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Chemiluminescence Enzyme Immunoassay for Monitoring Hepatitis C Virus Core Protein Durin Interferon-α2b and Ribavirin Therapy in Patient: With Genotype 1 and High Viral Loads

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This study evaluated an updated chemiluminescence enzyme immunoassay (CLEIA) for hepatitis C virus (HCV) core protein for monitoring viral kinetics during treatment with interferon (IFN)-α and ribavirin. Using the CLEIA, serum levels of HCV core protein were measured in 17 patients with genotype 1 and high baseline viral loads during the first 4 weeks of combination therapy. HCV RNA was measured by the Amplicor Monitor test for comparison. At the start of therapy, the median HCV level (interquartile range) was 700 (540–940) kIU/ml of viral RNA and 11,310 (5,528–14,238) fmol/L of core protein. HCV RNA was above the upper limit of the linear range of the Amplicor Monitor test in 13 of the 17 patients, while the core protein level was within the linear range of the CLEIA in all patients. During therapy, the proportion of patients with HCV levels below the cutoff values at each time point was less with the Amplicor Monitor test than with CLEIA. Serum HCV core protein level decreased rapidly during the first 24 hr of therapy and more slowly thereafter, with median exponential decays of 1.08 and 0.046 log10/day, respectively. In the second phase, between day 1 and 28, the median decrease in HCV core protein level was higher in four patients with sustained virologic response (0.13 log10/day) than in 13 patients with no response (0.028 log10/day, $P = 0.042$). The wide linear range of the HCV core protein assay is appropriate for measuring viral loads during therapy with IFN-α and ribavirin. ***J. Med. Virol. 77:77–82, 2005.*** © 2005 Wiley-Liss, Inc.

KEY WORDS: core protein; enzyme immunoassay; hepatitis C; interferon; ribavirin; viral kinetics

INTRODUCTION

Hepatitis C virus (HCV) is a major causative a chronic liver disease worldwide [Lauer and V 2001]. Persistent infection with HCV often progr chronic hepatitis, liver cirrhosis, and hepatoc carcinoma over the course of several decades. Si report by Hoofnagle et al. [1986], describing the e interferon (IFN) therapy on chronic hepatitis drug has been approved for the eradication of HC may reduce the incidence of hepatocellular car [Nishiguchi et al., 1995; Yoshida et al., 1999; Kub 2001]. Ribavirin is a synthetic guanosine nuc analog that inhibits replication of various RN DNA viruses. In patients with chronic hepatitis combination of IFN-α and ribavirin yields a high of sustained virologic response than IFN-α alone et al., 1998; McHutchison et al., 1998; Poynard 1998; Reichard et al., 1998]. However, the sustained eradication of HCV achieved by comb therapy remains unsatisfactory for patients wit genotype 1 and high baseline viral loads.

Analysis of the dynamics of HCV during th phase of IFN-based therapy is important for mon the response to therapy, and sometimes for mo treatment regimens. Beginning 7–10 hr after in of IFN administration, the serum level of HCV d

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rapidly, with an estimated half-life of 5.0–7.2 hr during the first 1 or 2 days of therapy, and then declines more slowly [Neumann et al., 1998; Yasui et al., 1998]. This second-phase decrease in HCV level is especially important when analyzing viral kinetics for prediction of long-term response to therapy [Enomoto et al., 2002a,b, 2004].

A simple and inexpensive method with a wide linear range of quantitation is needed for frequent measurement of serum HCV levels to assess responses to antiviral therapy. Several assays for measurement of serum HCV levels are available commercially, including reverse-transcription polymerase chain reaction (PCR) for HCV RNA [Zeuzem et al., 1994; Lee et al., 2000] and enzyme immunoassay (EIA) for HCV core protein [Tanaka et al., 1995, 1996; Nishiguchi et al., 2002]. Although the sensitivity of the conventional EIA for HCV core protein was previously inferior to that of PCR, EIA has been improved in its analytical sensitivity [Aoyagi et al., 1999; Tanaka et al., 2000; Zanetti et al., 2003]. In particular, a chemiluminescence enzyme immunoassay (CLEIA) has been developed recently utilizing a partially automated system with specialized equipment that is both simple to perform and can yield results rapidly. However, the usefulness of this assay for monitoring serum HCV levels during IFN-α and ribavirin therapy has not been assessed clinically.

