Combination thymosin α_1 and lymphoblastoid interferon treatment in chronic hepatitis C

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Abstract

***Background*—Monotherapy for chronic hepatitis C using interferon (IFN) results in a very small proportion of patients exhibiting a sustained response. Clinical trials assessing the benefit of combination drug therapy may provide evidence of improved treatment response over that seen with single drug treatment.**

***Aim*—To assess the response in patients with chronic hepatitis C to one year of combination treatment: thymosin α_1 ($T\alpha_1$), 1 mg twice weekly, and lymphoblastoid (L)-IFN, 3 MU thrice weekly.**

***Patients and Methods*—Fifteen patients with serum HCV RNA positive chronic hepatitis C were studied. Eleven patients were treatment naive and four had failed previous standard IFN therapy. Thirteen patients were HCV RNA serotype 1b. All patients were given combination $T\alpha_1$ and L-IFN therapy for one year with a six month follow up period.**

***Results*—Six months after initiation of treatment seven patients (47%) were sera HCV RNA negative and at completion of the one year treatment 11 (73%), including two who had failed previous standard IFN treatment, had negative serum HCV RNA. Six months after treatment, six patients (40%), including five with HCV type 1b, showed a sustained response characterised by a negative serum HCV RNA.**

***Conclusions*—The results of this open label trial suggest that there may be a potential benefit to combining an immune modulator ($T\alpha_1$) with an antiviral (IFN) in the treatment of chronic hepatitis C. Verification of the observations in this study require completion of a randomised controlled study.**

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Keywords: chronic hepatitis, HCV RNA, thymosin α_1, interferon, combination treatment.

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Most subjects acutely infected with the HCV will develop a persistent infection, with nearly 70% exhibiting a chronic necroinflammatory process in the liver.[1 2] Chronic hepatitis C is also associated with a significant risk for the development of cirrhosis and hepatocellular carcinoma.[3]

Standard treatment for chronic hepatitis C consists of interferon (IFN) α_{2b} at a dose of 3 MU thrice weekly for six months. Although approximately half of patients show an initial response to treatment with a decrease in alanine transaminase (ALT) values to normal or near normal levels and an improvement in liver histology, the majority of these initial responders will experience relapse of disease.[4–6] The response to IFN seen in patients with HCV genotype 1b (HCV 1b) is more dismal with 20–37% of patients experiencing an initial response and only 6–17% demonstrating a sustained response.[7–9]

Different treatment strategies using IFN have been devised to improve response rates including longterm treatment,[8–11] higher or escalating doses,[6 12 13] and combination therapy with other antiviral[14] or anticholestatic drugs.[15 16]

Thymosin α_1 ($T\alpha_1$) is a 28 amino acid peptide that has been shown to exert a modifying influence on a variety of cellular and humoral immune responses both in vitro and in vivo.[17] The effect of $T\alpha_1$ used as single drug therapy in the treatment of chronic hepatitis C is controversial. A pilot trial in which $T\alpha_1$ was used as single drug therapy for six months in 10 patients with chronic hepatitis C did not show any treatment benefit,[18] whereas a group of six patients unable to undergo standard IFN treatment administered $T\alpha_1$ for 12 months resulted in a sustained response in three patients (G Rasi, unpublished observations).

A clinical trial is in progress assessing the effect of six months treatment in a randomised controlled study with three arms: combination $T\alpha_1$ and IFNα_{2b}, IFNα_{2b} alone, or placebo.[19] Preliminary reports from this study suggest that there may be an additive effect of $T\alpha_1$ in decreasing HCV RNA titres during the early phases of treatment.[19]

The objective of this open label study was to determine the response to combination $T\alpha_1$ and lymphoblastoid IFNα (L-IFN) therapy in patients with chronic hepatitis C. The treatment regimen, unlike similar trials,[19] called for administration of a loading dose of $T\alpha_1$ prior to initiation of L-IFN followed by a maintenance schedule for both drugs for a total of 12 months. In addition, correlation of treatment response to HCV 1b and histopathological findings was investigated.

