Journal of Hepatology, 1993; 19: 408–412

HEPAT 01452

Ribavirin in the treatment of chronic hepatitis C unresponsive to alfa interferon

Joan Camps, Nicolás García, José I. Riezu-Boj, María P. Civeira and Jesús Prieto

Liver Unit, Department of Medicine, Clínica Universitaria and School of Medicine, University of Navarra, Pamplona, Spain

(Received 5 June 1992)

For the 30–50% of patients with chronic hepatitis C who do not respond to α-interferon therapy there is no alternative treatment. Some previously untreated patients have shown a biochemical response to ribavirin, but the antiviral effects of this substance on α-interferon-resistant cases is largely unknown. Twelve patients with chronic hepatitis C who had not responded to a 6–12 month course of α-interferon were included in this study. Oral ribavirin was administered at a dose of 16 mg/kg per day for 6 or 9 months. Aminotransferase levels had not significantly changed during interferon therapy but decreased significantly during ribavirin treatment (mean alanine aminotransferase at baseline, 102 ± 18 IU/l vs. 55 ± 14 IU/l at 6 months; P = 0.0001). Aminotransferase levels became normal in 6 cases (50%), significantly decreased in 3 patients (25%), and did not significantly change in the remaining 3 cases (25%). All patients with normalized aminotransferase values relapsed after ribavirin was discontinued and aminotransferase activity returned to pretreatment levels. Before therapy serum hepatitis C virus RNA was detected by polymerase chain reaction in 10 cases. None of them had cleared viral RNA when tested following 3, 6 and 9 months of ribavirin therapy. Side-effects were mild and reversible. In conclusion, about half of the patients with chronic hepatitis C who are unresponsive to α-interferon show a clear-cut biochemical response after 6–9 months of ribavirin administration. However, ribavirin does not clear circulating hepatitis C virus RNA and relapses occur after withdrawal. Therefore, since ribavirin alone does not appear to suppress viral replication, despite its biochemical effect, further studies should be made in combination with other antiviral substances.

Key words: Hepatitis C virus RNA; Viral replication; Aminotransferases; Cirrhosis

Hepatitis C virus (HCV) is the etiological agent of most, if not all, parenterally acquired cases of non-A non-B hepatitis (1–4). Acute infection usually remains subclinical, but unfortunately the disease evolves to chronicity in more than 50% of the patients (1,5,6). Chronic carriers of HCV have a high risk of developing cirrhosis, and some will eventually develop hepatocellular carcinoma (5,7,8). Serological studies in volunteer blood donors suggest that 0.5–1.2% of the general population in the developed world may be infected with HCV (1,3,4,9). At present α-interferon (αIFN) is the only therapy which has been extensively evaluated in the treatment of chronic hepatitis C (10). Of the patients with chronic hepatitis C, 50–70% respond to αIFN and show a normalization of aminotransferase levels and an improvement in the liver lesion. Response to αIFN is usually accompanied by a clearance of serum HCV RNA (11). However, since 50% of the responders relapse after αIFN discontinuation, the long-term benefit from this treatment remains limited to 20–30% of the patients. At present, there is no alternative therapy for chronic hepatitis C cases unresponsive to αIFN.

Correspondence to: Prof. J. Prieto, Departamento de Medicina Interna, Clínica Universitaria, Apartado 92, 31008 Pamplona, Spain.

Ribavirin(1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a synthetic nucleoside analogue structurally similar to guanosine, xanthosine, and pyrazofurin (12). Ribavirin displays antiviral activity against a wide range of DNA and RNA viruses, including some members of the flaviviridae family, to which HCV has been provisionally assigned (12). This substance also inhibits the in vitro replication of hepatitis A, B, and D (delta) viruses (13–15). Ribavirin induces an alteration of the nucleotide pools and inhibits the normal transcription of messenger RNA. It is rapidly transported into cells and converted by cellular enzymes, to mono-, di- and triphosphate derivatives, which then inhibit viral nucleic acid synthesis (16,17). Ribavirin has been approved for use as an aerosol for infants with serious infections due to respiratory syncytial virus. It has also been used orally or intravenously in the treatment of chronic hepatitis B (18), human immunodeficiency virus infection (19), influenza virus infection (20), and Lassa fever (21). Recently, a pilot study of ribavirin in 10 patients with chronic hepatitis C showed a significant decrease in aminotransferase levels during ribavirin administration (22). Unfortunately, all responders relapsed shortly after ribavirin withdrawal. Side-effects were mild and reversible.

