

• BRIEF REPORTS •

Ribavirin monotherapy increases sustained response rate in relapsers of end treatment virologic responders

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Abstract

AIM: To assess the efficacy of ribavirin monotherapy in patients with biochemical relapse after combination therapy.

METHODS: Twenty-four weeks of ribavirin monotherapy was given to biochemical relapsers of end treatment biochemical responders within 6 mo after combination therapy, including non-responders with HCV-RNA level ≤ 0.2 Meq/mL and end treatment virologic responders (ETVRs) with or without reappearance of HCV-RNA.

RESULTS: Sixty-two chronic HCV-infected patients completed 24 wk of interferon- α plus ribavirin combination therapy. Fifty patients (80%) achieved end treatment biochemical response including 16 non-responders and 34 of 36 ETVRs. Twenty-six patients (41.9%) were non-responders. Ribavirin monotherapy was given to 20 biochemical relapsers including 12 non-responders with HCV-RNA levels ≤ 0.2 Meq/mL, four of eight HCV-RNA reappearing ETVRs, and four HCV-RNA negative ETVRs. After 24 wk of ribavirin monotherapy, one of 12 non-responders, two of four HCV-RNA reappearing ETVRs and all four RNA-negative biochemical relapsers of ETVRs showed sustained virologic response. Two of 12 monotherapy treated non-responders showed persistent normalization of liver function test. In total, 50% (31/62) of patients achieved sustained virologic response.

CONCLUSION: Resumption of ribavirin monotherapy in ETVRs at signs of viral rebound and recurrent biochemical abnormalities rather than continuation of monotherapy appears to be the key to success of ribavirin monotherapy after interferon-related combination therapy.

Key words: Ribavirin monotherapy; Interferon- α plus ribavirin combination therapy; Relapser; End treatment virologic responder

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INTRODUCTION

Interferon- α monotherapy for chronic hepatitis C virus (HCV) infection has been disappointing with a sustained response rate of only 20-25% after 24-48 wk of treatment^[1,2]. Combination therapy with interferon- α and ribavirin has increased the sustained response rate to about 40%^[3,4]. With the advent of pegylated interferon- α , the sustained response rate of pegylated interferon- α plus ribavirin combination therapy was further advanced to 54-56%^[5,6].

In spite of these recent advances, patients with HCV genotype 1 infection have lower response rate to interferon- α related therapy than patients infected with HCV genotypes 2 and 3^[7]. Patients with HCV genotype 1b are recommended to receive 48 wk of interferon- α -related combination treatments^[5,8,9], which often include the pegylated interferon- α as a part of the treatment regimen. The side effect of pegylated interferon- α was reported to be similar^[5] or milder than interferon- α ^[6], but the cost is much higher.

Ribavirin monotherapy has been unsuccessful in treating patients with chronic hepatitis C infection^[10-13]. Ribavirin as an inhibitor of HCV viral replication, its mechanism of action in mono- or combination therapy of chronic hepatitis C remains unclear.

This study is designed to assess the efficacy of ribavirin monotherapy in treating chronic hepatitis C infected patients with low viral load after interferon- α plus ribavirin combination therapy, in the hope of reducing the medical cost and undesirable side effects due to resumption of interferon-related therapy.

MATERIALS AND METHODS

Materials

This study was conducted from March 1999 to July 2003. Sixty-five patients were recruited for the investigation. Eligible patients were previously untreated chronic C hepatitis patients with elevation of alanine transaminase (ALT) level 2-10 times over the normal upper limit for more than

six months. Patients were excluded if they had decompensated liver cirrhosis, alcoholism, hepatitis B virus superinfection, uncontrolled diabetes mellitus, autoimmune disorders, human immunodeficiency virus infection, uremia, or organ transplantation. Liver biopsies were done before and after the combination therapy and were read and given a Knodell histologic activity index (HAI) numeric score^[14] by the same pathologist. Patients, who needed ribavirin dosage reduction, were excluded from the study.

Methods

Patients who fulfilled the above criteria were given combination therapy consisting of 3 million *U* alpha-2a interferon three times per week administered subcutaneously or intramuscularly plus ribavirin 1 000 or 1 200 mg/d based on the body weight (1 000 mg if body weight <70 kg, 1 200 mg if body weight ≥70 kg). Liver function test and hemogram were performed monthly during the treatment. After the combination treatment, the liver function test was followed up monthly over the first 3 mo then bimonthly in all groups. The HCV-RNA level was measured again when the liver function test became abnormal during the follow-up period after the combination therapy.

