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Original article

Histological improvement in chronic hepatitis C patients treated with combination of Alpha-Interferon and ribavirin who failed previously to respond to interferon monotherapy

N.C. Tassopoulos[1], G.D. Anagnostopoulos[1], Johanna Delladestima[2], Paraskevi Angelopoulou[1], Vana Sypsa[3], A.E. Hantzakis[3]

SUMMARY

***Background/Aim*: Half of the patients infected with hepatitis C virus (HCV) show no response to alpha-interferon (a-IFN) and no alternative modality has so far proven to be effective. Combination of a-IFN with ribavirin has shown promising results in naive patients and relapsers. The aim of the present study was to assess the sustained virological and histological response after combination in 20 chronic hepatitis patients, non-responders (NR) to a previous a-IFN monotherapy. *Methods*: Twenty NR patients were treated with a-IFN (3MU three times a week subcutaneously) and ribavirin (1000-1200 mg daily peros) for 48 weeks. Serum levels of HCVRNA and ALT levels were tested at the end of treatment and 24 weeks post-treatment while histological evaluation was done at the end of follow-up. *Results*: Genotype was 1a in 2, 1b in 11, 3a in 5 and 4 in 2 patients. Serum ALT levels normalized in 14 (70%) and HCVRNA became seronegative in 8 (40%) patients by the end of treatment. However, six months posttreatment, ALT levels remained normal in 4(20%) non-responder patients. Serum HCVRNA was positive in 15 and remained negative in 5 (25%) sustained responder patients. Sustained response rate was 0% in genotype 1, 60% in genotype 3a and 100% in genotype 4. Necroinflammatory activity improved in 68,4%, showed no change in 26,3% and worsened in 5,3% cases (ranked assessment). Treatment was well tolerated and ribavirin was decreased in 5 (25%) patients. *Conclusions*: Combination treatment was well tolerated and the sustained response rate was 25%, being higher particularly in the non-1 genotype patients. Induction therapy with a-IFN in combination with ribavirin should be further evaluated in this group of patients.**

Key Words: Chronic hepatitis C, A-interferon, Cirrhosis, Ribavirin

[1]First Dept of Medicine, Western Attica General Hospital Athens, Greece, [2]Laboratory of Pathology, Laikon General Hospital, Athens, Greece, [3]Department of Hygiene and Epidemiology, Athens University Medical School, Athens, Greece

Author for correspondence:

Nicholas C. Tassopoulos, MD, Liver Unit, First Dept of Medicine, Western Attica General Hospital, 1 Dodecanissou str., 123 51 Athens, Greece, Tel.: 00301-6844046, Fax: 00301-5301307, e-mail: ntass53@otenet.gr

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Alpha-interferon (a-IFN) has been shown to induce a sustained biochemical response in approximately 10% to 20% of chronic hepatitis C (CHC) patients treated for 24 weeks.[1-4] A high rate of relapse, however, after initial biochemical and virological response has been shown in approximately half of the CHC patients after discontinuation of treatment. Consequently, a small subset (10-15%) of CHC patients had sustained biochemical and virological response to a-IFN monotherapy.[1-4] Moreover, a remarkable proportion of patients (50%) do not respond to treatment at all. This group of patients is considered to be non-responders (NR) to a-IFN monotherapy. Ribavirin, a synthetic nucleoside analogue, exhibits a broad spectrum of antiviral activity against a range of DNA and RNA viruses. Ribavirin monotherapy has been shown to decrease serum alanine aminotransferase

(ALT) levels and, to some degree, improve histology, but it has no effect on serum HCVRNA levels.[6-8] The combination of ribavirin with a-IFN has been proven to increase the sustained virological response (SR) rate in either naive or relapsed patients in several large-scale randomized controlled trials. The mechanism by which this enhancement of activity occurs in the combination regimen is unknown. The aim of this study was to evaluate the efficacy and tolerance of a-IFN in combination with ribavirin in NR patients to a-IFN monotherapy.