Methods

Patients

Patients with confirmed chronic hepatitis C for a minimum of one year underwent pretreatment screening. Candidates were required to have biopsy confirmed chronic hepatitis within

one year of inclusion, raised ALT values no less than 1·5 times the upper normal limit, positive serum HCV RNA by PCR, and compensated liver disease. Additional inclusion criteria included albumin levels ≥3·0 g/dl, total bilirubin ≤1·5 mg/dl, and prothrombin time less than 4·5 seconds prolonged over control values. Patients were excluded when the haemoglobin was ≤10 g/dl, platelet count ≤50 000 per ml, white cell count ≤3000/mm^3, and granulocyte count ≤1500/mm^3. Other exclusion criteria were a recent history (two years) of intravenous drug abuse, alcohol consumption in excess of 80 g/day within the preceding five years, presence of HBsAg or antibody to HIV, other causes of liver disease, creatinine >2·5 mg/dl, malignancy, autoimmune diseases, poorly controlled insulin dependent diabetes mellitus, haemophilia, cardiac decompensation, or other illnesses that might preclude completion of the study. Candidates must not have received immunosuppressive or antiviral therapy within 12 months of inclusion.

The majority of patients evaluated for entry into the study had not received any previous treatment. Four of 15 patients entered into the study phase had initially undergone six months' treatment with IFNα_{2b} (3 MU thrice weekly) without responding. After a six month follow up, they were enrolled in a 12 month treatment programme using IFNα_{2b} (3 MU thrice weekly) and once again, failed to respond. These patients were considered treatment non-responders on the basis of persistent increases in ALT values (≥1·5 ULN) at the conclusion of the second IFN treatment. They were followed up for an additional six months before entering a six month screening phase in the current study. Candidates who were IFN naive before entry were screened for three months. Of the 17 patients screened, two were excluded for non-compliance with the screening protocol and removed from the study before treatment initiation. Both patients were IFN naive.

TREATMENT PROTOCOL

In this open label study, patients were given combination L-IFN (Wellferon, Welcome Research Laboratories, Beckenham, UK) and synthetic Tα_1 (Sclavo SpA, Siena, Italy). A loading dose, maintenance dose regimen was used. Patients received Tα_1 (1·0 mg) by subcutaneous (SC) injection daily for four days followed by the first L-IFN injection (IM) on the evening of the fourth day. Beginning with the second week, and for the subsequent 51 weeks, patients injected themselves with Tα_1 on each Monday and Thursday morning. L-IFN (3 MU) was self administered on Mondays, Wednesdays, and Fridays in the evening. Patients were followed up for six months after completion of the one year treatment period (18 months total). Patients were seen monthly during treatment and every three months during the follow up period. Clinical and laboratory assessments were accomplished on each visit and included serum ALT, complete blood counts (WBC, PMN, lymphocytes, platelets) prothrombin time, and urine analysis. PCR determination for serum HCV RNA was done at inclusion, six months, 12 months, and at six months after completion of treatment (18 months follow up). Assay for antibody to IFNα was accomplished at the start of screening in the four patients previously treated with IFN (all were negative). A response to treatment was defined at 12 months as a negative serum HCV RNA. A sustained response was determined as a negative serum HCV RNA six months after completion of treatment.

This study was approved by the Comitato Etico, University of Rome, Tor Vergate, Italy, and informed written consent was obtained from all patients.

Biochemical, serological, and immunological evaluation

All laboratory studies were conducted at a single institution. Anti-HCV was initially tested by ELISA (Abbott Laboratories, North Chicago, IL) followed by RIBA II (Ortho Diagnostics). Serum HCV RNA was detected by nested PCR reverse transcription using primers derived from the 5'UTR of the HCV.[20] Genotype identification was accomplished on specific second round PCR products of the 5'UTR as previously described.[20]

Liver biopsy

Percutaneous liver biopsy specimens were obtained within one year of initiating treatment in all patients and within two months of completion of the injections in 13 of 15 patients. Thus 13 paired samples were available for analysis. Liver biopsy specimens were reviewed under code by a single pathologist (M Amini) who graded the specimens according to the Knodell histological activity index (HAI).[21]

Statistical analysis

Changes in laboratory values, and Knodell HAI scores were compared by Student's two tailed *t* test and Student's two tailed paired *t* test.