The aim of this study was to assess the activity of ribavirin in patients with chronic hepatitis C unresponsive to αIFN. The biochemical and virological responses were assessed with serial determination of aminotransferase levels, and by the detection of serum HCV RNA.

Methods

Twelve patients (4 men and 8 women) with histologically proven chronic hepatitis C (10 with active cirrhosis) were included in this study. Mean age was 57.1 years (range, 35–70 years). All patients had raised aminotransferases levels for more than 6 months, and antibodies against HCV were repeteadly detected in all patients by a second-generation enzyme-linked immunosorbent assay (Ortho Diagnostic Systems, Raritan, NJ). Four patients had previously received transfusions, and the remainder had no known percutaneous risk factor. Other causes of chronic active hepatitis were carefully excluded. All were negative for antibodies to the human immunodeficiency virus, and other causes of immunodeficiency were also excluded. All 12 patients had been previously treated with lymphoblastoid αIFN (Wellferon, Gayoso-Wellcome, Madrid, Spain) at doses of 3×10^6 units daily or 3 times/week for 6 ($n = 5$) or 12 months ($n = 7$). Aminotransferases were unchanged during and after αIFN therapy in all cases. The minimum αIFN-free period before ribavirin administration was 6 months. Ribavirin (Virazole, ICN-Hubber SA, Barcelona, Spain) was taken orally every 8 h for a total daily dose of 16 mg/kg. Due to the absence of significant side-effects all patients completed the treatment schedule. Nine patients received ribavirin for 6 months, and the remaining 3 patients received it for 9 months. Patients were followed-up for a mean of 4.6 ± 1.9 months (range, 3–9) after treatment was stopped. Aminotransferases (upper normal limit (UNL) of alanine aminotransferase (ALT): 30 IU/l), alkaline phosphatase, γglutamyltranspeptidase, bilirubin, hemoglobin, leucocytes, platelets, serum creatinine, and uric acid were assessed every 10 days for the first 2 months, and monthly thereafter, including follow-up. Presence of serum HCV RNA was investigated by reverse transcription of RNA extracted from 50-μl samples and amplification of the cDNA by 'nested' polymerase chain reaction (PCR) (see below), before ribavirin administration (month 0), and at months 3, 6, and 9 of ribavirin therapy. Serum samples were stored at −80°C until PCR testing.

The PCR technique was performed as described elsewere (23,24) using primer sequences derived from the highly conserved 5′ non-coding region of a cDNA clone of a Japanese isolate (25) (outer primers: C15, sense, 5′GTATCTCGAGGCGACACTCCACCATAGAT and C16, antisense, 5′ATACTCGAGGTGCACGGTCTACGAGACCT corresponding to nucleotide positions 1–19 and 323–303, respectively, and inner primers: C17, sense, 5′CCACCATAGATCTCTCCCCTGT and C18, antisense, 5′CACTCTCGAGCACCCTATCAGCGAGT, corresponding to nucleotide positions 10–31 and 296–271, respectively). The second PCR round enhances sensitivity of the technique, theoretically allowing the detection of one single copy of the virus, while a unique PCR round followed by gel electrophoresis and ethidium bromide staining detects approximately 100–200 copies. Positivity in the first round PCR, or only after the second round was used as a semiquantitative method to assess the antiviral effect of ribavirin. Carry over contaminations were avoided by strictly applying the measures described by Kwok and Higuchi (26).

All patients gave informed consent and the trial was approved by the Local Ethics Committee.

Statistical analysis was performed using paired and unpaired Student's *t*-tests, and the Wilcoxon test. Results are presented as mean ± S.E.M.

Results

As shown in Fig. 1, mean ALT levels had not changed significantly during αIFN therapy, (mean ALT: 103 ± 12, 119 ± 23, and 114 ± 27, at months 0, 3, and at 6–12 months, respectively). In contrast, ALT levels significantly decreased during ribavirin administration; month 0: 102 ± 18, month 3: 69 ± 18, month 6: 55 ± 14; month 0 vs. month 3, $P < 0.01$ and month 0 vs. month 6, $P = 0.0001$ (Fig. 2). Aminotransferase values became normal in 6 patients (50%) (complete response), decreased to less than twice the UNL in 3 cases (25%) (partial response), and did not substantially change in the remaining 3 patients (25%) (no response). The mean interval to normalization of aminotransferases in the 6 complete responders was 2.6 months, (range, 1–5 months). Pre-treatment aminotransferase levels were lower in complete responders ($n = 6$) than in non-responders and partial responders ($n = 6$) (79 ± 16 vs. 123 ± 25), although this did not reach statistical significance (Fig. 2).