The aim of this study was to evaluate whether the CLEIA for HCV core protein can be used to examine viral kinetics in the early phase of treatment with IFN-α and ribavirin. CLEIA was used to monitor serum levels of HCV core protein in patients with genotype 1 and high viral loads during the first 4 weeks of combination therapy. HCV RNA was measured by the PCR-based Amplicor Monitor test for comparison.

MATERIALS AND METHODS

Patients

There were 17 patients with chronic hepatitis C (10 men and 7 women; mean age, 54 ± 10 years) who began combination therapy with IFN-α2b and ribavirin at our hospital between March 1999 and December 2002. The inclusion criteria were as follows: persistent elevation of serum alanine aminotransferase for at least 6 months before therapy; presence of genotype 1 of HCV in serum; presence of serum HCV RNA at levels above 200 kIU/ml as determined by the Amplicor Monitor test; absence of serum hepatitis B surface antigen and of signs of other likely causes of chronic liver disease; histological features of chronic hepatitis in liver biopsy specimens obtained within 6 months before the start of therapy; absence of anemia (hemoglobin concentration less than 12 g/dl in women and less than 13 g/dl in men); and no evidence of hepatocellular carcinoma on ultrasonographic or computed tomographic examinations. Serum samples were obtained from the patients before administration of the drug(s) on the first day of therapy (day 0) and on day 1, 7, 14, and 28. The samples were the study were in accord with the Declai Helsinki of 1975 (1983 revision) and were app the ethics committee of our hospital.

Treatment

Patients received recombinant IFN-α2b (I Schering-Plough, Kenilworth, NJ) by intrai injection at a dosage of 6 MU every day for followed by 6 MU three times a week for 4 Ribavirin (Rebetol, Schering-Plough) was giv twice a day for the first 24 weeks at a total dail 600 mg in the nine patients who weighed 60 k and 800 mg in the remaining eight patie weighed more than 60 kg. This protocol was c used in this country at the time of this st response to therapy was assessed virologi repeated PCR assays for serum HCV RNA. A s virologic response was defined as one in whic HCV RNA was not found more than 6 months end of therapy. Patients who did not meet thes were considered to have no response to therap

Assays

Routine hematological and biochemical te performed using standard procedures. Ser RNA was measured by the Amplicor Monitor te Diagnostics, Branchburg, NJ) [Zeuzem et al., 1 et al., 2000], which exhibits good linearity 0.5 and 500 kIU/ml. When HCV RNA was not by this method, the serum was tested again more sensitive, qualitative Amplicor test [L 2000]. Genotypes of HCV were identified l sequencing of the amplification products g during the Amplicor Monitor test with an A DNA sequencer (Perkin Elmer Corp./Appliec tems, Foster City, CA) [Kuboki et al., 2000].

Serum HCV core protein was measured b (Lumipulse Ortho HCV Antigen, Ortho-Clini nostics, Tokyo, Japan) according to the manuf instructions. In brief, 200 µl of serum sample w with 100 µl of a pretreatment solution contain Triton X-100 and 15% sodium dodecyl sulfa incubation at 56°C for 30 min, 100 µl of the pret solution was added to a well, coated with m antibodies to the HCV core antigen, and filled w of reaction buffer. The mixture was incuba agitation for 10 min at 37°C and then wasl buffer. Alkaline phosphatase-conjugated m antibodies to HCV core antigen were then add well, which was then incubated for 10 min After washing, 200 µl of a substrate buffer w and the mixture was incubated for 5 min at 3 reactive chemiluminescence unit was measu the concentration of HCV core antigen was de according to a standard curve generated u recombinant HCV core antigen. All steps of t after the first incubation (at 56°C for 30 m performed on a fully automated chemilumi

Japan). The total assay time was 30 min. The linear range of the assay was 15–50,000 fmol/L.