Results

All 15 patients completed the 12 month treatment period and six month follow up. Table I shows the clinical, biochemical, and serological characteristics at inclusion. All patients were heterosexual, two had a previous history of intravenous drug abuse, three had received infusion of blood products in the past. Nine of 10 patients with community acquired disease reported previous extensive dental procedures. The duration of illness was determined as the estimate of the time interval between either an episode of a non-A non-B acute hepatitis, the recognition of increased aminotransferase, or identification of high risk exposure. Six months after starting combination therapy seven

TABLE I *Patient characteristics at initiation of treatment*

Characteristic	*Treatment group*
Number	15
Male:female	7:8
Age (years)	47 (15)* (22–68)†
Duration (years)	3·3 (2·8) (1–13)
ALT (IU/L)‡	163 (56) (95–275)
Total bilirubin (mg/dl)	0·9 (0·4) (0·6–1·3)
Albumin (g/dl)	3·3 (0·5) (3·0–5·0)
Prothrombin time (s)	11·5 (0·4) (10·0–13·4)
Histological findings:§	
CAH	9
AC	6
HCV Genotype	
1b	13
3a	1
4	1

*Values are means (SD). †Range. ‡Normal values: ALT <48 IU/l, total. bilirubin <1·5 mg/dl, albumin 3·5–5·2 g/dl. §CAH, chronic active hepatitis; AC, active cirrhosis.

TABLE III *HCV genotype, histological findings, and response to combination therapy*

Patient	*Genotype*	*Initial liver biopsy findings**	*HCV-RNA (12 months)*	*HCV-RNA (18 months)*
1	1b	CAH	–	+
2	1b	CAH	+	+
3	1b	CAH	–	–
4	1b	CAH	–	–
5	1b	CAH	–	–
6†	1b	CAH	–	+
7	1b	CAH	–	+
8	1b	CAH	–	+
9†	1b	CAH	–	–
10	1b	AC	–	–
11†	1b	AC	+	+
12†	1b	AC	+	+
13	1b	AC	+	+
14	3a	AC	–	–
15	4	AC	–	+

*CAH, chronic active hepatitis; AC, active cirrhosis.
†Previous treatment failures with IFN.

patients (47%), including two who failed previous IFN treatment, became serum HCV RNA negative. At the completion of the 12 month treatment, 11 patients (73%), including two who failed earlier IFN therapy, responded to treatment with negative serum HCV RNA. A sustained response was observed in six (40%) of the responders, including one patient who had failed previous IFN treatment (Table II). Normalisation of ALT values was achieved in the majority of patients responding to treatment (Table II).

Three of six patients with active cirrhosis on initial liver biopsy were responders at completion of treatment with two showing a sustained response after six months of follow up. Eight of nine patients (89%) with chronic active hepatitis responded to treatment, with four relapsing during the six month follow up. Table III shows the relation between HCV genotype, histological findings, and treatment response. Nine of 13 patients (69%) with HCV 1b responded to treatment, with five (39%) demonstrating a sustained response. One of four patients with active cirrhosis and HCV 1b had a sustained response to treatment.

Paired liver biopsy specimens were available for 13 patients. The Knodell HAI was calculated to include or omit fibrosis from the score. Patients with a sustained response showed significant improvement in the HAI score after treatment. No significant improvement was seen in the group of non-responders/relapsers (Table IV).

No significant adverse events were identified with combination treatment other than mild influenza-like symptoms associated temporally with the L-IFN injections. No patients required dose reduction or withdrawal from treatment.

TABLE II *Response to combination therapy*

	Treatment group (n=15)		*Previous IFN treatment failures (n=4)*	
Time interval (months)	*HCV RNA (–)*	*HCV RNA(–) with normal ALT*	*HCV RNA(–)*	*HCV RNA(–) with normal ALT*
6	7 (47)*	5 (33)	2 (50)	2 (50)
12	11 (73)	8 (53)	2 (50)	1 (25)
18	6 (40)	5 (33)	1 (25)	1 (25)

*Percentage of total.

Discussion

The most striking observation in this study was the high rate of response to combination therapy (nine of 13 patients, 69%) as well as the sustained response in patients infected with HCV 1b (five of 13 patients, 39%). When all genotypes are considered, the response rate was 73% with a sustained response of 40%. Furthermore, two of six patients with active cirrhosis demonstrated a sustained response to treatment. Two of four patients who had previously failed a total of 18 months IFNα_{2b} treatment responded to the combination therapy, with one patient (HCV 1b) maintaining a sustained response. In future studies patients with sustained response would require follow up beyond the six months after treatment period to determine if the response is truly sustained or if a relapse has merely been delayed.