Non-responder is defined as detection of HCV-RNA level at the end of 24 wk of combination therapy. End treatment biochemical response is defined as normalization of liver function test at the end of combination treatment with or without detectable HCV-RNA. End treatment virologic response is defined as normalization of liver function test and undetectable HCV-RNA (<100 copies/mL) at the cessation of combination treatment. Sustained virologic response is defined as normalization of ALT and undetectable HCV-RNA level (<100 copies/mL) at the cessation of the combination therapy and over the following 12 mo. Biochemical relapse is defined as elevation of ALT ≥1.5× normal upper limit after combination therapy with or without detectable HCV-RNA within 6 mo after end treatment biochemical response.

Patients who experienced biochemical relapse within 6 mo after combination therapy, according to their intention and consent to be treated, received 24 wk of ribavirin monotherapy, and in dosages described above. Patients diagnosed as having diabetes mellitus, fatty liver, other viral infections, and other possible causes of abnormal liver function test were excluded. During ribavirin monotherapy, liver function test was followed up monthly over the first 3 mo, then bimonthly until the end of one year. HCV-RNA level was re-checked at the beginning of monotherapy, at 12 wk into the therapy and at the end of the monotherapy.

Virologic method

Quantification of HCV-RNA was first performed with a reverse transcription-polymerase chain reaction and a digoxigenin detection system^[15]. The titer of HCV-RNA was expressed in million genome equivalent (Meq) per milliliter. The lower limit of detection of the test is 0.01 Meq/mL. For HCV-RNA titers less than 0.01 Meq, the specimens were reexamined using Amplicor qualitative HCV-RNA assay (Roche Diagnostics, Branchburg, NJ), which has a

lower limit of sensitivity of 100 copies/mL. Bayer Versant 3.0 (bDNA) Quantitative Assay (Bayer Diagnostics, Emeryville, CA) was used to examine HCV-RNA levels between 100 and 10 000 copies/mL, the lowest level of detection is 3 200 copies/mL. The genotyping of HCV was carried out using reverse hybridization assay (Inno-LiPA HCV-II; Innogenetics, Gent, Belgium).

Statistical analysis

Baseline data of quantitative variables were expressed as mean±SD. Pair data of post-treatment response in groups were compared with *t* test, *F* test or χ^2 test. *P* values <0.05 were considered statistically significant.

RESULTS

Three of 65 patients failed to complete the study, including two patients who were unable to tolerate the side effect of interferon- α and one patient who experienced severe insomnia and dyspnea caused by ribavirin. Among 62 patients who completed the study, 26 (41.9%) were non-responders. The remaining 36 patients (58.1%) achieved end treatment virologic response. Fifty patients (80%) including 16 non-responders and 34 of 36 end treatment virologic responders (ETVRs) achieved end treatment biochemical response. The HCV-RNA levels in 12 of 16 non-responders who achieved end treatment bio-chemical response were ≤0.2 Meq/mL as ALT relapsed. They were included in the monotherapy. Two among 36 ETVRs demonstrated persistent abnormal liver function test resulting from fatty liver as HCV-RNA levels were undetectable (<100 copies/mL) in four consecutive follow-up tests for one year and obvious fatty liver was demonstrated by ultrasonography. They were excluded from the monotherapy. Mild and transient elevations of ALT levels (<1.5 normal upper limit), which returned to normal one month later, were observed in four ETVRs at the fifth month after combination therapy. They were also excluded from the monotherapy. In total, 12 of 36 ETVRs demonstrated biochemical relapse within 5 mo after combination therapy including eight patients with HCV-RNA reappearance and four patients without detectable serum HCV-RNA levels (HCV-RN<100 copies/mL). Four of HCV-RNA reappearing ETVRs and all four RNA-negative ETVRs were included in the study. In total, ribavirin monotherapy was administered to 20 biochemical relapsers including four of eight HCV-RNA reappearing ETVRs, four HCV-RNA negative ETVRs, and 12 HCV-RNA levels ≤0.2 Meq/mL non-responders. The baseline characteristics and virologic profiles of these 62 patients are divided into three groups according to treatment responses as presented in Table 1. No significant difference is seen in gender, age, HCV genotype, cirrhosis, patient number of HCV-RNA >2 Meq/mL, and HAI scores in pairwise comparisons among three treatment groups.