Histological Evaluation

Liver biopsy was performed for each patient within 6 months before the start of therapy. Histopathological findings were assessed by grading inflammatory activity and staging fibrosis according to the classification of Desmet et al. [1994]. All evaluations were done by an experienced pathologist blinded to the clinical data.

Statistical Analysis

Statistical analysis was performed with the Statview SE + Graphics program, version 5.0 (SAS Institute, Cary, NC). The significance of correlations was evaluated by Spearman's rank analysis. Distributions of continuous variables were analyzed by the Mann–Whitney U-test. A two-tailed P-value of less than 0.05 was taken to indicate statistical significance.

RESULTS

Baseline Characteristics of Patients

Of the 17 patients, 9 had a history of IFN monotherapy. All patients were infected with genotype 1b of HCV, which is the most common kind in Japan. At the start of treatment with IFN-α2b and ribavirin, the median alanine aminotransferase activity (interquartile range) was 80 (46–114) IU/L. The median HCV level (interquartile range) was 700 (540–940) kIU/ml of viral RNA and 11,310 (5,528–14,238) fmol/L of core protein. The baseline HCV RNA level was above the upper limit of linear range of the Amplicor Monitor test in 13 of the 17 patients, but the core protein level of all patients was within the linear range of the CLEIA. The grade of inflammatory activity was mild in nine patients and moderate in seven. The stage of fibrosis was mild in eight patients, moderate in six, severe in one, and cirrhotic in one. In one patient, the biopsy sample was too small to evaluate.

Relationship Between HCV RNA Level and Core Protein Concentration

The relationship between the results of measuring HCV RNA by the Amplicor Monitor test and those of measuring HCV core protein by CLEIA is shown in Figure 1. In three samples taken from one patient during therapy, HCV core protein was above the cutoff value for the CLEIA (534, 221, and 55.4 fmol/L, respectively), while HCV RNA was below the cutoff value for the Amplicor Monitor test. In eight samples taken from five patients, HCV RNA was above the cutoff value for the Amplicor Monitor test (median, 2.7 kIU/ml; range, 0.6–20 kIU/ml), while HCV core protein was below the cutoff value for CLEIA. Samples below the cutoff value of each assay were assigned the viral load of the cutoff value for calculation. There was a significant correlation between the results obtained with the two

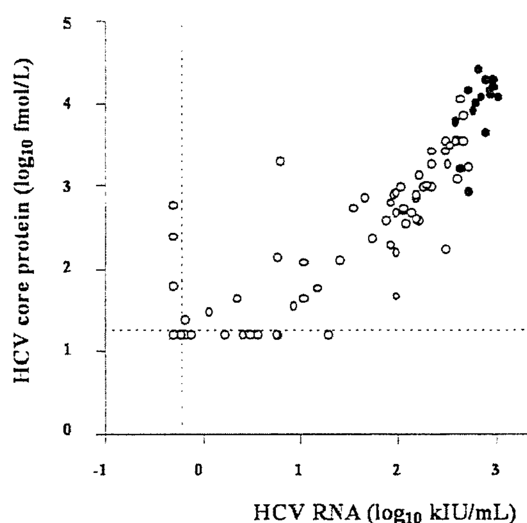

Fig. 1. Correlation between serum HCV RNA level measure Amplicor Monitor test and HCV core protein level measure CLEIA. Broken lines show the cutoff value for each assay. ●, taken at the start of therapy with IFN-α2b and ribavirin. ○, taken during therapy.

