varies considerably (Tataryn & Chochinov,

**Toshio Makie, MD, PhD; Department of Biometry and Epidemiology; Medical University of South Carolina; 135 Cannon Street, Suite 302G; PO Box 250835; Charleston, SC 29425, U.S.A;.makie@musc.edu (email).*

2002; Morris, Suissa, Sherwood, Wright, & Greer, 1986), and this is especially true when the patient is burdened with an incurable disease. Consequently, in order to administer the most humane care possible, attending medical staff must recognize the factors that affect terminal patients. In this study, demographic and timeframe factors were examined in end-stage acquired immunodeficiency syndrome (AIDS) patients. This patient group was selected because they had lived under individually and socially complicated circumstances for the duration of their illnesses (Nichols 1985; Jones, Garsia, Wu, Job, & Dunn, 1995; Drotar, Agle, Eckl, & Thompson, 1995) and because the debilitating effects of these circumstances almost always reduce the quality of life (Ragsdale & Morrow, 1990; Wachtel et al., 1992; Schag, Ganz, Kahn, & Petersen, 1992; Lenderling et al., 1994). Yates, Chalmer, St. James, Follansbee, and McKegney (1981) discussed how demographic factors are related to acceptance of death. However, the timeframe over which patients' psychological status is measured has received little attention, likely because patients at the end-stage of disease have difficulty participating in interviews and psychological tests due to their deteriorating physical and psychological status. In the present study, our objective was to examine demographics in relation to acceptance of dying and how the timeframe affects acceptance of dying in end-stage AIDS patients. To do so, we examined the nursing records of conversations between nurses and end-stage AIDS patients during the final week of the patients' lives.

Method

Participants and Materials

The participants were 42 end-stage AIDS patients who had been hospitalized for more than one week prior to death at one of eight hospitals (Kyushu University Hospital, Hiroshima University Hospital, Fukuoka University Hospital, Kansai Medical University Hospital, Nara Medical University Hospital, Nagoya National Hospital, Higashi-Saitama National Hospital, and Tokyo Metropolitan Fuchu Hospital) between July 1987 and April 1999. After excluding seven cases of HIV encephalopathy, the data from 35 patients (20-29 years (8); 30-39 years (9); 40-49 years (9); and 50 and over (9)) were analyzed. Of these 35, 20 were hemophiliacs and 15 were non-hemophiliacs (33 males and 2 females). The causes of death were respiratory failure (16), organ hemorrhage (10), waste syndrome (5), lymphoma, (2) and infectious encephalitis (2).

Design and Procedure

The nursing care provided to these end-stage patients was grouped into three time blocks (block 1, 0000–0800 hours (h); block 2, 0800–1600 h; and block 3, 1600–2400 h). The time of death was operationally defined as the time of the final conversation before death. Twenty-one nursing records (7 days × 3 records/day) for each patient over the week before the final conversation were used in the analysis. Two physicians independently judged each entry of all 21 records as either "having accepted dying" (displaying positive affect or behavior

suggesting that the patient was facing death willingly) or as "not having accepted dying" (displaying negative affect or behavior suggesting that the patient was not facing death willingly) (Felton and Revenson, 1984). However, some nursing records were excluded from this study because the physicians regarded the records as incomplete. Missing records for patients who had left the hospital or remained at home were also excluded from this study.

To evaluate the reliability of the nursing records, two nurses chosen at random from the attendant nurses simultaneously observed the same patients, independently created nursing records of them, and judged their records regarding the patients' acceptance of dying.

Analyses

The demographic factors used in the statistical analysis were the patients' age at death (years), marital status (0 for unmarried and 1 for married), parental status (0 for no children and 1 for one or more children), whether the patient had experienced a family member's death (0 for no and 1 for experienced), the length of illness (years), the final CD4 lymphocyte (CD4) counts (1 for<10, 2 for 10–99, and 3 for 100+), the date of death (1 for<1990, 2 for 1990–1993, and 3 for 1994–1999), and the infection route (0 for non-hemophilia and 1 for hemophilia). In this study, "family" was defined as people who lived with the patient.

The timeframe factors included as explanatory variables were nursing time blocks (0–1 dummy variables) and number of days before death (6, 5, 4, 3, 2, 1, 0). Hereafter, for the sake of brevity, we define a dummy variable "Day to death" as taking a value n at Day n, for n = 6, 5, 4, 3, 2, 1, or 0. A piecewise linear logistic regression analysis (Gallant and Fuller, 1973; Gathery 1974; Ertel and Forkles, 1976; Nakamura 1986) was performed to determine the demographic and timeframe factors that were significantly associated with acceptance of dying. The response variable was acceptance of dying (0 for non-acceptance and 1 for acceptance). To perform this piecewise linear regression analysis, candidate change points, or knots, for intersecting line segments were specified. For age at death, the intersecting line segments were described as 10-year intervals, and for Day n, they were each distinct value 5, 4, 3, 2, or 1. The piecewise linear variables corresponding to the change points were denoted by MAX (Age-30,0), MAX (Age-40,0), MAX (Age-50,0) and MAX (Day-n, 0), for n = 5, 4, 3, 2, and 1, respectively, (Nakamura 1986; Akazawa, Nakamura, & Palesch 1997). The logistic regression analysis was performed in a stepwise manner.

A two-sided $p<0.05$ was considered statistically significant. The statistical computation was carried out by SAS (1999) on a UNIX workstation (Sun Microsystems Sparc Station 20, OS; Japanese Solaris 2.5.1).

Results

There were 648 (158 acceptance and 490 non-acceptance) valid records, excluding 14 missing and 73 incomplete records. The percentage of agreement

and the Kappa statistics between the two nurses was 90.7% and r = 0.81 (n = 107, p<0.001), respectively. According to Landis and Koch's report (1977), the agreement indicated by the Kappa statistics is excellent for the nursing records of two nurses chosen at random. The judgment of the nursing records by the two physicians were 92.1% and r = 0.85 (n = 735, p<0.001). The agreement shown by the Kappa statistics for the judgment of the two physicians is also excellent.