The initial HCV-RNA levels of 12 non-responders who were eligible for monotherapy ranged from 0.0046 to 0.2 Meq/mL at biochemical relapse. Three patients failed to normalize the liver function tests. Nine patients (75%) experienced normalization of ALT during the monotherapy. One of these nine patients achieved sustained virologic

Table 1 Base-line characteristics of patients who underwent combination therapy

	Total	Non-responder	SVR	Relapser
Sex (m/f)	25/37	10/16	10/14	5/7
Age (yr)	51.5±11.1	50.2±12.5	52.6±9.6	52.9±10.7
HCV genotype (%)				
1a	-	-	-	-
1b	49 (79)	22/26 (84.6)	18/24 (75)	9/12 (75)
2a	5 (8)	1/26 (3.8)	3/24 (12.5)	1/12 (8)
2b	8 (13)	3/26 (11.5)	3/24 (12.5)	2/12 (17)
Cirrhosis (%)	16/62 (26)	9/26 (35)	5/24 (21)	2/12 (17)
HCV-RNA (%)	6/62 (10)	2/26 (8)	2/24 (8)	2/12 (17)
titer >2 Meq copies/mL				
HAI score	9.5±3.8	10.6±4.3	8.6±2.4	8.7±3.1

No statistical difference in pair comparisons of sex, age, genotypes, cirrhosis, patient number of HCV-RNA titer >2 Meq/mL and HAI scores in non-responders, sustained virologic responders and relapsers.

response when the monotherapy ended. The HCV-RNA level was 0.011 Meq/mL at the start of the monotherapy. This patient sustained the virologic response one year after monotherapy. Two of these nine patients, although HCV-RNA levels were 0.0046 and 0.08 Meq/mL, respectively at the beginning of the monotherapy, experienced 2 and 3 years of biochemical remission after the monotherapy. The HCV-RNA levels were 2.6 and 0.868 Meq/mL, respectively at the end of follow-up. The ALT levels of the remaining six patients became abnormal again and the HCV-RNA levels remained detectable at the end of the monotherapy.

In four HCV-RNA negative biochemical relapsers of ETVRs, the ALT levels normalized and the HCV-RNA levels remained undetectable for 2 years after monotherapy. In two of four HCV-RNA reappearing biochemical relapsers of ETVRs, ribavirin monotherapy was given at 1 and 3 mo after the detection of ALT elevation. The HCV-RNA levels in both patients became undetectable (<100 copies/mL) again at the end of the monotherapy and remained undetectable for over one year. The HCV-RNA levels were 3.8 and 0.061 Meq/mL, respectively at the start of monotherapy. The remaining two of four HCV-RNA reappearing biochemical relapsers of ETVRs received ribavirin monotherapy as late as 6 mo after the cessation of combination treatment and failed to respond to ribavirin monotherapy. The HCV-RNA levels were 1.06 and 2.7 Meq/mL, respectively at the beginning of monotherapy, and remained detectable at the end of monotherapy.

The demography of patients who had sustained virologic response after monotherapy is shown in Table 2. With seven additional sustained virologic responders after the monotherapy, the number of patients with sustained virologic response increased from 24 to 31 (50% of 62 enrolled patients) at the conclusion of the study.

DISCUSSION

In the present study, we have shown that adjuvant ribavirin monotherapy given early at the signs of recurrent liver function abnormalities following the completion of interferon-ribavirin combination therapy achieved sustained

virologic response in seven of 20 patients. These results contrast with previous studies^[10-12] wherein ribavirin monotherapy, used at the start of the anti-viral treatment or administered in non-responders when the viral loads were well established, showed little efficacy. Our results further contrast with a previous randomized controlled study by Shiffman *et al.*^[13], wherein no enhanced anti-viral response was observed in virologic responders of combination therapy who continued ribavirin monotherapy.

We think the different clinical outcome in our study may be attributable to the following. First, the patients studied here are treatment-naïve, unlike Shiffman's study in which the patients were relapsers after interferon monotherapy. Second, we employed ribavirin in a different treatment strategy-under the condition when the viral loads of the patients were low and soon after the combination therapy. The ribavirin monotherapy in our study was administered 1 to 3 mo after liver function abnormalities instead of continuing immediately after the combination or interferon therapy as carried out by Shiffman *et al.*

Mathematical analyses of viral load changes have provided critical insight into the pathogenesis of the chronic viral infection of HCV^[16,17]. Dose-dependent exponential decline in viral load has been demonstrated by Lam *et al.*^[18] in interferon treatment as well as Buti *et al.*^[19] in pegylated interferon treatment. The decline is slower in patients infected by HCV genotype 1 and in African Americans^[18,20]. After the rapid clearing of the viral RNA in the first phase, viral decline slows and becomes variable in the second phase^[21]. The effect of the first phase is believed to be predominantly due to the inhibition of de novo infection of susceptible cells by interferon. The second phase of viral decline is thought to depend on the clearing of the virus from the infected cells with or without cell turnover^[22]. To explain our results, we hypothesize that the significant reduction in viral load after the initial interferon-based mono- or combination-therapy may have created a genetic bottleneck wherein only attenuated HCV variants escaped and persisted. When reemerged, these attenuated HCV variants may have provided a boost to the immune system that was accompanied by liver function abnormalities. Indeed,