The correlation between results was also sign when all serum samples taken during therap included in analysis ($r = 0.859$, $P < 0.0001$).

Changes in HCV Levels During the First 4 Weeks of Treatment

On day 1, 7, 14, and 28 of treatment with IFN-α ribavirin, HCV RNA was below the cutoff value Amplicor Monitor test in zero (0%), one (6%), four and four (24%) patients, respectively, while HC protein was below the cutoff value by CLEIA in (1 (6%), 5 (29%), and 8 (47%) of the 17 patient proportion of patients with HCV levels belo respective cutoff values during therapy was lowe the Amplicor Monitor test than with CLEIA.

Changes in serum HCV core protein in all pa monitored by the CLEIA are shown in Figure 2. Ch in HCV RNA as measured by the Amplicor Monit paralleled those in core protein (data not show reported previously, HCV core protein decreased r during the first 24 hr of therapy and more thereafter. We defined the period between 0 and 2 therapy (day 0) as "the first phase," and the perio day 1 to 28 (day 14 if core protein was below the value on day 28, or day 7 if core protein was the cutoff value on day 14) as "the second phase median rate of exponential decay of serum HC protein (interquartile range) in the first and in phases were 1.08 (0.69–1.34) and 0.046 (0.016 log10/day, respectively.

Of the 17 patients treated with combination th 4 had a sustained virologic response and 13 h response. The median rate of decay in the first

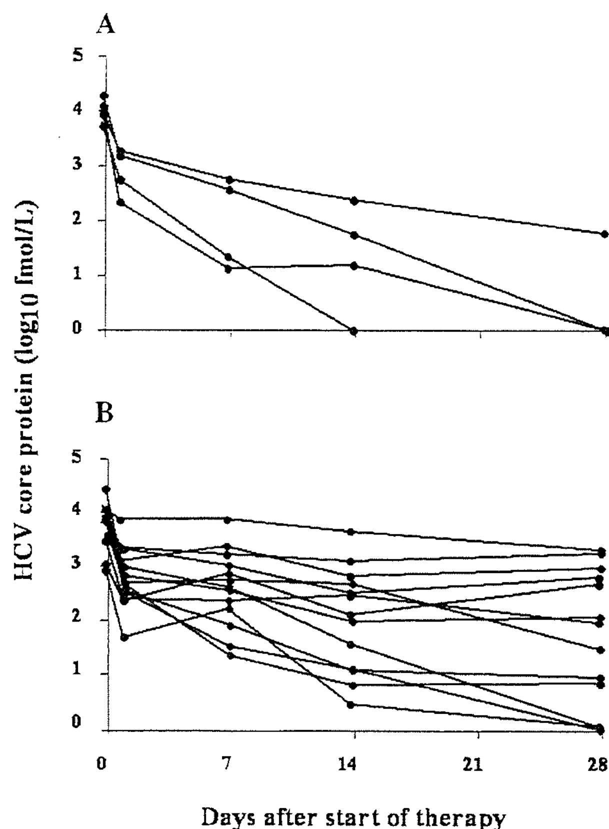

Fig. 2. Time course of serum HCV core protein level monitored by CLEIA during the first 4 weeks of IFN-α2b and ribavirin treatment (A) in the four patients with sustained virologic response, and (B) in the 13 patients with no response. In the second phase between day 1 and 28, the median decrease in HCV core protein was larger in patients with sustained virologic response (0.13 log10/day) than in patients with no response (0.028 log10/day, $P = 0.042$).

patients with sustained virologic response and 1.11 (0.69–1.34) log10/day in patients with no response; the differences in first-phase viral decline were not significant ($P = 0.73$). The median rate of decay in the second phase (interquartile range) was 0.13 (0.045–0.21) log10/day in patients with sustained virologic response and 0.028 (0.0048–0.11) log10/day in patients with no response; the differences in second-phase viral decline between the sustained-response and no-response groups were significant ($P = 0.042$).