One complete responder and two non-responders received ribavirin for 9 months, and the remaining patients for 6 months. All six complete responders relapsed after therapy and aminotransferase activities returned to pretreatment levels (month 1 (after stopping ribavirin): 48.16 ± 9.45; month 2: 53.5 ± 9.44; month 3: 77.66 ± 23.65). Aminotransferases did not change after treatment in non-responders and increased to pretreatment levels in partial responders.

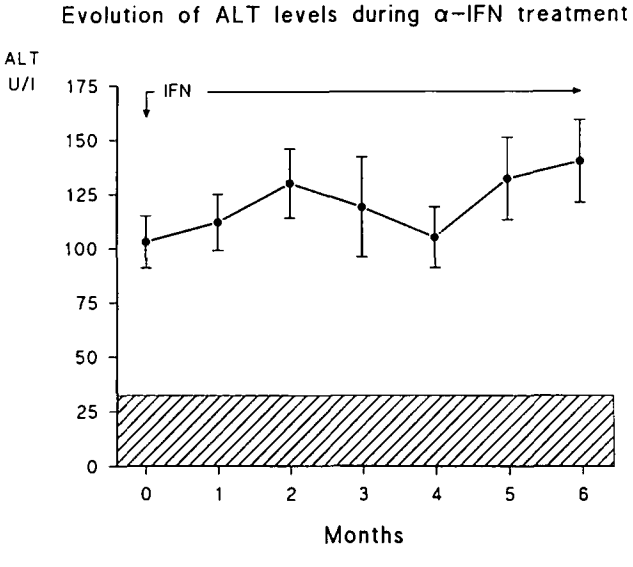

Fig. 1. Evolution of ALT levels during the first 6 months of αIFN therapy. No significant changes were observed. The shadowed area corresponds to the normal range of ALT. Results are presented as mean ± S.E.M.

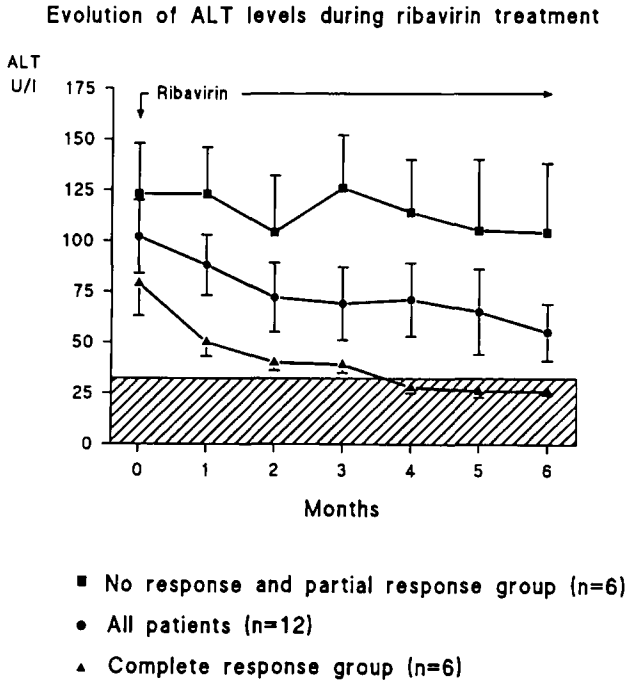

Fig. 2. Evolution of ALT concentrations during ribavirin therapy. The whole group of patients, the complete response group, and the group of non-responders and partial responders are separately represented.

Before ribavirin therapy, HCV RNA was detected in 10 cases (83%). None of these patients cleared HCV RNA during therapy when tested 3, 6 and 9 months after beginning treatment. HCV RNA was detectable at months 3 and 6 in 1 of the 2 cases without detectable viral RNA before treatment. One patient with von Willebrand's disease who had been transfused during delivery of a normal baby, and developed clear-cut post-transfusion non-A, non-B hepatitis, but in whom HCV RNA was never detected despite seroconversion to anti-HCV, also showed a complete biochemical response to ribavirin administration. Seventy percent of the baseline samples showed a positive band on ethidium bromide staining after the first PCR round, while the remainder were detected only after the second round of amplification. This percentage did not change significantly when samples obtained at 3 and 6 months after ribavirin administration, were tested for HCV RNA (Table 1). Response to ribavirin was not related to baseline PCR results; 4 out of the 5 non-responders or partial responders were positive by the first round PCR and 3 out of the 5 complete responders were also positive by first round PCR (Table 1).