Table 1
Results of Stepwise Logistic Regression Analysis

Background factors	Initial step χ^2 score	Last step χ^2 score	Odds ratio (95% confidence limits)	P value
Age at death (Age)	4.74	4.79	1.002–1.032	0.029
MAX (Age–30, 0)	4.44	0.09	—	0.767
MAX (Age–40, 0)	2.20	2.11	—	0.146
MAX (Age–50, 0)	2.34	0.05	—	0.817
Length of illness	2.067	0.35	—	0.554
Final CD_4 counts	0.65	0.20	—	0.657
Date-of-death	2.40	0.91	—	0.340
Infection route	2.77	0.44	—	0.508
Marital status	0.65	0.04	—	0.834
Children	0.10	0.36	—	0.546
Family member's death	1.58	0.38	—	0.538
Nursing time, 1st block	reference	reference	1.000–1.000	
Nursing time, 2nd block	0.77	0.81	—	0.369
Nursing time, 3rd block	2.78	2.81	—	0.094
Day n (DAY)	6.44	13.57	1.142 - 1.542	<0.001
MAX (DAY –1, 0)	5.13	0.36	—	0.550
MAX (DAY –2, 0)	3.77	0.03	—	0.875
MAX (DAY –3, 0)	1.49	0.00	—	0.974
MAX (DAY –4, 0)	0.08	7.74	0.383-0.847	0.005
MAX (DAY –5, 0)	0.11	0.07	—	0.817

Note 1: $P(\chi^2>10.83) = 0.001$, $P(\chi^2>6.63) = 0.01$, $P(\chi^2>3.84) = 0.05$

Figure 1

The Observed Incidence of Acceptance of Dying by Day n and Age at Death

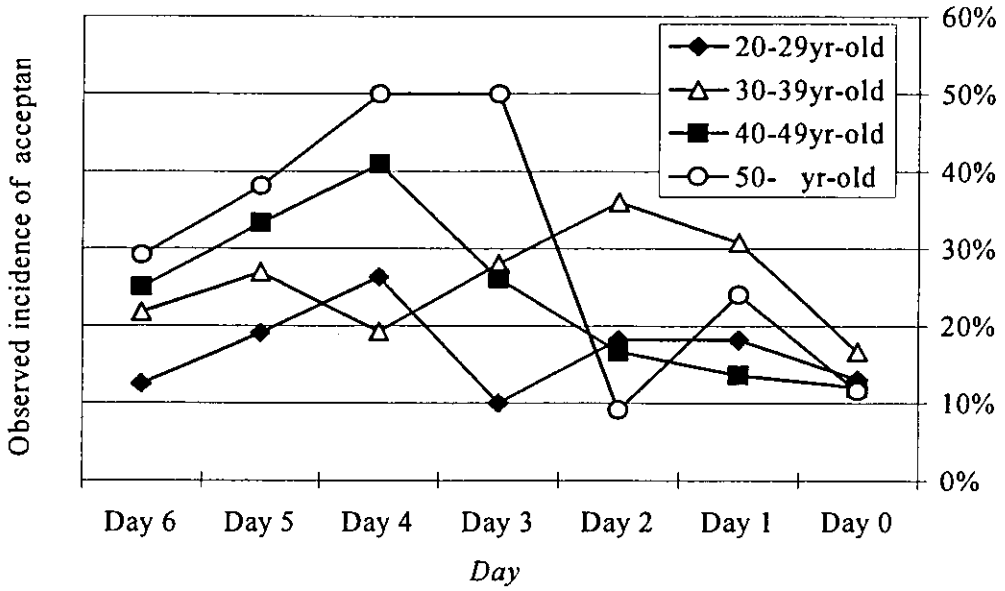
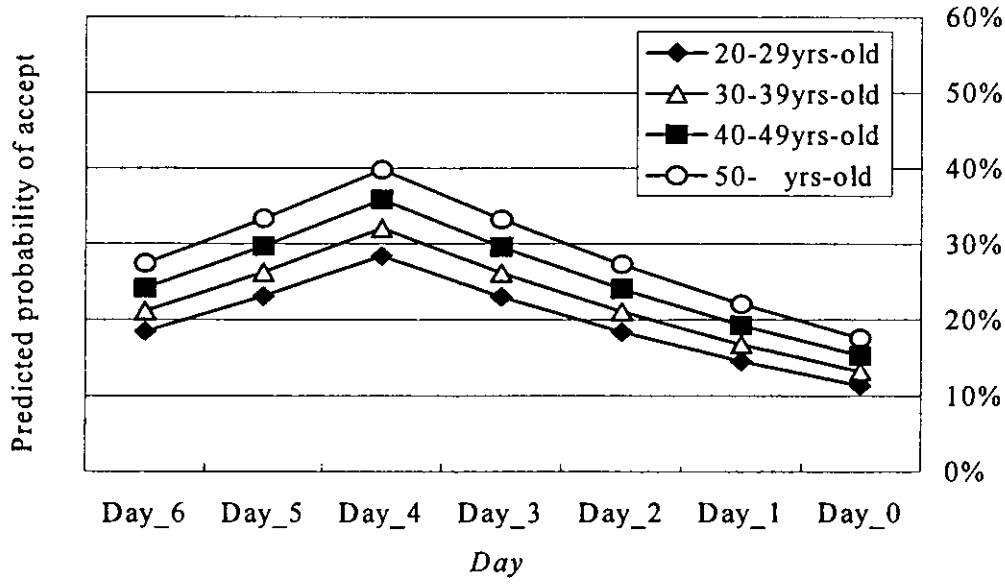


Figure 2

The Predicted Probability of Acceptance of Dying by the Logistic Equation with Significant Factors in Table 1 and Significant Interaction Term



Note: Predicted probability = exp(Y)/(1+exp(Y)). Y = -2.482-0.017(Age) + 0.283(DAY) - 0.563 MAX(DAY-4,0).

Figure 1 demonstrates the observed incidence of acceptance of dying by Day and age at death. Between Days 6 and 4, there was a clear trend relating acceptance of dying with patient's age. That is, early in their final week, older

patients were more likely to be judged as having accepted that they were dying. However, age effects were not apparent in the last three days. From Figure 1, it is also evident that three age groups, 20-29 years old, 40-49 years old and 50 or older, showed a similar trend. For all three groups, the incidence of acceptance of dying increased between Days 6 and 4, and then decreased to approximately 15% for the last three days. The remaining age group, 30-39 years old, did not show any clear trend in terms of the incidence of acceptance of dying, which remained over 20% for all days except the final day.

