ORIGINAL ARTICLE

Hepatitis Monthly 2006; 6(1): 11-18

Efficacy and safety of Interferon-alpha (PDferon B®) and Ribavirin Combination Therapy in patients with Chronic Hepatitis C in Iran

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Background and Aims: **There are different treatment regimens for chronic HCV (CHC). The regimen that combines interferon α (IFN α) and ribavirin (RIBA) is one of the best known effective regimens. Aim: to assess the therapeutic efficacy (as end treatment and sustained response) and safety of a particular brand of IFN α (PDferon B®) in combination with RIBA on Iranian patients with CHC considering genotype characteristics of subjects.**

Methods: **A quasi-experimental study of 176 adult patients with chronic hepatitis C, regardless of previous IFN α treatment carried out between December 2002 and February 2006 in Tehran Hepatitis Center and then divided in two comparison groups: group 1 with genotype (1a, 1b, non-typable) treated for 48 weeks and group 2 with genotype 2,3 (2a,3a,3b) treated for 24 weeks. Treatments consisted of 48 or 24 weeks of 3 million units subcutaneous PDferon B® three times weekly plus 1000-1200 mg oral RIBA twice daily depending on body mass index (BMI). The Main outcomes were the end treatment virologic response (EVR), biochemical response (EBR) and sustained virologic response (SVR) and their influencing factors.**

Results: **115 patient with genotype 1 (male/female: 91/24) and 61 patients with genotype 2,3 (male/female: 50/11) were included and matched for age, BMI and cirrhosis. The rate of EVR, EBR and SVR for all patients were 76.1%, 83.5% and 68.2% respectively and EVR and SVR were significantly higher in group 2 than group 1 (71.3% and 61.7% for group 1 and 85.2% and 80.3% for group 2 respectively; P<0.05). In 5.7% and 9.1% of patients adverse drug reactions led to dose modification of PDferon B® and RIBA respectively.**

Conclusions: **The genotype and age of patients are the only two independent factors influencing efficacy of treatment either as end treatment or sustained response. The particular brand of IFN in this study (PDferon B®) in combination with RIBA had comparable adverse effects with other reports and a somewhat higher rate end treatment and sustained responses.**

Keywords: **Chronic Hepatitis C, Interferon α, Ribavirin, Iran, Treatment, Adverse Drug Effects**

Introduction

The Hepatitis C Virus (HCV) is one of the leading known causes of chronic liver diseases including cirrhosis and hepatocellular carcinoma [1,2,3]. New human practices together with global expansion of the human population have increased its spread during last decades from about 100 million infected persons in 1997 [4] to prevalence rate averagely 3% worldwide resulting in about 210 million HCV-infected persons in 2002 [1,5]. Reports showed prevalence of HCV in Iran blood donors below 1% [6-13].

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Hep Mon 2006; 6 (1): 11-18

HCV has a positive-sense, single-stranded RNA genome that presents a high mutation rate (1.44 x 10-3 substitutions/site/year). HCV strains isolates worldwide have been classified into six different genotypes (1 to 6), each comprising multiple subtypes (designated as a, b, c, etc) [14-16].

The genotype determination is a relevant predictive parameter of response to antiviral treatment since genotype 1 is associated with a lower sustained virologic response (40 - 45%) compared to genotypes 2 and 3, whose sustained virologic response is 70-80% with peginterferon and Ribavirin combination therapy for 48 and 24 weeks, respectively [2,17]. In addition, the various genotypes have distinct geographical distributions. Although HCV genotypes 1, 2 and 3 appear to have a worldwide distribution, their relative prevalence varies from one geographic area to another. HCV genotype 4 is found in the Middle East and North Africa; and genotypes 5 and 6 in South Africa and Asia, respectively [14,18,19]. Recent reports from Tehran and five cities from different locations of Iran, showed that genotype 1a was predominant (47%), and 3a, 1b, and 4 were 36%, 8%, and 7%, respectively [20]. The patterns of our genotypes are similar to those of England, but different from other Middle-East countries such as Republic of Yemen, Kuwait, Iraq, and Saudi Arabia, where genotype 4 is the most prevalent [3, 21-24].