HCV 1b is more likely to be associated with cirrhosis, hepatocellular carcinoma, and lack of response to standard or escalating doses of IFN.[13 22–25] Both genotype and viral load at initiation of IFN therapy are predictors of treatment response, and are independent variables,[8] but HCV RNA levels may be the more important factor predictive of IFN response.[26]

The sustained response in patients given standard IFN treatment (3 MU, thrice weekly for six months) and followed up for at least six months is much lower with HCV 1b than it is for other HCV genotypes (reviewed in 27). In one study, IFN therapy increased the rate of sustained response in HCV 1b infected

TABLE IV *Histological score of liver biopsy specimens before and after treatment using the HAI**

Specimen	*Total group (n=13)*	*Sustained responders (n=5)*	*Non-responders (n=8)*
Inclusion			
Total score	11·2 (2·8)†	10·8 (4·0)†	11·5 (2·1)
Excluding fibrosis	9·5 (2·1)‡	9·4 (2·7)†	9·6 (1·8)
Post-treatment			
Total score	8·0 (3·7)	6·2 (3·0)	9·1 (3·8)
Excluding fibrosis	6·5 (2·9)	4·8 (1·9)	7·5 (3·0)

*HAI, histological activity index, reference 21; The score for the extent of necroinflammation alone is provided by subtracting the score for fibrosis. Data shown as mean (SD).
†$p<0·05$ compared with corresponding post-treatment value.
‡$p<0·01$ compared with corresponding post-treatment value.

patients from the 27% observed in patients treated for 28 weeks, to 39% after one year of treatment.[23] In the latter study, however, patients were given higher doses of IFN (5 MU, thrice weekly). Furthermore, a sustained response was predicated on maintenance of normal ALT values. Serum HCV RNA status at the conclusion of the follow up period was not provided.[23]

Using ALT normalisation as an indicator of response, patients with chronic hepatitis C treated with IFNα_{2b} for 60 weeks (3 MU, thrice weekly) showed response to therapy at treatment end in 24 of 40 patients (60%) with a sustained response 24 weeks after treatment in 15 (38%). All sustained responders had also cleared serum HCV RNA. However, HCV genotypes were not provided.[11] In additional studies, a virological sustained response to IFNα_{2b} in 61 patients 12 months after a 12 month treatment period of 3 MU thrice weekly was 16%.[9]

Immune mechanisms are believed to play a part in HCV mediated hepatocellular damage.[28] These mechanisms include HCV specific cytotoxic T cells requiring HLA class I recognition and perhaps natural killer cells.[28-30] Furthermore, increased levels of cytokines including TNFα, TNFB, and IFNγ have been described in the livers of patients with chronic hepatitis C and may play a part in liver injury.[31] Tα_1 is present in and secreted by lymphocytes and therefore can be designated a cytokine. This peptide may be a component of the cytokine cascade that is influenced by IFNα and in turn may serve to promote IFNα effects.[31-33]

Thymic extracts and isolated peptides including thymostimulin, thymic factor X, and thymic humoral factor γ_2 (THFγ_2) have been used to treat patients with chronic hepatitis B or C.[17 34 35] Although there is no homology between the 28 amino acid sequence of Tα_1 and the octapeptide THFγ2,[17 36] these peptides share immunomodulatory activities.[37] Furthermore both Tα_1 and THFγ_2 in combination with IFNα appear more effective in the treatment of chronic hepatitis B than IFNα when given as monotherapy.[34 38]

A loading dose of Tα_1 was given for four days before the first L-IFN injection followed by a maintenance schedule for both Tα_1 and L-IFN. This regimen is similar to that used in the application of chemotherapeutic agents. One of several drugs is given first with the objective of enhancing conditions for promoting the efficacy of a second drug given at a later time. We have previously shown that Tα_1 increases in vitro production of a variety of cytokines by activated lymphocytes including IFNα and IFNγ.[17 39] Furthermore, the biological effects of L-IFN may be enhanced by Tα_1.[33]

Combination drug therapy in chronic hepatitis C is currently undergoing investigation using two potentially toxic antiviral agents (IFN and ribavirin) or a combination of a non-toxic immune modulator and an antiviral (Tα_1 and IFN). The results of this study do not prove that combination Tα_1 and IFN therapy is superior to IFN monotherapy. Such a conclusion must await completion of a randomised controlled study in patients with chronic hepatitis C.

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