The results of the logistic regression analysis with the chi-square scores for each of the factors considered in the initial and last steps are shown in Table 1. Acceptance of dying was significantly associated with age at death, DAY *n*, and MAX (DAY–4, 0). The chi-square score for both Length of illness and Infection route differed drastically between the initial and last steps (2.06 → 0.35 and 2.77 → 0.44, respectively). The changes indicate that these factors did not emerge as significant when the effects of the three significant factors are adjusted in the logistic regression analysis (see Table 1). Conversely, the scores for Nursing time, the 3rd block, changed very little with the significant factors (2.78 → 2.81). A large sample size required to consider the significance of this factor. The predicted probability based on the equation also indicated that older patients accepted dying on Day 6. The trend of the probability with Day based on the equation reproduces those seen in Figure 1 well, except for the age group 30-39 years old (see Figure 2). The probability of acceptance of dying also changed drastically from an upward to a downward trend on Day 4. After Day 4, the probability decreased rapidly to approximately 15% on Day 0.

Discussion

Considerable psychological research has been conducted with patients in the process of developing AIDS (Ragsdale & Morrow, 1990; Wachtel et al., 1992; Schag et al.,1992; Lenderking et al., 1994); however, from the perspective of medical staff, psychological factors are also critical for helping patients to die with dignity, stay as comfortable and calm as possible, and accept their approaching death. Examination of these end-stage psychological factors posed two main difficulties in this study. First, HIV infection by non-heated blood products has been a serious social issue in Japan (Ross 1993; Swinbanks 1994; Hoshino 1995). Consequently, most hospitals in which HIV patients were treated are hesitant to disclose clinical records, making it difficult to obtain sufficient reliable data on the psychological status of patients under special care for statistical analysis. Second, we were not allowed to risk any additional psychological or physical examinations of patients, which might have worsened their psychological or physical status. Therefore, we chose nursing records for our analysis, as each patient's psychological status was observed by nurses as part of their routine work.

Despite the possible biases incidental to these subjective observations, our findings that age at death and timeframe are significantly related to acceptance

of dying are what most attending physicians would expect, and this may lend support to the reliability of our model. The results of this study should be regarded as the first step toward psychological research using statistical methods to understand and benefit HIV patients.

Our first main finding was that acceptance of dying is significantly associated with age at death; one week before death, there was a strong correlation between the two. Similar results have been reported in research on end-stage cancer patients (Yates et al. 1981; Morris, Mor et al. 1986; Morris, Suissa, 1986; Felton & Revenson 1987). Confirmatory studies would be possible in any hospital caring for end-stage patients where medical records similar to the nursing records that we used are available.

Our second main result was that in the dying process, acceptance of dying often has a fixed timeframe. This timeframe is indicative of a pattern in patients' capacity to communicate with persons close to them. The optimal day for this communication is typically followed by days during which communication is virtually impossible. Once a doctor feels that a patient's death is near, staff need to observe the patient carefully, and be conscious of this timeframe. In the week preceding death, it may be difficult to recognize a patient's improving psychological status; however, the likelihood of identifying this improving status can be increased by examining serial information received from staff. If medical staff understand the timeframe, they will then be aware of the timing for administering appropriate intervention for the patient. The persons closest to the patient also need to understand the patient's final timeframe, since this "normalization" can help them to cope with the situation.

The research design described in this study is potentially helpful to detect the treatment effects of psychotherapeutic drugs and psychological intervention, since piecewise-linear logistic analysis can reveal not only its effectiveness but also its timeframe, after the administration of psychotherapeutic drugs or intervention. The potential bias introduced by the subjective nature of nurses' notes and doctor's judgments is a limitation of this method, and this issue will be addressed in a carefully designed prospective study. It is also expected that acceptance of death may be dependent on the cause of death, the medical treatment, and whether the patient has hemophilia, and we leave these questions for future research.

Author Notes

The authors gratefully acknowledge members of the hospitals that cooperated in this study. Specifically, we thank A. Kimura and N. Takata (Hiroshima University Hospital); Y. Ueda (Kansai Medical University Hospital); K. Tamura (Fukuoka University Hospital); M. Konishi (Nara Medical University Hospital); M. Utsumi, M. Yonekura, and E. Kikuchi (National Nagoya Hospital); T. Toyota (National Higashi-Saitama Hospital); A. Fujita (Tokyo Metropolitan Fuchu Hospital); K. Hasegawa, T. Taniguchi, and Y. Harada (Kofu-kai Munakata Hospital); Y. Inada (St. Luke's Hospital of Columbia University) and his

colleague (Yorigonomi-kai) for supplemental data. We would also like to express our gratitude to S. Tominaga (Dept. General Medicine, Kyushu University Hospital) for the aggregation of data and J. Ariail, PhD (Medical University of South Carolina (MUSC)) for technical support with the writing. This work was completed at MUSC, with biostatistical support provided by David Hoel, PhD.

References

Akazawa, K., Nakamura, T., & Palesch, Y. (1997). Power of logrank test and Cox regression model in clinical trials with heterogeneous samples. *Statistics in Medicine, 16*, 583-597.

Drotar, D. D., Agle, D. P., Eckl, C. L., & Thompson, P. A. (1995). Psychological response to HIV positivity in hemophilia. *Pediatrics, 96*, 1062-9.

Ertel, J. E., & Forkles, E. B. (1976). Some algorithms for linear spline and piecewise multiple linear regression. *Journal of the American Statistical Association, 71,* 640-8.

Felton, B.J., & Revenson, T. A. (1987). Age differences in coping with chronic illness. *Psychology of Aging, 2*, 164-70.

Felton, B.J., & Revenson, T. A. (1984). Coping with chronic illness: a study of illness controllability and the influence of coping strategies on psychological adjustment. *Journal of Consulting and Clinical Psychology, 52*, 343-53.

Gallant, A. R., & Fuller, W. A.(1973). Fitting segmented polynomial regression models whose join points have to be estimated. *Journal of the American Statistical Association, 68*, 144-7.

Gathery S. B. (1974). Partition regression. *Journal of the American Statistical Association, 69*, 945-7.

Hoshino S. (1995). HIV+/AIDS-related bioethical issues in Japan. *Bioethics, 9*, 303-8.

Jones Q. J., Garsia, R. J., Wu, R. T., Job, R. F., & Dunn, S.M. (1995). A controlled study of anxiety and morbid cognitions at initial screening for human immunodeficiency virus (HIV) in a cohort of people with haemophilia. *Journal of Psychosomatic Research, 39*, 597-608.

Landis, J.B., Koch, G.G. (1977). The measurement of observer agreement for categorical data. *Biometrics, 33*, 159-74.

Lenderking, W. R., Gelber, R. D., Cotton, D. J., Cole, B. F., Goldhirsch, A., Volberding, P. A., & Testa, M.A. (1994). Evaluation of the quality of life associated with zidovudine treatment in asymptomatic human immunodeficiency virus infection. The AIDS Clinical Trials Group. *New England Journal of Medicine, 330*, 738-43.