Genotype is also a factor in the period of time required to treat with current HCV medications. Generally, genotype 1 is treated for 48 weeks and genotype 2 and 3 are treated for 24 weeks. However, there are studies underway to determine the most optimal treatment duration based on certain factors. For instance, some experts believe that people with genotype 1, high viral load should be treated for 72 weeks instead of 48 weeks to maximize treatment response rates. There are also studies evaluating treating people with genotype 2 for 12 weeks and genotype 3 for 48 weeks [3,19].

Most risk factors associated with transmission of HCV included blood transfusion, injecting-drug use, employment in patient care or clinical laboratory work, exposure to a sex partner or household member who has had a history of hepatitis, exposure to multiple sex partners, and low socioeconomic level [25-31].

For a long time interferon alpha (IFN α) was the only unique effective treatment for chronic hepatitis C (CHC). But during recent years combination therapy of CHC patients with IFN α and Ribavirin (a synthetic nucleoside analogue) have yield better treatment and sustained responses [6,2]. Previous studies have reported that addition of RIBA to the standard treatment with interferon (IFN) alpha led to an improvement in sustained virologic response (SVR) from less than 20% with IFN monotherapy to 40-45% in combination therapy.

The aim of this study is to assess the therapeutic efficacy (as end treatment and sustained response) and safety of a particular brand of IFN alpha (PDferon B®) in combination with ribavirin (RIBA) on Iranian patients with CHC considering genotype characteristics of subjects.

Methods and Patients

Patients

Adult patients (18 years old and older) with chronic hepatitis C with or without a history of previous treatment with recombinant or lymphoblastoid IFN α (IFN α-2b, or IFN α-n1) were eligible for the study. The following were reasons for exclusion: decompensated cirrhosis, a hemoglobin concentration of less than 12 g per deciliter in women and less than 13 g per deciliter in men, a white-cell count of less than 3000 per cubic millimeter, a neutrophil count of less than 1500 per cubic millimeter, a platelet count of less than 100,000 per cubic millimeter, human immunodeficiency virus and/or hepatitis B virus co-infection, prior organ transplantation, severe psychiatric conditions, a seizure disorder, cardiovascular disease, renal insufficiency, hemoglobinopathy, hemophilia, poorly controlled diabetes mellitus, and immunologically mediated diseases.

Study Design and Treatment Regimens

The study was a quasi-experimental design, according to single group time series of HCV infected patients referred to Tehran Hepatitis Center (THC). The patients categorized regarding genotyping result in two main groups: group 1 with genotypes (1a, 1b, non-typable) receiving 48 weeks treatment regimen and group 2 with genotypes 2,3 (2a,3a,3b) receiving 24 weeks treatment regimen. The treatments consisted of 48 or 24 weeks of subcutaneous interferon alfa-2b (PDferon B®, Pooyesh Darou, Tehran, Iran) at a dose of 3 million units three times per week plus oral ribavirin, administered twice daily at a total daily dose of 1000 mg (for patients who weighed 75 kg or less) or 1200 mg (for those who weighed more than 75 kg). A total of 290 patients were screened, of whom 207 met the inclusion criteria and received the treatment regimen as described. Thirty one patients were lost

in follow up. Therefore 176 patients received the studied treatment regimen. The patients were evaluated after 1, 2, 4 and 8 weeks of treatment and then monthly thereafter and 12 and 24 weeks after the treatment was discontinued. All biochemical and hematological tests were performed in Tehran Blood Transfusion center and Keyvan laboratories in Tehran, Iran. Serum was collected and stored under conditions known to optimize the detection of HCV RNA [32]. Qualitative serum HCV RNA was measured before treatment, 12/24 weeks after the initial dose, at the end of treatment, and at the last follow-up visit 24 weeks after treatment stop by a qualitative reverse-transcription-polymerase-chain-reaction assay with a lower limit of detection of 100 copies per milliliter (Amplicore II, Roche).