Table 2 Demography of patients with sustained virologic response after ribavirin monotherapy

Gender, age (yr)	Genotype (b)	HCV-RNA	ALT elevation (mo)	Monotherapy (mo)	Cirrhosis
F53 (Meq/mL)	1	3.81	2	3	+
F43 (Meq/mL)	2	0.061	1	1	-
F49 ¹ (Meq/mL)	1	0.011	2	1	-
F57 (copies/mL)	2	<100	1	2	-
F50 (copies/mL)	1	<100	2	1	-
M50 (copies/mL)	1	<100	1	2	-
M58 (copies/mL)	1	<100	5	2	-

Note: ALT elevation denotes the onset of ALT $\geq 1.5 \times$ normal upper limit after combination therapy in months. Monotherapy denotes the onset of ribavirin monotherapy after biochemical relapse in months. ¹ Non-responder at the cessation of combination therapy.

a previous report by Balladini *et al*^[23] showed that serum ALT level is correlated with the number of lobular CD8+ cells and histologic manifestation. ALT elevation, in our cases, may coincide with a rebound of immune response to the reemergence of virus. The timely administration of ribavirin at this point may have reduced the viral load and/or impaired the virus further by introducing mutations into the viral genome. These conditions may have mimicked a booster effect in vaccination procedure and thus favor the induction of an effective host immune response against residual HCV, leading to the eventual viral clearance. This would explain why continuous ribavirin monotherapy is ineffective, while restarting ribavirin monotherapy at the signs of reemergence of viral RNA and/or liver function abnormalities as described here shows significant efficacy. Continuing ribavirin immediately after the interferon therapy may reduce HCV replication, but at the same time eliminate the immunological booster effect provided by the reemergence of low-level replication of "attenuated" HCV.

The mechanisms of action of ribavirin in the treatment of HCV infection remain incompletely understood. Ribavirin monotherapy has been shown previously to induce transient improvement of liver function test in chronic HCV-infected individuals, but had no sustained response^[10-12]. While the added benefit of ribavirin to interferon treatment is obvious, the mechanism of its action is unclear, as it did not show significant inhibition of viral production^[24]. Ribavirin has been shown to decrease HCV replicon RNA sequence moderately and can cause a significant reduction in viral infectivity in a single round of poliovirus infection by increasing the viral mutation rate^[25,26]. Ribavirin has also been shown to enhance antiviral Th1 and suppress Th2 cytokines expression in human T cells^[27]. Further studies to clarify the role of ribavirin in improving the efficacy of our treatment protocol are needed.

In agreement with the previous report where 5% of the patients with sustained virologic response had persistent rise of ALT^[28], two of 36 ETVRs (5%) in this study were found to have fatty liver and persistently abnormal ALT. Critical to the significance of this study is the inclusion of those four biochemical relapsers who showed elevated ALT levels (≥ 1.5 - $2.0 \times$ normal upper limit) but were serum HCV RNA-negative at the start of the ribavirin monotherapy. These four patients were able to normalize their ALT levels after the monotherapy. We think the conditions of these four patients would have worsened if had they been left untreated. Their hepatic abnormalities are clear indications of viral reemergence despite negative serum HCV levels. Indeed, a recent report using quantitative hepatic HCV-RNA measurement found that intrahepatic HCV RNA was detectable in 2% of sustained virologic responders, among them, two of five patients had reappearance of HCV-RNA^[29]. Two of four patients whose HCV-RNA recurred after end treatment virologic response responded to monotherapy is most intriguing in this study because monotherapy was minimally effective in non-responders. The observation requires a control and larger number of patients to clarify its role.

In conclusion, combination treatment with 24 wk of interferon and ribavirin achieved 40% of sustained virologic

response in the previous study in this island^[3]. In the present study, we show that the sustained virologic response rate can be increased up to 50% by adjuvant ribavirin monotherapy following the combination therapy upon recurrence of ALT abnormalities. The present result further indicates 24 wk of interferon and ribavirin combination treatment followed by adjuvant ribavirin monotherapy when signs of HCV-RNA and ALT abnormalities appear can achieve nearly the same treatment goal as 48 wk of combination treatment especially in biochemical relapsers of ETVRs before or on HCV-RNA reappearing, thus reducing the suffering associated with the latter treatment.

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