DISCUSSION

Among commercial assays for the measurement of serum HCV levels, the Amplicor Monitor test is used widely, as a sensitive PCR-based method [Zeuzem et al., 1994; Lee et al., 2000]. However, it yields results with limited reproducibility and requires expensive equipment. The cumbersome procedures make testing of many samples difficult and increase the risk of con-

estimated with it, and, in addition, its ε sensitivities may be affected by the genotyp resulting in underestimation of viral loads of 2 or 3.

The serum concentration of HCV core p correlated with HCV RNA titer [Tanaka et . 1996]. Viral protein is more resistant to freezing and thawing than viral RNA. In version of the EIA, HCV core antigens are rele the virion and antibodies to HCV core are inact pretreatment with sodium dodecyl sulfate. ' treatment step increases sensitivity by appro 100-fold. Aoyagi et al. [1999] demonstrated hi assay precision and inter-assay reproducibili EIA over a wide range of values. This metho uses specific monoclonal antibodies specifi conserved region of core protein, can evaluate loads of different genotypes with equal sensi particular, the partially automated CLEI requires less than 1 hr to yield results. Clinica availability of results allows viral loads to be a monitored and treatment regimens to be modi quired. The low cost of the assay enables serum to be frequently tested to evaluate viral kineti

The cutoff value of the CLEIA was set at 15 HCV core protein, based on the results of meas of serially diluted standard samples. This equivalent to 1–2 kIU/ml of viral RNA (unp observations). Although the proportions of pati HCV levels below the cutoff values during ther smaller with the Amplicor Monitor test than CLEIA, the analytical sensitivity of the CLEIA the same as that of the Amplicor Monitor addition, the baseline HCV core protein le patients with genotype 1 and high viral loads w the linear range of the CLEIA, whereas in a of the patients the viral RNA was above the up of the linear range of the Amplicor Monitor wide linear range permitted by the HCV cor assay is appropriate for accurate measureme high viral loads present before and during the days after the start of therapy.

Davis [2002] showed that a decrease in ser level by 2 log10 units within the first 12 weeks o with IFN-α and ribavirin can be used as the definition of an early virologic response whi achieved, is associated with a low likelihood of virologic response. Discontinuation of therapy recommended for patients with genotype 1 w achieve an early virologic response. This typ pective assessment also requires methods w linear ranges to precisely evaluate viral loads b during antiviral therapy.

Previous studies of the dynamics of HCV in few weeks of IFN treatment have revealed biph decline [Neumann et al., 1998; Yasui et al., 19 also observed. Neumann et al. [1998] suggeste rapid viral decrease in the first phase reflects dependent effects of IFN on HCV production,

death of hepatocytes infected with HCV. It was found previously that the second-phase decrease in HCV RNA monitored by quantitative PCR was correlated with the long-term effects of IFN therapy [Enomoto et al., 2002a,b]. In the present study, CLEIA also exhibited significant correlation between the second-phase decrease in HCV core protein and the sustained virologic response to treatment with IFN-α and ribavirin. The changes in serum HCV core protein monitored by the CLEIA early during IFN-α and ribavirin treatment can be used to predict long-term therapeutic response.

Randomized controlled trials have shown that weekly treatment with pegylated IFN-α plus ribavirin yields higher rates of sustained virologic response than treatment with unmodified IFN-α plus ribavirin [Manns et al., 2001; Fried et al., 2002]. Owing to advances in treatment, IFN is now indicated even for difficult-to-treat patients with HCV genotype 1 and high baseline viral loads. Evaluation of HCV levels by assays with wide linear ranges will most likely become more important in the future.

In summary, the CLEIA is a simple, sensitive, specific, reproducible, and inexpensive method for the measurement of HCV core protein. The wide linear range of the HCV core protein assay is appropriate for monitoring viral loads in patients with genotype 1 and high viral loads during therapy with IFN-α and ribavirin.

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