The study was conducted between December 2002 and February 2006. The protocol was approved by the regulation of Islamic republic of Iran, regulation affair of Tehran University of medical sciences research deputy and all patients provided written informed consent.

Main outcomes

The primary outcome was disappearance of HCV RNA from serum and biochemical response defined as normal serum alanine aminotransferase (ALT) levels. In patients who have undetectable serum HCV RNA levels for 24 weeks after treatment, the concentrations remain undetectable indefinitely [33]. For consistency with other reports and current clinical practice, we also reported conventional end points as defined by the National Institutes of Health Consensus Development Conference on management of Hepatitis C [34]. These include a response at the end of treatment (defined as normal serum alanine aminotransferase concentrations and undetectable serum HCV RNA levels at the end of therapy) and a sustained response (defined as a response that persists for at least six months after treatment).

Liver-biopsy specimens obtained before treatment were interpreted by a single pathologist. The degree of hepatic inflammation and fibrosis were scored with the Knodell Histologic Activity Index [35] and the Metavir system [36] respectively. The inflammation score was obtained by combining the scores for the first three components of the Knodell index (portal, periportal, and lobular inflammation). The scores can range from 0 to 18, and higher scores indicate more severe abnormalities. Liver biopsy sample had histopathological confirmation of chronic hepatitis and abnormal serum aminotransferase concentration for at least 6 months before the start of protocol or normal enzymes while Knodell score is exceeding or equal to 4 and fibrosis stage is more than 2.

Treatment safety was assessed considering treatment related complication in different organ systems and patients' adherence and tolerance to treatment was evaluated considering discontinuing treatment before treatment end.

Statistical Analysis

The analysis was based on the 176 patients who received treatment using SPSS® 11.5 for windows software (SPSS Inc. Chicago, Illinois, USA). The demographic information, viral characteristics, and treatment responses for the all patients was recorded. The base-line characteristics of the patients were reported as frequency percentage. Treatment responses regarding genotype compared with use of the Cochran-Mantel-Haenszel test. The relatedness of various pretreatment characteristics to the response was examined using stepwise logistic-regression analysis. All statistical tests were two-tailed at the significance level of $P<0.05$.

Results

Characteristics of patients

Two distinct groups of patients included: 115 HCV genotype 1 (1a, 1b, nontypable) infected patients and 61 HCV genotype 2, 3 (2a, 3a, 3b) infected patients receiving the same treatment regiment for 48 and 24 weeks respectively (Table 1). The frequency of distinct HCV genotypes were 83, 29,2,57,2 and 3 patients for genotypes of 1a,1b,2a,3a,3b and non-typable respectively.

The patients with different genotypes matched well for baseline characteristics except for frequency of intravenous drug abuse (IVDA) and unsafe sex that were higher in patients with genotype 2,3 of HCV infection. All genotype 1 HCV infected patients with previous IFN therapy received 12 month standard duration while this duration was shorter for 2, 3 genotype HCV infected patients.

Qualitative serum HCV RNA

Serum HCV RNA levels became undetectable by the end of treatment in 134 of the 176 patients (76.1 percent), where it became undetectable by the end of treatment in 82 out of the 115 patients (71.3 percent) with genotype 1 HCV infection who were treated for 48 weeks with interferon and ribavirin and in 52 out of the 61 patients (85.2 percent) with genotype 2,3 HCV infection who were treated for