PATIENTS AND METHODS

Twenty adult patients (13 males, 7 females) were included in the study (Table 1). Non-response to the first a-IFN monotherapy was defined as the persistence of at least 3 abnormal ALT levels and detectable serum HCVRNA by polymerase chain reaction (PCR) during a course of a-IFN for at least 12 weeks. The mean age of the patients was 44.0±11,9 (26-63) years; twelve (60%) aged above 40 years. The possible source of infection was blood transfusion in 3 (15%), parenteral drug abuse in 4 (20%) and unknown in 13 (65%) patients. Serum ALT levels were 117±39 (range: 75-199) IU/L.

All patients were treated with a combination of a-IFN (Intron-A, Schering Plough) at a standard dose of 3MU three times a week subcutaneously and ribavirin (Rebetol, Schering Plough) at a daily dosage of 1000-1200 mg according to the body weight (≤ or ≥ 75 kg respectively) for 48 weeks.

Inclusion criteria were persistently elevated serum ALT levels (as defined by at least 3 abnormal values during the treatment and three abnormal values after the first course of a-IFN), presence of HCVRNA by PCR and biopsy-proven chronic hepatitis. Exclusion criteria were HBsAg and antibodies to human immunodeficiency virus positivity, autoimmune hepatitis, chronic alcoholism, use of hepatotoxic drugs in the last six months.

Serum antibodies to HCV (anti-HCV) were detected with the second generation HCV enzyme-linked immunosorbent assay (ELISA 2, Abbott Diagnostics).

Table 1. Baseline characteristics of the patients with chronic hepatitis C non responders to a previous course of α-IFN alone.

#	Gender	Age	**Genotype**	**HCVRNA*** **(Meq/ml)**	**ALT*** **(IU/L)**	**Necroinflammatory activity**
1	F	62	1b	1,9	86	8
2	M	26	4c/d	ND	75	4
3	M	38	1b	11,9	199	6
4	M	53	1b	0,6	129	6
5	M	37	1b	1	157	5
6	F	48	1b	1,4	78	11
7	M	57	1b	ND	106	7
8	M	58	1b	19,1	92	7
9	M	36	1a	0,3	78	7
10	M	26	1a	11,8	100	8
11	M	33	3a	5,6	124	4
12	M	33	3a	5,8	168	7
13	F	43	3a	2,3	89	4
14	F	51	1b	ND	80	8
15	F	45	1b	0,3	139	4
16	M	37	3a	27,8	97	7
17	M	35	4h	ND	128	6
18	M	49	3a	ND	94	10
19	F	49	1b	3,1	177	8
20	F	63	1b	15,6	141	7

ND denotes not detectable.

*The cut off for HCVRNA was 0.2 Meq/ml and the upper limit of normal ALT 40 IU/L.

Qualitative detection of serum HCVRNA was done by RT-PCR (Amplicor HCV, Roche Diagnostics Systems, 1st gen.). Quantitative detection of serum HCVRNA was done by a branched-DNA technology (Quantiplex HCVRNA 2.0, Chiron corp.). HCV genotype was performed by a reverse hybridization assay (InnoLiPa, Innogenetics).

Histological evaluation of pretreatment in all patients and post-treatment liver biopsies in 19 patients were performed according to the Knodell Histologic Activity Index (HAI).[13] This allocates a numerical score for the histological activity of hepatitis on a scale of 0 to 22, with higher scores indicating more severe abnormalities. The overall Knodell score is the sum of the scores for periportal bridging necrosis (0-10), intralobular degeneration and focal necrosis (0-4), portal inflammation (0-4), and fibrosis (0-4). Response rates were based on necroinflammatory activity, i.e. the sum of the first three components of the score. Histological response was defined as a ≥ 2-point reduction in the necro-inflammatory score between the pre-treatment and the post-treatment biopsies. Using a ≥ 2-point reduction in the necro-inflammatory score as the response definition should minimize the impact of the 1-point variation, which would be anticipated owing to observer variability and liver sampling.[14] Fibrosis and necro-inflammatory activity were also compared for each biopsy pair (blinded for sequence) to assess whether one showed more severe hepatitis and/or more fibrosis (ranked response).

The primary efficacy end point was defined as loss of detectable serum HCVRNA at week 24 post-treatment (sustained virological response, SR).