Morris, J. N., Mor V., Goldberg, R. J., Sherwood, S., Greer, D.S., & Hiris, J. (1986). The effect of treatment setting and patient characteristics on pain in terminal cancer patients: a report from the National Hospice Study. *Journal of Chronic Diseases, 39*, 27-35.

Morris, J. N., Suissa, S., Sherwood, S., Wright, S. M., & Greer, D. (1986). Last days: a study of the quality of life of terminally ill cancer patients. *Journal of*

Chronic Diseases, 39, 47-62.

Nakamura, T. (1986). BMDP program for piecewise-linear linear regression. *Computer Methods and Programs in Biomedicine, 23*, 53-55.

Nichols, S. E. (1985). Psychosocial reactions of persons with the acquired immunodeficiency syndrome. *Annals of Internal Medicine, 103*, 765-7.

Ragsdale, D., & Morrow, J. R. (1990). Quality of life as a function of HIV classification. *Nursing Research, 39*, 355-9.

Ross, C. (1993) An apology for AIDS. *Lancet, 8860*, 1585-1586.

SAS Institute Inc. SAS/STAT User's Guide, Version 8. (1999). Cary, NC: SAS Institute Inc.

Schag. C. A., Ganz, P.A., Kahn, B., & Petersen, L. (1992). Assessing the needs and quality of life of patients with HIV infection: development of the HIV Overview of Problems-Evaluation System (HOPES). *Quality of Life Research, 1*, 397-413.

Swinbanks, D. (1994). Haemophiliacs seek charges in AIDS scandal. *Nature, 368*, 680.

Tataryn, D., Chochinov, H. M. (2002). Predicting the trajectory of will to live in terminally ill patients. *Psychosomatics, 43*, 370-7.

Wachtel, T., Piette, J., Mor, V., Stein, M., Fleishman, J., Carpenter, C. (1992). Quality of life in persons with human immunodeficiency virus infection: measurement by the Medical Outcomes Study instrument. *Annals of Internal Medicine, 116*, 129-37.

Yates, J. W., Chalmer, B. J., St. James, P., Follansbee, M., McKegney, F. P. (1981). Religion in patients with advanced cancer. *Medical and Pediatric Oncology, 9*, 121-8.

Received 09/30/2003; Revision Received 12/08/2003; Accepted 02/03/2004

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Hepatology Research 28 (2004) 36–40
Hepatology Research
www.elsevier.com/locate/ihepcom

Efficacy of early retreatment with interferon β for relapse in patients with genotype Ib chronic hepatitis C

Hideyuki Nomura[a,*], Suketo Sou[a], Takashi Nagahama[a], Jun Hayashi[b], Seizaburo Kashiwagi[c], Hiromi Ishibashi[d]

[a] *Department of Internal Medicine, Shin-Kokura Hospital, 1-3-1 Kanada, Kokurakitaku, Kita-Kyushu 803-8505, Japan*
[b] *Department of Environmental Medicine and Infectious Diseases, Faculty of Medical Sciences, Kyushu University, 3-1-1 Maedashi, Higashiku, Fukuoka 812-8582, Japan*
[c] *Fukuoka Red Cross Blood Center, 232-11, Kamikoga Chikushino, Fukuoka 818-8588, Japan*
[d] *Clinical Research Center, National Nagasaki Medical Center, 2-1001-1 Omura, Nagasaki 856-8562, Japan*

Received 28 February 2003; received in revised form 3 September 2003; accepted 24 September 2003

Abstract

Background: Interferon (IFN) retreatment for hepatitis C virus (HCV) relapsers has been effective under some conditions. We conducted a randomized, controlled trial of IFN β retreatment for HCV relapsers after IFN α. *Patients and methods:* We gave IFN β 6MIU therapy to 43 patients who had relapse of HCV after the 24 weeks IFN α monotherapy. The 43 patients were randomly assigned to two groups: Group A started retreatment within 4 weeks after relapse; and Group B started retreatment 24 weeks or more after relapse. *Results:* Nine patients showed sustained virological response (SR) to the retreatment. All of these patients were in a low viral load subgroup. The SR rate in Group A (8/22, 36%) was significantly higher than in Group B (1/21, 5%) ($P = 0.0128$). Among patients with lower viral load, the SR rate in Group A (8/10, 80%) was also significantly higher than in Group B (1/8, 13%) ($P = 0.0076$). *Conclusion:* The retreatment with IFN β is effective for patients with HCV low viral load, and the sooner after the relapse the retreatment is started, the better the clinical results will be.

Keywords: Interferon α; Interferon β; Retreatment; Chronic hepatitis C; Genotype 1b

1. Introduction

Chronic hepatitis due to persistent infection with the hepatitis C virus (HCV) often progresses to cirrhosis and hepatocellular carcinoma [1]. Interferon (IFN) represents an effective therapy for many patients with chronic hepatitis C [2,3]. A sustained virological response (SR) with IFN treatment depends on many factors, including viral genotype, viral load, presence of hepatic fibrosis, age, gender, and mutations in IFN susceptibility determinants in the nonstructural 5A (NS5A) region of the virus [4–7]. HCV genotype and viral load are particularly important factors in predicting treatment response [2,4,5,8].

Resistance of the HCV genotype 1b to treatment with IFN has been frequently reported in Japan [8–10]. Many patients infected with genotype 1b fail to eliminate HCV after treatment with IFN monotherapy. In addition, many patients eliminate HCV during the treatment but can not prevent later relapse. Alberti et al. reviewed several prospective studies [11]. Their report generally described patients other than genotype 1b (genotype 1b, 11%; other, 41%), significantly higher alanine aminotransferase (ALT) levels at time of retreatment, treatment with IFN for at least 12 months, and retreatment with IFN in patients who relapsed.

Recently, a combination of IFNα2b and ribavirin has been used for retreatment of patients who relapsed after initial treatment with IFN alone. This combination has been proven more effective than IFNα2b monotherapy [12]. However, this combination therapy is limited because ribavirin is contraindicated in many patients [13].

This study is suggesting an effective new retreatment regimen. The study evaluated differences in efficacy of reattempting therapies, one was starting immediately after the relapse and the other was starting after 24 weeks, in patients

* Corresponding author. Tel.: +81-93-571-0553; fax: +81-93-591-1031.
E-mail address: h-nomura@shin-kokura.gr.jp (H. Nomura).