TABLE 1
Results of the detection of serum HCV RNA before and during ribavirin treatment in patients with chronic HCV infection resistant to alfa interferon

Complete responders				Non-responders and partial responders			
Patient No.	Baseline	Month 3	Month 6	Patient No.	Baseline	Month 3	Month 6
1	++	++	++	7	+	+	+
2	++	++	++	8	++	++	+
3	++	+	++	9	−	+	+
4	−	−	−	10	++	++	++
5	+	++	+	11	++	+	++
6	+	+	++	12	++	++	+

Plus and minus marks indicate a positive or negative PCR result: +, positive only after second round PCR (see Methods section); ++, positive after first round PCR; −, negative PCR result.

Side-effects were always mild and reversible. Two patients complained of mild abdominal discomfort during the first week of therapy, and most (10/12, 83%) developed mild asymptomatic hemolytic anemia (hemoglobin levels between 10 and 12 g/dl), and 5 asymptomatic hyperuricemia (6.5–9 mg/dl). Hemoglobin and uric acid levels had returned to normal values in all cases 1 month after ribavirin therapy was discontinued.

Discussion

To our knowledge this is the first study which evaluates treatment for chronic hepatitis C resistant to αIFN. Ribavirin was well tolerated in all cases, and a significant decrease in ALT levels was observed in 75% of the patients. Although half the patients normalized aminotransferase concentrations, ribavirin did not seem to effect viral replication since none of the patients cleared serum HCV RNA as detected by PCR. Moreover, not even a partial inhibition of HCV replication could be demonstrated as determined by first and second round PCR results (27,28). Since response to αIFN is usually associated with serum HCV RNA clearance (11), the mechanism by which ribavirin induces a biochemical response is not known. However, quantitative PCR methods, (i.e., serial dilutions of the samples), should be made to examine a possible partial inhibition of HCV replication. Ribavirin could interfere with the mechanisms of liver cell injury (perhaps by reducing the cytopathic effect of HCV), without affecting viral replication. Ribavirin has also been shown to diminish the primary antibody responses and memory cell generation to T-dependent and T-independent antigens in vivo (29). Thus, ribavirin, in addition to its antiviral effect, might induce a biochemical response by acting as an immunosuppressor drug. However, this immunosupressant effect has not been consistently demonstrated in vivo, and at low doses ribavirin could enhance certain immunological responses (30). The reason why half the patients showed a complete biochemical response to ribavirin is not known, since viral replication was persistant in all cases. Since mutations of the plus-stranded RNA Sindbis virus have been shown to lead to resistance to ribavirin therapy (31), some mutants of HCV could also be resistant to ribavirin.

The results of this study suggest that some chronic hepatitis C patients who are unresponsive to αIFN may benefit from ribavirin therapy. Ribavirin could therefore constitute an alternative treatment for these patients. However, whether the biochemical response is accompanied by an improvement of the liver necroinflammatory lesion must be studied. The relapse of all responders after therapy suggests that for long-term benefit patients should receive ribavirin for years or indefinitely. The persistence of viremia in all cases is consistent with the post-therapy relapses observed in all complete responders (22). A sinergistic in vitro effect of ribavirin and αIFN on the replication of infectious feline peritonitis virus has been described (32). Therefore, a combination of ribavirin with αIFN or other antivirals should be investigated in these patients.

Acknowledgments

We are very grateful to Edurne Elizalde and Celia Asensio for excellent technical assistance, and we thank Dr. Juan I. Esteban for helpful comments on the manuscript. This work was supported in part by a grant from the Fundación Ramón Areces, Spain.

References

1 Alter HJ. The hepatitis C virus and its relationship to the clinical spectrum of NANB hepatitis. J Gastroenterol Hepatol 1990; (Suppl 1): 78–94.