RESULTS

All patients were HCVRNA seropositive by qualitative PCR, while 5 (25%) were seronegative by quantitative bDNA (cut off 2×10^5 copies/ml). Serum HCVRNA was above 2×10^6 eq/ml in 9 (45%) and below 2×10^6 eq/ml in 6 (30%) patients. HCVRNA ranged from 0,2 to $27,8\times10^6$ eq/ml. Genotype 1a was detected in 2, 1b in 11, 3a in 5 and 4 in 2 patients. High viral load ($>2\times10^6$ eq/ml) was found in 1 genotype 1a, 4 genotype 1b and 4 genotype 3a patients. The serum ALT levels were 116.8±39.4 (75-199) IU/L.

At the end of treatment ALT became normal in 14 (70%) and remained abnormal in the remaining 6 patients. The abnormal serum ALT levels ranged from 50 to 168 IU/L and were <1.5×ULN in 3 of the 6 non-biochemical responders.The mean ALT level was 45,8±46,3 (range10-168) IU/L. Qualitative serum HCVRNA became negative in 8 (40%) and remained positive in 12 (60%) patients. Quantitative HVCRNA was positive in 9 (45%) patients; serum HCVRNA was $>2\times10^6$ eq/ml in 3 and $<2\times10^6$ eq/ml in 6 patients. Six months post-treatment, 3 of the 8 HCVRNA-negative patients at the end of treatment relapsed and the SR was 25% (5/20). Serum ALT levels also increased in 11 (55%) and remained normal in 4 (20%) non-responder (NR) patients. The 5 SR patients had normal liver enzymes. Consequently, the sustained biochemical response was 45% (9 of 20 patients) (Table 2).

At the end of treatment, virological response was in genotype 1a: 0 of 2, 1b: 3 (27,3%) of 11, 3a: 3 (60%) of 5, and 4: 2 (100%) of 2 patients. Thus, SR was significantly higher in patients with genotype non-1 than in patients with genotype 1 (5/7 or 71% vs. 0/13 or 0%, p=0.001). Serum HCVRNA reappeared in the 3 genotype 1b patients and consequently the SR in genotype 1b was 0 of 11 patients.

Baseline liver histology showed that the mean score of necroinflammatory activity was 6,7±1,9 (range 4-11) and the stage 3.0±2,1 (range 0-4). Three (15%) had cirrhosis (stage 4) and 3 (15%) patients incipient cirrhosis (bringing fibrosis, stage 3). The posttreatment mean score of necroinflammatory activity was 3,9±1,9 (1-7) (p<0.001) and the stage 2,5±2,2 (0-4). In the ranked assessment of necroinflammatory activity between the pretreatment and posttreatment slide pairs 63% (12 of 19) improved, 31% (6 of 19) showed no change and 5% (1 of 19) worsened. In particular, necroinflammatory activity improved in 80% (4 of 5) SR, 100% (2of 2) relapsers and 50% (6 of 12) NR, remained unchanged in 20% (1 of 5) SR and 42% (5 of 12) NR and worsened in 8% (1 of 12) NR patients (Figure 1). The ranked assessment of fibrosis showed that 95% (18 of 19) had no change in fibrosis and only 5% (1 of 19) of the patients showed a remarkable improvement (from stage 3 to stage 1).

Both drugs doses were decreased in three patients

Table 2. End of treatment (EOT) and sustained response (SP) in 20 chronic hepatitis C patients who failed previously to respond to IFN monotherapy