1386-6346/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.hepres.2003.09.005

who relapsed after initial treatment. All patients were diagnosed as chronic hepatitis C with genotype 1b and were initially treated with natural IFNα. Patients with relapse were then randomly assigned to two groups. One group began retreatment with a 6 week course of natural IFNβ starting immediately after relapse. The other began retreatment with the same regimen starting 24 weeks after relapse. We now report on the significant differences in the treatment responses.

2. Patients and methods

2.1. Selection of patients

The patients involved in this study were treated at ShinKokura Hospital from January 1997 to December 1999. Each patient was positive for HCV-RNA based on reverse transcription nested polymerase chain reaction (RT-nested PCR) test [14] and diagnosed as chronic hepatitis C from findings on a liver biopsy performed within 3 months. Only adult patients with HCV genotype 1b were eligible for this study. Patients with cirrhosis, autoimmune hepatitis, alcoholic liver diseases, or hepatitis B surface antigen positives by an enzymelinked immunosorbent assay (Abbott Japan, Co. Ltd., Tokyo, Japan) were also excluded. Patients ranged in age from 20 to 69 years. The study design and IFN treatment were explained, and written informed consent was obtained from all of the patients prior to enrollment. All procedures in this study were conducted in accordance with the Helsinki Declaration of 1975 (1983 revision).

2.2. Study design and treatment regimens

Natural IFNα (Sumiferon, Sumitomo Pharmaceutical Industries Inc., Osaka, Japan) was used as the initial treatment. The dose was 6 million international units (MIU) by intramuscular injection, administered once daily for 2 weeks, followed by three times a week for 22 weeks (total dose: 480 MIU). Patients who were HCV negative at completion of the initial treatment but who later experienced relapse were randomized into one of two treatment groups by using an enveloped enrollment sheet method. In Group A, retreatment was initiated within 4 weeks after relapse. In Group B, retreatment was started 24 weeks or more after relapse. Twenty two of the 43 relapsed patients after initial treatment were assigned to Group A (low viral load: 10 patients) and the others were assigned to Group B (low viral load: 8 patients). Table 1 shows the baseline of clinical profiles of the patients. No statistically significant differences in any baseline profiles were observed between Groups A and B. Measurement of viral load prior to retreatment demonstrated low viral load in 18 of the 43 patients. The retreatment regimen was natural IFNβ (6 MIU per day) (Feron, Toray Industries Inc., Tokyo, Japan), administered once daily by intravenous injection for 6 weeks (total dose: 252 MIU).

Table 1
Clinical characteristics of patients receiving retreatment

	Group A ($n = 22$)	Group B ($n = 21$)	Total ($n = 43$)
Age (years)			
21–49	13	11	24
50–69	9	10	19
Gender			
Male	15	13	28
Female	7	8	15
Viral loads			
<100 (kcopies/ml)	10	8	18
≥100 (kcopies/ml)	12	13	25
Liver tissue			
Mild hepatitis	11	16	27
Severe hepatitis	11	5	16
Serum ALT value			
<2 × ULN	14	16	30
≥2 × ULN	8	5	13

This table shows the clinical characteristics of patients before retreatment with IFNβ. Patients were randomly assigned to Group A or Group B, and classified into subgroups based on liver histology: mild hepatitis (HAI score ≤ 10) and severe hepatitis (HAI score ≥ 11). ULN: the upper limit of the normal range (40 IU/l). HAI: histological activity index.

SR was defined as serum ALT levels below the upper limits of the normal level (ULN: 40 IU/L) and negative HCV-RNA at 24 weeks after retreatment. Any clinical findings other than SR were considered nonresponse (NR). The subsequent followup period was 1 year.

HCV viral load was measured just before the start of retreatment. Viral load was measured with Amplicor-HCV monitor assay (Roche Molecular Diagnostics, Tokyo, Japan) [15]. Low viral load was defined as <100 kcopies/ml, and high viral load as ≥100 kcopies/ml. Serum ALT levels, hemoglobin concentration, white blood cell count, and platelet count were measured at 4-week intervals from the start of treatment until 24 weeks after completion of retreatment. If serum ALT levels again increased to ≥2 × ULN after completion of treatment, HCV-RNA was assayed. Hepatic inflammation and fibrosis were assessed using the Knodell histological activity index (HAI). Patients were classified into two subgroups: mild hepatitis (HAI ≤ 10) and severe hepatitis (HAI ≥ 11) [16]. Treatment with IFN was discontinued if the hemoglobin concentration decreased below 9.0 g/dl, if the white blood cell count decreased below 1500 mm^{-3}, if the platelet count decreased below 50,000 mm^{-3}, or if symptoms such as severe malaise was observed.

2.3. Statistical analysis

Differences between the groups were analyzed with Fisher's exact test. In the determination of predictive factors for IFN retreatment efficacy, simple logistic regression analysis was performed. Logistic analysis was performed

for age (21–49 years versus 50–69 years), gender (male versus female), time of starting retreatment (Group A versus Group B), HCV-RNA level (<100 kcopies/ml versus ≥100 kcopies/ml), liver histology (HAI score 2–10 versus 11–22), and serum ALT value (< 2 × ULN versus ≥ 2 × ULN). Probability values of <0.05 stands for statistically significant.

3. Results

3.1. Relationship between predictive factors and efficacy of IFN retreatment

The results of univariate analysis for predictive factors associated with SR in the 43 retreated patients were: age (P = 0.2607), gender (P = 0.2185), time of starting retreatment (P = 0.0482), viral load (P = 0.0020), hepatic histology (P = 0.3910), and serum ALT level (P = 0.6043). Both "viral load" and "time of starting retreatment" were significant predictive factors (Table 2).

3.2. Results of IFN retreatment by viral loads

Table 3 shows the results of IFN retreatment by viral loads. SR was observed in nine retreated patients (21%), all of whom had low viral load prior to retreatment. The SR rate was significantly higher in Group A (8/22, 36%) than in Group B (1/21, 5%) (P = 0.0128). Among the patients with low viral load, SR was observed in 8 of 10 patients in Group A (80%) but only in 1 of 8 patients in Group B (13%, P = 0.0076). Serum ALT at 24 weeks after retreatment was below ULN in 10 patients in Group A (45%), but only in 3 patients in Group B (14%, p = 0.0279). In evaluation after retreatment (24 weeks after completion of retreatment), among the HCV-RNA positive patients, 4 of 5 with serum ALT below ULN eventually had a rise in serum ALT to above ULN the during followup for 1 year. However, no patients with SR had a relapse during 1 year of followup after retreatment.

No discontinuations or withdrawals occurred during the 6 weeks of retreatment with IFNβ.