Table 1. Baseline characteristics of the patients†

Characteristic	total n=176	genotype 1 (1a,1b, nontypable) n=115	genotype 2,3 (2a,3a,3b) n=61	p.value
Sex, M/F	141/35	91/24	50/11	N.S.§
Age; years				
Mean±SE	38.9±0.9	40.8±1.2	38.3±1.4	N.S.
Range	18-76	18-76	19-62	
BMI‡; kg/m2	24.9±0.4	24.9±0.5	24.8±0.5	N.S.
Rout of diagnosis				
Blood Donation, n(%)	61 (34.7)	45 (39.1)	16 (26.2)	N.S.
Screening, n(%)	90 (51.1)	51 (44.3)	39 (63.9)	
Clinical Symptoms, n(%)	24 (13.6)	19 (16.5)	5 (8.2)	
Unknown, n(%)	1 (0.6)	0 (0)	1 (1.6)	
Previous Interferon therapy				
n(%)	17 (9.7)	10 (8.7)	7 (11.5)	N.S.
no response / relapse	10/7	6/4	4/3	N.S.
<12 mo duration, n(%)	5 (2.8)	0 (0)	5 (71.4)	
duration, mo	10.2±0.7	12±0.0	7.6±1.3	0.016
Transfusion Hx, n(%)	44 (25)	28 (25)	16 (26.7)	N.S.
IVDA£ n(%)	51 (29)	27 (23.6)	24 (39.3)	0.027
Hejamat¥ Hx n(%)	26 (14.8)	20 (17.4)	6 (9.8)	N.S.
Unsafe sex n(%)	54 (30.7)	29 (25.2)	25 (41)	0.031
smoking n(%)	83 (47.2)	48 (41.7)	35 (57.4)	0.048
cirrhosis n(%)	18 (10.2)	13 (11.3)	5 (8.2)	N.S.
Histological stage	2.3±0.15	2.2±0.15	2.7±0.3	N.S.
Histological grade	6.0±0.3	5.8±0.4	6.8±0.6	N.S.
Knodell score€	8.3±0.4	7.9±0.5	9.5±0.8	N.S.

†. Plus-minus values are means ±SE. P.values show comparision between genotype 1 and 2,3 patient Because of rounding, percentages may not total 100.
‡. Body mass index defined as weight as Kg devided by height square as meters.
§. Not Significant
£. Former or current intravenous drug abuse.
¥. Hejamat (Phlebotomy): A procedure in Iranian traditional medicine which is done by making shallow cuts by razor on the upper trunk and drawing blood.
€. Scores could range from 0 to 18, with higher scores indicating more severe abnormalities.

24 weeks with interferon and ribavirin (P=0.039). Among the patients in whom serum levels of HCV RNA were undetectable at the end of treatment, the levels became undetectable during the first 12 weeks of treatment in 117 out of the 134 patients (87.3 percent), which became undetectable in 74 out of the 82 (90.2 percent) and 50 out of the 52 (96.1 percent) in patients with genotype 1 and genotype 2,3 HCV infection respectively (P=0.013). Serum HCV RNA levels remained undetectable throughout the follow-up period for six months (defined as a sustained virologic response, SVR) in 110 patients (62.5 percent), which remained undetectable in 63 patients (54.8 percent) and 47 patients (77 percent) patients with genotype 1 and genotype 2,3 HCV infection respectively (P=0.004) (Table 2).

Table 2. Rates of End Treatment Virological (EVR) and Biochemical Response (EBR), Sustained Virologic Response (SVR) and Sustained Biochemical Response (SBR) to Treatment According to the HCV genotypes.

Characteristic	total n=176	genotype 1 (1a,1b, nontypable) n=115	genotype 2,3 (2a,3a,3b) n=61	p.value
EVR				
n(%)	134 (76.1)	82 (71.3)	52 (85.2)	0.039
response in 12 weeks, n(%)	124 (70.5)	74 (64.3)	50 (82)	0.013
response in 24 weeks, n(%)	------	81 (70.4)	------	------
EBR				
n(%)	147 (83.5)	95 (82.6)	52 (85.2)	N.S. §
response in 2 weeks, n(%)	102 (58)	61 (53)	41 (67.2)	N.S.
response in 4 weeks, n(%)	146 (83)	92 (80)	54 (88.5)	N.S.
response in 12 weeks, n(%)	152 (86.4)	100 (87)	52 (85.2)	N.S.
response in 24 weeks, n(%)	------	100 (87)	------	------
response in 36 weeks, n(%)	------	98 (83.2)	------	------
SBR				
n(%)	120 (68.2)	71 (61.7)	44 (80.3)	0.012
SVR				
n(%)	110 (62.5)	63 (54.8)	47 (77)	0.004