	Biochemical		Virological	
	No.	%	No.	%
EOT	14	70.0	8	40.0
SR	9*	45.0	5	25.0

*4 were non-responders and 5 patients were sustained responders

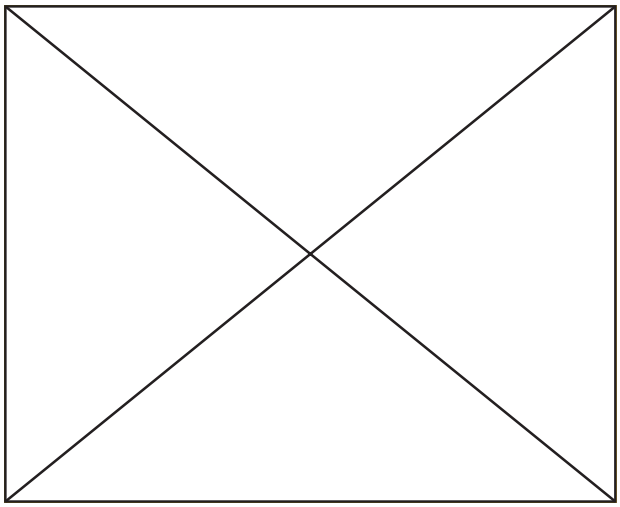

Figure 1. In the ranked assessment of necroinflammatory activity between pretreatment and posttreatment slide pairs 63% (12 of 19) improved, 31% (6 of 19) showed no change and 5% (1 of 19) worsened. SR denotes sustained responder REL, relapsers and NR, non-responder patients. By subgroup of patients improvement was found in 80% (4 of 5) SR, 100% (2 of 2) REL and 50% (6 of 12) NR, and worsening in 8% (1 of 12) NR patients.

while the ribavirin dose was decreased in two additional patients. Hemoglobin (Hb) decreased by 1,0 to 3,9 gr/dl in 19 (95%) patients. In particular, Hb decreased less or equal to 2 gr/dl in 10 (50.0%), 2,1-3 gr/dl in 8 (40.0%) and 3,9 gr/dl in 1 (5%) of the treated patients. In total, ribavirin decreased in 5 (25%) treated patients.

DISCUSSION

The combination therapy with a-IFN and ribavirin for either 24 or 48 weeks has been found to be superior to therapy with α-IFN alone with respect to biochemical, virological and histological end points.[10,11] Logistic regression analysis identified five independent factors significantly associated with a favorable response: genotype 2 or 3, viral load less than $2X10^6$ copies/ml, age 40 years or less, minimal fibrosis stage and female sex.[10] The mechanism of the apparently synergistic antiviral effect achieved by the combination of a-IFN and ribavirin is not well characterized, but ribavirin has been postulated to inhibit viral-dependent RNA polymerase and inosine monophosphate dehydrogenase.[5] However, other immunomodulatory actions may also contribute to the drug's beneficial effects.[15]

Our current study suggests that there may be a beneficial effect of a-IFN combined with ribavirin in these difficult to treat NR to a-IFN monotherapy patients. Thirty percent of our patients had cirrhosis or extensive fibrosis, an ominous prognostic factor in the treatment of CHC patients. However, the SR rate was 71% in patients with non-1 HCV genotype similar to that observed in naive and relapsed patients.[10-12] All the SR patients were males, genotype 3a or 4 and two of them had a high ($>2\times10^6$ eq/ml) viral load, an unfavorable prognostic factor in CHC patients.[10] The 3 relapsed patients had genotype 1b and viremia was high in one of them. Overall, the SR was 0% in genotype 1 patients confirming its unfavorable effect in this subgroup of patients. Similar results have been published by Schvarcz et al.[16] A meta-analysis of nine controlled trials (789 patients) showed that the number needed to treat was 14 suggesting that approximately 14 patients would need to be treated with 6 months of combination therapy for 1 patient to have a SR.[17] However, the 25% SR who were on our cohort (65% genotype 1) with α-2b interferon and ribavirin appear to be higher than the less than 20% in the non-responsive subgroup in the metanalysis of randomized trials.[18]

Liver histology showed significant improvement of the necroinflammatory activity in 63% and worsening in only 5% of the patients ($p<0.001$). Consequently, the combined regimen not only had a SR rate of 25% but also delayed the progression of the necroinflammatory activity. The combination therapy of a-IFN and ribavirin was well tolerated and no serious adverse events were encountered. Hemolytic anemia was rapidly reversible in the five patients whose was decreased.

These data, in association with the low toxicity of ribavirin, appears to promise better results for these patients. Induction therapy with a-IFN combined with ribavirin should be further evaluated, particularly in genotype 1 patients, irrespective of sex, age and viral load.

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