Table 2
Results of univariate analysis for predict factor of retreatment

Factor	Chi-Square	P-value	Odd ratio	95% Confidence limits
Age	1.2648	0.2607	0.385	0.073–2.034
Gender	1.5039	0.2185	3.356	0.488–23.084
Time of starting retreatment	3.9016	0.0482	5.970	1.014–35.147
Virus loads	9.5104	0.0020	18.86	2.915–125.102
Histology	0.7358	0.3910	1.959	0.421–9.110
Serum ALT level	0.2686	0.6043	1.527	0.308–7.569

Cutoff values for liver histology were HAI score of 11 and viral load of 100 kcopies/ml. Patients were divided into Group A or Group B based on time of starting retreatment.

Table 3
Results of sustained virologic response rate for interferon retreatment by viral loads

	Group A (n = 22) SR/n (%)	Group B (n = 21) SR/n (%)	Total (n = 43) SR/n (%)
Viral loads			
<100 (kcopies/ml)	8/10 (80)**	1/8 (13)**	9/18 (50)
≥100 (kcopies/ml)	0/12 (0)	0/13 (0)	0/25 (0)
Total	8/22 (36)*	1/21 (5)*	9/43 (21)

This table summarized analysis of data from Fisher's exact test. SR: Sustained virologic response, (%):Sustained virologic response rate.
** P < 0.01 (Group A vs. Group B).
* P < 0.05 (Group A vs. Group B).

3.3. Safety

No clinically significant decreases in hemoglobin concentrations, white blood cell counts or platelet counts were observed during initial treatment and retreatment. No symptoms such as severe malaise or adverse reactions requiring discontinuation of IFN treatment were observed.

4. Discussion

Our study was characterized by [1] time of starting retreatment [2], change to a different types of IFN for retreatment [3], short duration of retreatment and small total dose (252 MIU), and [4] absence of any treatment discontinuations or withdrawals. Based on data from previous studies evaluating efficacy of retreatment [11–13,17], the present study targeted retreatment in HCV relapsers after IFN therapy.

Genotype 1b chronic hepatitis C is often resistant to IFN treatment. All patients in this study were initially treated with IFNα. Only the HCV relapsers were retreated with IFNβ. For patients with low viral load, the SR rate with retreatment early after relapse (Group A) was about 80%. Unfortunately, as in previous reports [18], the response rate was poor when retreatment was initiated after 24 weeks or more from the relapse. Our data supports that retreatment should be started after HCV recurrence as soon as possible for patients with low viral load. HCV load may rise from 4 to 8 weeks after completing treatment with IFN but then decrease [19]. Retreatment in Group A was probably started at a time of decreased HCV load, thus resulting in a high SR rate. In Group B, however, retreatment was started 24 weeks or more after HCV recurrence. This resulted in a low SR rate, like those reported with other retreatment regimens.

Not only IFNα, but IFNβ is also used for treatment of chronic hepatitis C in Japan. The IFNβ standard regimen is daily administration for 6–8 weeks. The IFNα regimen is a combination of daily administration followed by intermittent administration for a total of 24 weeks. SR rates for each

regimen are almost the same in chronic hepatitis C treatment [17]. Barbaro et al. compared IFNβ monotherapy to a combination of IFNα and ribavirin for retreatment of IFNα nonresponders, and found that the HCV negative rate was higher with IFNβ [21]. In another study, patients who did not convert to HCV-RNA negative during treatment with IFNα later did convert to HCV-RNA negative after treatment was switched to IFNβ [22]. Thus, a switch to a different type of IFN (e.g., IFNβ) can be effective in patients with no response to IFNα.

IFNβ demonstrates a potent antiviral effect against HCV. A short 6-week course of IFNβ shows an HCV-RNA negative turning ratio of about 90%, but the rate of recurrence is high [20]. Therefore, a longer course of therapy has been tried in genotype 1b patients resistant to IFN [23,24]. Watanabe et al. reported that IFNβ and IFNα sequential therapy has better results than IFNα monotherapy [25]. However, IFNβ costs more than IFNα in Japan, and longterm treatment can become a considerable financial burden on patients [24]. Our results show that higher efficacy can be achieved over a short period (6 weeks) and with a small total dose (252 MIU) for patients with low viral load if retreatment is started sooner after relapse. In addition, the shorter duration of therapy increases patient quality of life and helps to reduce the financial burden from the perspective of insurance coverage.

A combination of IFN and ribavirin has been used for retreatment of patients with genotype 1b HCV. However, the use of ribavirin in many patients is limited because of contraindications and adverse effects. In our study on IFNα and IFNβ, no clinically significant decreases in hemoglobin concentrations, white blood cell counts or platelet counts were observed, and no serious adverse reactions requiring discontinuation of treatment were noted.

In this randomized controlled study, combination use of two types of IFN provided effective treatment for patients with low viral load HCV genotype 1b, if we start sooner after the relapse of HCV. Retreatment in our study was relatively short term, using a small total dose, and was highly tolerable. This retreatment regimen shows promise for effective treatment and improving quality of life in patients with low viral load HCV genotype 1b.

5. Conclusion

In this study, we show that retreatment with IFN-β is effective in low viral load genotype 1b patients, if we start sooner after the relapse of HCV.

References

[1] Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, et al. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. Hepatology 1990;12:671–5.

[2] Davis GL, Balart LA, Schiff ER, Lindsay K, Bodenheimer HC, Perrillo RP, et al. Treatment of chronic hepatitis C with recombinant interferon alpha: a multi-center randomized, controlled trial. N Engl J Med 1989;321:1501–6.

[3] Di Bisceglie AM, Martin P, Kassianides C, Lisker-Melman M, Murray L, Waggoner J, et al. Recombinant interferon alfa therapy for chronic hepatitis C: a randomized, double-blind, placebo-controlled trial. N Engl J Med 1989;321:1506–10.

[4] Yoshioka K, Kakumu S, Wakita T, Ishikawa T, Itoh Y, Takayanagi M, et al. Detection of hepatitis C virus by polymerase chain reaction and response to interferon alfa therapy: relation to genotypes of hepatitis C virus. Hepatology 1992;16:293–9.

[5] Tsubota A, Chayama K, Ikeda K, Yasuji A, Koida I, Saitoh S, et al. Factors predictive of response to interferon-alpha therapy in hepatitis C virus infection. Hepatology 1994;19:1088–94.

[6] Davis GL. Prediction of response to interferon treatment of chronic hepatitis C. J Hepatol 1994;21:1–3.