§. Not Significant

The response was more likely to be sustained in patients in whom serum HCV RNA levels became undetectable during the first 12 weeks of treatment than in those with a later response (79.7 percent vs. 9.8 percent in the genotype 1 group, 88 percent vs. 33 percent in the genotype 2,3 group) (P<0.001).

Biochemical response

Serum alanine aminotransferase concentration was normal by the end of treatment in 147 of the 176 patients (83.5 percent), which was normal in 95 patients (82.6 percent) and 52 patients (85.2 percent) patients with genotype 1 and genotype 2,3 HCV infection respectively (table 2). The serum alanine aminotransferase concentration remained normal throughout follow-up in 120 patients (68.2

percent), which remained normal in 71 patients (61.7 percent) and 44 patients (80.3 percent) patients with genotype 1 and genotype 2,3 HCV infection respectively (P=0.012).

Correlation between baseline characteristics and response

The HCV genotype grouped as 1 and 2,3 and age of patients influence the initial and sustained response to treatment specially the virologic responses but the other factors such as liver injury level (defined by existence of cirrhosis, Knodell score and pretreatment ALT level), BMI and risk factors such as IVDA, transfusion, Hejamat, unsafe sex had no significant effect on patients responses either in bivariate or multivariate analysis (table 3). There was also no significant differences between genotypes subgroups (i.e. 1a, 1b, 2a, 3a, 3b and non typable) overall and in each patients group regarding the initial and sustained response to treatment. Patient with previous interferon therapy had lower rate of end treatment virological response (58.8 percent in patients with interferon therapy vs. 78 percents in other patients) that was not statistically significant after adjustment for other factors such as genotype (P=0.078).

Table 3. correlation between baseline characteristics and responses to treatment as end treatment virologic response and sustained virologic response for six months after treatment.†

	End-Treatment Virologic Response; n (%)	Sustained virologic response; n (%)
number of subjects	134 (76.1)	110 (62.5)
Smoking		
yes	60 (72.3)	47 (56.6)
no	74(79.6)	63(67.7)
IVDA		
yes	41 (80.4)	37 (72.5)
no	93 (74.4)	73 (58.4)
Tranfusion		
yes	33 (75)	24 (54.5)
no	97 (75.8)	82 (64.1)
Hejamat		
yes	21 (80.8)	113 (75.3)
no	18 (69.2)	92 (61.3)
Unsafe sex		
yes	41 (75.9)	37 (68.3)
no	93 (76.2)	73 (59.8)
Cirrhosis		
yes	13 (72.2)	10 (55.6)
no	121 (76.6)	100 (63.3)
Previous interferon therapy		
yes	10 (58.8)	9 (52.9)
no	124 (78)	101 (63.5)

†. All comparisons are statistically not significant.

Adverse events

The adverse events, subjective and objective symptoms and laboratory tests results were evaluated. All adverse events were checked by the patients using questionnaires, double-checked, and recorded by the investigators. All adverse events were graded for severity based on the World Health Organization (WHO) criteria. General symptoms and signs were more frequent than other adverse events. In 143 patients (81.3%) there were adverse events related to treatment regimen and decrease in PDferon® and ribavirin doses was done in 10 and 16 patients (5.7% and 9.1%) respectively due to intolerance or severe adverse events, where just 10 patients have excluded from study due to adverse event induced intolerance. A total of 286 adverse events were recorded during the treatment period (averagely 1.6 adverse events for each patient). The most frequent adverse events were general symptoms (59.7% of all patients) including Flu like syndrome (48.8%), fatigue (21.6%), fever (19.3%), myalgia (2.3%), weakness (0.5%) (table 4).