2 Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989; 244: 359–61.
3 Kuo G, Choo QL, Alter HJ, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 1989; 244: 362–4.
4 Esteban JI, Gonzalez A, Hernandez JM, et al. Evaluation of antibodies to hepatitis C virus in a study of transfusion-associated hepatitis. N Engl J Med 1990; 323: 1107–12.
5 Alter HJ. Chronic consequences of non-A, non-B hepatitis. In: Seeff LB, Lewis JH (eds.). Current Perspectives in Hepatology. New York: Plenum, 1989; 83–97.
6 Dienstag JL. Non-A, non-B hepatitis. I. Recognition, epidemiology, and clinical features. Gastroenterology 1983; 85: 439–62.
7 Colombo M, Kuo G, Choo QL, et al. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. Lancet 1989; ii: 1006–8.
8 Bruix J, Barrera JM, Calvet X, et al. Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. Lancet 1989; ii: 1004–6.
9 Esteban JI, Esteban R, Viladomiu L, et al. Hepatitis C virus antibodies among risk groups in Spain. Lancet 1989; ii: 292–7.
10 Hoofnagle JH, Di Bisceglie AM. Treatment of chronic type C hepatitis with alpha interferon. Semin Liver Dis 1989; 9: 259–63.
11 Shindo M, Di Bisceglie AM, Cheung L, et al. Decrease in serum hepatitis C viral RNA during alpha-interferon therapy for chronic hepatitis C. Ann Intern Med 1991; 115: 700–4.
12 Sidwell R, Hoffman J, Kharp L, et al. Broad-spectrum activity of virazole: 1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide. Science 1972; 117: 705–6.
13 Crance JM, Biziagos E, Passagot J, van Cuyck Gandre H, Deloince R. Inhibition of hepatitis A virus replication in vitro by antiviral compounds. J Med Virol 1990; 31: 155–60.
14 Choi SS, Rasshofer R, Roggendorf M. Inhibition of hepatitis delta virus RNA replication in primary woodchuck hepatocytes. Antiviral Res 1989; 12: 213–22.
15 Civitico G, Wang YY, Luscombe C, et al. Antiviral strategies in chronic hepatitis B virus infections. J Med Virol 1990; 31: 90–7
16 Patterson JL, Fernandez-Larsson R. Molecular mechanisms of action of ribavirin. Rev Infect Dis 1990; 12: 1139–46.
17 Rankin JT, Eppes SB, Antczak JB, Joklik WK. Studies on the mechanism of the antiviral activity of ribavirin against reovirus. Virology 1989; 168: 147–58.
18 Patki S, Gupta P. Evaluation of ribavirin in the treatment of acute hepatitis. Chemotherapy 1982; 28: 298–303.
19 Roberts RB, Hollinger FB, Parks WP, et al. A multicenter clinical trial of oral ribavirin in HIV-infected people with lymphadenopathy: virologic observations. AIDS 1990; 4: 67–72.
20 Ray CG, Icenogle TB, Minnich LL, Copeland JG, Grogan TM. The use of intravenous ribavirin to treat influenza virus-associated acute myocarditis. J Infect Dis 1989; 159: 829–36.
21 McCormiak JB, King IJ, Webb PA, et al. Lassa fever — effective therapy with ribavirin. N Engl J Med 1986; 314: 20–6.
22 Reichard O, Andersson J, Schvarcz R, Weiland O. Ribavirin treatment for chronic hepatitis C. Lancet 1991; 337: 1058–61.
23 Garson JA, Tedder RS, Briggs M, et al. Detection of hepatitis C viral sequences in blood donations by 'nested' polymerase chain reaction and prediction of infectivity. Lancet 1990; 335: 1419–22.
24 Garson JA, Ring C, Tuke P, Tedder RS. Enhanced detection by PCR of hepatitis C virus RNA. Lancet 1990; 336: 878–9.
25 Okamoto H, Okada S, Sugiyama S, et al. The 5'-terminal sequence of the hepatitis C virus genome. Jpn J Exp Med 1990; 60: 167–77.
26 Kwok S, Higuchi R. Avoiding false positives with PCR. Nature 1989; 339: 237–8.
27 Wang J-T, Wang T-H, Sheu J-C, Lin J-T, Chen D-S. Hepatitis C virus RNA in saliva of patients with posttransfusion hepatitis and low efficiency of transmission among spouses. J Med Virol 1992; 36: 28–31.
28 Inchauspe G, Abe K, Zebedee S, Nasoff M, Prince AM. Use of conserved sequences from hepatitis C virus for the detection of viral RNA in infected sera by polymerase chain reaction. Hepatology 1991; 14: 595–600.
29 Peavy DL, Powers CN, Knight V. Inhibition of murine plaque-forming cell responses in vivo by ribavirin. J Immunol 1981; 126: 861–4.
30 Ribavirin. Staff editorial. In: McEvoy GK, ed. AHFS Drug Information. Bethesda, MD. 1992, 8(18): 383.
31 Scheidel LM, Stollar V. Mutations that confer resistance to mycophenolic acid and ribavirin on Sindbis virus map to the nonstructural protein nsP1. Virology 1991; 181: 490–9.
32 Weiss RC, Oostrom-Ram T. Inhibitory effects of ribavirin alone or combined with human alpha interferon on feline infectious peritonitis virus replication in vitro. Vet Microbiol 1989; 20: 255–65.