[7] Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, et al. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996;334:77–81.

[8] Hayashi J, Ohmiya M, Kishihara Y, Tani Y, Kinukawa N, Ikematsu H, et al. A statistical analysis of predictive factors of response to human lymphoblastoid interferon in patients with chronic hepatitis C. Am J Gastroenterol 1994;89:2151–6.

[9] Nomura H, Tsuchiya Y, Maruyama T, Miki K, Yokota T, Okubo H, et al. The effects of a high dose, short course of interferon on hepatitis C. J Gastroenterol Hepatol 1999;14:85–9.

[10] Nomura H, Kimura Y, Tada H, Hisano C, Morita C, Okamoto O, et al. Predictive factors of a response to interferon therapy in chronic hepatitis C. J Clin Gastroenterol 1996;23:185–90.

[11] Alberti A, Chemello L, Noventa F, Cavalletto L, De Salvo G. Therapy of hepatitis C: re-treatment with alpha interferon. Hepatology 1997;26:137S–42S.

[12] Davis GL, Esteban-Mur R, Rustgi V, Hoefs J, Gordon SC, Trepo C, et al. Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. N Engl J Med 1998;339:1493–9.

[13] Iyoda K, Yuki N, Kato M, Sugiyasu Y, Komori M, Fujii E, et al. Retreatment with interferon for chronic hepatitis C after transient response. J Clin Gastroenterol 2000;31:297–301.

[14] Detmer J, Lagier R, Flynn J, Zayati C, Kolberg J, Collins M, et al. Accurate quantification of hepatitis C virus (HCV) RNA from all HCV-genotypes by using branched-DNA technology. J Clin Microbiol 1996;34:901–7.

[15] Lau JYN, Davis GL, Kniffen J, Qian K-P, Vrdea MS, Chan C, et al. Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. Lancet 1993;341:1501–4.

[16] Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981;1:431–5.

[17] Brillanti S, Garson J, Foli M, Whitby K, Deaville R, Masci C, et al. A pilot study of combination therapy with ribavirin plus interferon alfa for interferon alfa-resistant chronic hepatitis C. Gastroenterology 1994;107:812–7.

[18] Montalto G, Tripi S, Cartabellotta A, Fulco M, Soresi M, Di Gaetano G, et al. Intravenous natural β-interferon in white patients with chronic hepatitis C who are nonresponders to alpha-interferon. Am J Gastroenterol 1998;33:950–3.

[19] Arase Y, Ikeda K, Chayama K, Murashima N, Tsubota A, Suzuki Y, et al. Increased response rate to interferon therapy after a second course in hepatitis C patients who show relapse after the initial course. J Gastroenterol 2000;35:607–12.

[20] Furusyo N, Hayashi J, Ohmiya M, Sawayama Y, Kawakami Y, Ariyama I, et al. Differences between interferon-alpha and -beta treatment for patients with chronic hepatitis C virus infection. Dig Dis Sci 1999;44:608–17.

[21] Barbaro G, Di Lorenzo G, Soldini M, Giancaspro G, Pellicelli A, Grisorio B, et al. Intravenous recombinant interferon-beta versus interferon-alpha-2b and ribavirin in combination for short-term treatment of chronic hepatitis C patients not responding to interferon-alpha. Multicenter Interferon Beta Italian Group Investigators. Scand J Gastroenterol 1999;34:928–33.

[22] Oketani M, Higashi T, Yamasaki N, Shinmyozu K, Osame M, Arima T. Complete response to twice-a-day interferon-beta with standard interferon-alpha therapy in acute hepatitis C after a needle-stick. J Clin Gastroenterol 1999;28:49–51.

[23] Izumi N, Kumada H, Hashimoto N, Harada H, Imawari M, Zeniya M, et al. Rapid decrease of plasma HCV RNA in early phase of twice daily administration of 3(MU doses interferon-beta in patients with genotype 1b hepatitis C infection: a multicenter randomized study. Dig Dis Sci 2001;46:516–23.

[24] Asahina Y, Izumi N, Uchihara M, Noguchi O, Tsuchiya K, Hamano K, et al. A potent antiviral effect on hepatitis C viral dynamics in serum and peripheral blood mononuclear cells during combination therapy with high-dose daily interferon alfa plus ribavirin and intravenous twice-daily treatment with interferon beta. Hepatology 2001;34:377–84.

[25] Watanabe H, Iwata K, Sohda T, Sakisaka S. Interferon beta induction/interferon alpha therapy in patients with interferon-resistant chronic hepatitis C. Hepatol Res 2002;24:355–60.

Constitutive nitric oxide production in bovine aortic and brain microvascular endothelial cells: a comparative study

Chiwaka Kimura[1], Masahiro Oike[1], Keizo Ohnaka[2], Yoshiaki Nose[3] and Yushi Ito[1]

[1]*Department of Pharmacology,* [2]*Department of Geriatric Medicine and* [3]*Department of Medical Information Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, 812-8582 Japan*

Vascular endothelium constitutively generates nitric oxide (NO) in large vessels and induces a relaxation of smooth muscle cells. However, little is known about the production of NO in microvessels, where smooth muscle layers are thin or absent. In this study, we have compared the constitutive production of NO in bovine brain microvascular endothelial cells (BBECs) with that in bovine aortic endothelial cells (BAECs). ATP, acetylcholine (ACh) and A23187 induced Ca^{2+} transients both in BBECs and BAECs. In contrast, although ATP and A23187 evoked a similar degree of $[Ca^{2+}]_i$ increase in both types of cell, they failed to induce NO production in BBECs, as measured with an NO-sensitive fluorescent dye DAF-2, whereas in BAECs there was an increase in DAF-2 fluorescence. Hypotonic stress induced ATP release and subsequent NO production in BAECs, but not in BBECs. We have developed an *in vitro* model vessel system that consists of aortic smooth muscle cells embedded in a collagen gel lattice and overlaid with endothelial cells. Precontracted gels showed relaxation in response to ACh, when BAECs were overlaid. However, ACh-induced relaxation was not observed in BBEC-overlaid gels. Expression of eNOS protein as well as cellular uptake of L-[^{3}H]arginine were significantly lower in BBECs than in BAECs. These results indicate that Ca^{2+}-dependent NO production is at an undetectable level in BBEC, for which at least two factors, i.e. low levels of eNOS expression and L-arginine uptake, are responsible.