Table 4. Episodes of Different Adverse Events Types categorized by body system in patients with chronic HCV infection receiving PDferon® and Ribavirin combination therapy.

Type of Adverse evnets	Frequency of adverse events	Percent of patients with adverse events
General	105	59.7%
GI	28	15.9%
Nervous	61	34.7%
Hematologic	6	3.4%
Cardiopulmonary	21	11.9%
Endocrine	8	4.5%
Skin	53	30.1%
Musculoskeletal	3	1.7%
Urology	1	0.6%
Total	286	162.5%

Total Number Of 176 patients showed 286 adverse events. Some patients showed more than one adverse events while there was no adverse events for 36 patients (18.7%).

Discussion

In about 40 percent of patients with chronic HCV infection interferon therapy alone may result in biochemical and virological responses but the main problem is that the majority of patients relapse shortly after treatment is stopped [15,37]. However

the higher doses of interferon or longer treatment period may result in further responses in relapsed patients but this regimen is costly and poorly tolerated[37]. Our study showed that the combination therapy of IFN and ribavirin in patients with either previous IFN therapy or new diagnoses chronic HCV infection results in favorable response at the end of treatment course and sustained response for six months. Despite the small sample of patients with no response to or relapse after previous interferon therapy, our findings showed no differences in response rate between these patients and patients initially treated with combination regimen. End treatment response to IFN-ribavirin combination therapy in our study patients with previous IFN therapy was lower than reports from other studies but sustained response was similar [15,17,38]. An open-label pilot study investigating the safety and tolerability of the combination therapy of PDferon B® and Ribavirin in 40 patients with CHC infection was done in Tehran University of medical sciences; Shariati hospital and THC in 2001-2002. Preliminary efficacy data had showed that 60% of patients had undetectable HCV RNA (<100 copies/ml) at the end of treatment [39-40].

The HCV genotype was strongly associated with the response to combination therapy. The genotype and age of patients are the only two independents factors influencing efficacy of treatment either as end treatment or sustained response. These findings are also reported is other studies [15, 19, 34, 41-42]. Older patients are less likely to response to treatment and are less likely to have sustained responses regardless of genotype and other baseline characteristics [42].

Most recent studies, performed in large numbers of naïve patients and relapsers after previous interferon therapy, have shown that combination therapy significantly increases the percentage of biological and virological sustained responses by reducing the rate of relapse after treatment discontinuation [15,38]. The rate of end treatment virologic and biochemical responses to combination therapy for 24-48 weeks varies between 52-90% and 55-76% respectively [15,17,38,43-46]. Our study showed the end treatment virologic and biochemical responses as 76.1% and 83.5% respectively; to somewhat higher rate of responses comparing to some of similar studies. The reason for this result may be related to different characteristics of HCV infection in Iranian patients or even the particular brand of interferon used, but these interpretations must to be evaluated in double blinded controlled trials. The mechanisms that might explain a lower rate of relapse (about 37.5% in our study) are not fully understood, but may ribavirin/interferon combination, may have a synergistic antiviral effect. Our study also demonstrated that BMI, patient's gender and severity of liver injury or fibrosis had no significant effect on patients virologic response to combination therapy.

In summary, the combination of interferon and ribavirin is effective for the treatment of patients with chronic hepatitis C regardless of previous IFN therapy status. Combination therapy offers a striking advantage over interferon monotherapy: it has a much higher rate of sustained response in comparison to higher doses or longer courses of IFN monotherapy [47-48].

The incidence of adverse events associated with PDferon B® was very similar to rates reported for other alpha interferon preparations and it seems that they were not constant. The most common adverse events were flu-like symptoms. Psychiatric disorders such as nervousness, anxiety, and depression also were frequent. Thyroid disorders, including hypothyroidism and hyperthyroidism, also were observed. Dose modification due to intolerable adverse events occurred in 13.1% of patients. Discontinuation of therapy was comparable or even lower than similar reports of 16.6%-31% dose modification rate due to drug related adverse events [43,49].

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