(Resubmitted 20 October 2003; accepted after revision 12 November 2003; first published online 14 November 2003)
Corresponding author M. Oike: Department of Pharmacology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, 812-8582 Japan. Email: moike@pharmaco.med.kyushu-u.ac.jp

Nitric oxide (NO) plays various physiological and pathological roles in variety of cell types (Moncada *et al.* 1991). It is well documented that vascular endothelium constitutively generates NO in response to the elevation of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Ca^{2+} binds to calmodulin thereby stimulating endothelial NO synthase (eNOS) to produce NO, in combination with other cofactors such as NADPH and tetrahydrobiopterin (Lopez-Jaramillo *et al.* 1990). Endothelium-derived NO induces a relaxation of vascular smooth muscle cells, and prevents atherosclerosis and cell adhesion (Moncada *et al.* 1991). However, this view has been mainly developed in larger conducting vessels, and the relatively greater importance of endothelium-derived hyperpolarizing factor (EDHF) rather than NO has been suggested in smaller resistance arteries (Garland *et al.* 1995). Furthermore, a neural rather than an endothelial source of NO has been considered to regulate vascular flow in rat mesenteric arterioles (Kashiwagi *et al.* 2002).

It has been reported in cultured human cerebral microvascular endothelial cells that an exogenously applied NO donor inhibits endothelin-1-induced Ca^{2+} transients and down-regulates actin reorganization (Chen *et al.* 2003). Other reports have also shown that eNOS protein is expressed in rat brain microvascular endothelium and its expression level is altered by pathophysiological stimuli such as oestrogen (McNeill *et al.* 1999), perinuclear EP3 receptor stimulation (Gobeil *et al.* 2002) or angiotensin II (Yamakawa *et al.* 2003). However, if NO is constitutively generated by eNOS in cerebrocortical microvascular endothelium, it would affect the functions of neighbouring neurones directly or by changing cerebrocortical blood flow. Actually there has been no direct evidence reported so far showing the constitutive production of NO in cerebral microvascular endothelium. Furthermore, a recent report has shown that control of cerebral microcirculation is obtained by neurone-to-glia signals but not by vascular signals (Zonta *et al.* 2003).

DOI: 10.1113/jphysiol.2003.057059

The aim of this study was to clarify whether NO is constitutively generated in bovine brain microvascular endothelial cells (BBECs) or not. We have used two methods to detect NO production in BBECs and bovine aortic endothelial cells (BAECs). Firstly we measured the intracellular NO production with an NO-sensitive fluorescent dye, DAF-2 (Kojima *et al.* 1998). Secondly, we have developed a novel method to detect cultured endothelium-dependent vasorelaxation. We have previously reported that vascular smooth muscle cells embedded in collagen gels show contraction in response to Ca^{2+} mobilizing stimuli (Kimura *et al.* 2002). We therefore considered that the endothelial functions could be examined by overlaying cultured endothelium onto smooth muscle cell-embedded collagen gel. The results obtained indicate that Ca^{2+}-dependent NO production is detectable using these methods in BAECs, but not in BBECs.

Methods

Cell culture

Thoracic aortas and brains of 1-year-old calves were obtained from the local slaughterhouse. BAECs were scraped off from the intima with the edge of a razor (Oike *et al.* 2000). BBECs were prepared following a Percoll gradient separation method as previously described (Kimura *et al.* 1998b). Both BAECs and BBECs were cultured in Dulbecco's modified Eagle's medium (DMEM, Life Technologies, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS, PAA Laboratories, Linz, Austria). Cells from primary cultures were subcultured at a split ratio of 1 : 3, and the harvested subcultured cells were used for the present experiments. Cells were grown on coverslips, which were coated with collagen type IA (Nitta Gelatin Inc., Osaka, Japan), for measuring $[Ca^{2+}]_i$ and NO production. Endothelial identification of BAECs was confirmed by the specific uptake of fluorescence-labelled acetylated low-density lipoprotein (Dil-Ac-LDL) as previously reported (Kimura *et al.* 2001a). BBECs exhibited immunohistochemical staining for antifactor VIII antibody, indicating their endothelial nature. Endothelial cells obtained from 12 aortas and four brains were used in the present study.

Bovine aortic smooth muscle cells (BASMCs) from thoracic aortas were cultured in DMEM with 10% FBS by the explant method as previously described (Kimura *et al.* 2002). Cells grown to confluency were harvested by trypsin digestion and stored at –80°C after one-step subculture. Smooth muscle α-actin was stained to confirm that the cells retained the nature of smooth muscle cells (not shown). The cells were embedded in collagen gel lattice for the gel contraction assay as described below. Smooth muscle cells from five aortas were used in the present study.

Measurement of $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ was measured from non-confluent BBECs and BAECs with fura-2 using an Attofluor digital fluorescence microscopy system (Atto Instruments, Rockville, MD, USA), as previously described (Oike *et al.* 2000).

Measurement of intracellular production of NO

For the measurement of NO with DAF-2, an NO-sensitive fluorescent dye (Kojima *et al.* 1998), non-confluent cells grown on coverslips were incubated with a diacetylated form of DAF-2 (10 μM, Daiichi Pure Chemicals, Co. Ltd, Tokyo, Japan) for 20 min at room temperature and for a subsequent 20 min at 37°C. DAF-2 was excited at a wavelength of 490 nm and emitted fluorescence at a wavelength of 515 nm was measured with an Attofluor fluorescence microscopy system. Since DAF-2 has single excitation and single emission wavelengths, conversion of DAF-2 fluorescence into intracellular NO concentrations is impossible (Kojima *et al.* 1998). However, since DAF-2 fluorescence increases almost linearly with NO concentration (Kojima *et al.* 1998), we have expressed the DAF-2 fluorescence relative to its initial values. Because NOS generates O_2^- instead of NO in the absence of L-arginine (Xia *et al.* 1998), we added an excess concentration of L-arginine (3 mM) to all solutions used for NO measurement, except for the experiment with N^{ω}-nitro-L-arginine methyl ester (L-NAME)-treated cells, where L-NAME (0.1 mM) was added during the last 30 min of the DAF-2 incubation period.

Gel contraction assay

Endothelial NO production was also assessed in an *in vitro* model vessel, which consists of a BASMC-embedded collagen gel and an overlaid endothelium. Cultured BASMCs were re-suspended in DMEM containing 0.2% collagen type IA at a density of 4×10^5 cells ml^{-1}, poured into a 24-well culture plate and allowed to form a gel for 10 min at 37°C, as previously described. DMEM with 10% FBS was then poured on to the gel. After culturing for 24 h at 37°C, BBECs or BAECs were overlaid on the gel at a density of 2×10^4 cells cm^{-2}.