LIVER

Effects of alpha interferon induction plus ribavirin with or without amantadine in the treatment of interferon non-responsive chronic hepatitis C: a randomised trial

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Background: Fifty per cent of chronic hepatitis C patients are non-responders to interferon. At present, there are no recommended therapeutic options for non- responders.
Aims: The safety and long term effect of alpha interferon induction plus ribavirin with or without amantadine in the treatment of interferon non- responsive chronic hepatitis C was evaluated.
Patients and methods: A total of 114 consecutive patients were randomly divided into three groups with a final 2:2:1 ratio: group A (44 patients) received interferon alfa 2b, 3 million units (MU), three times a week, and oral ribavirin (1000 mg/day); group B (46 patients) received interferon 3 MU daily for the first four weeks and subsequently 3 MU three times a week, and ribavirin as in regimen A; and group C (24 patients) received interferon and ribavirin as in regimen B, plus oral amantadine hydrochloride (200 mg/day). The duration of treatment was 12 months.
Results: The end of treatment response for groups A and B was 25% and 29%, respectively, and for group C, 68% ($p<0.005$). At the end of one year of follow up, a sustained response was observed for six (25%) patients in group C, one (2%) patient in group A, and two (4%) patients in group B ($p<0.002$). The triple regimen was well tolerated and did not increase the frequency or severity of side effects.
Conclusions: The study demonstrates that for the treatment of interferon non- responder hepatitis C patients, the association of interferon-ribavirin has a negligible long term effect whereas a triple regimen including interferon, ribavirin, and amantadine can be an effective and safe treatment.

Forty to fifty per cent of patients with chronic hepatitis C treated with interferon are non- responders.[1] The management of non-responders represents the most challenging of all aspects in the care of patients with chronic hepatitis C virus (HCV). At present, there is no therapeutic option available for these patients because re-treatment with interferon or with an interferon-ribavirin combined regimen, which may be effective in the treatment of relapse,[2 3] are not currently recommended for non-responders.[4] These patients therefore remain at risk of developing cirrhosis, liver failure, and even hepatocellular carcinoma.

It has been reported that daily administration of interferon (induction treatment) may induce favourable viral kinetics and a fast decline in viral load,[5] which may induce a better response to interferon. It has also been shown that amantadine may be a promising agent for the treatment of chronic hepatitis C patients, although conflicting data are reported on its efficacy.[6 7] Amantadine is an antiviral agent that is currently used in humans for the treatment of influenza A and dengue virus.[8 9] Recently, Brillanti and colleagues[10] showed promising results with α-interferon, ribavirin, and amantadine triple therapy in interferon non-responder chronic hepatitis C patients. At present, there are no data on the safety and therapeutic effects of a regimen of interferon induction and ribavirin with or without amantadine in the treatment of interferon non-responder chronic hepatitis C patients. In addition, little is yet known of the long term maintenance of response in chronic hepatitis C interferon non-responders who have been re-treated with interferon plus ribavirin, as all studies performed have reported only a six month follow up.

Accordingly, the aim of this study was to evaluate the safety and therapeutic role of alpha interferon induction plus ribavirin with or without amantadine for the treatment of interferon non-responder chronic hepatitis C patients and to assess maintenance of response after one year of follow up.

PATIENTS AND METHODS

From 1997 to 2000, all consecutive patients admitted to our Division of Internal Medicine and Hepatology, Second University of Naples, were asked to take part in this study if they had chronic hepatitis C, had previously received a course of recombinant or lymphoblastoid interferon alfa at a dose of 3–6 million units (MU) three times a week for at least four months, and were considered as non-responders—that is, on no occasion had they both serum HCV RNA clearance and normalisation of serum transaminase levels. The enrolled period was defined as the time during which it was possible to utilise interferon and ribavirin for compassionate use in interferon non-responders.

All patients were from southern Italy, had received previous treatment at our institute, had been followed up every two or three months, and had undergone a treatment washout period of at least six months. Patients were included in the study if they met the following criteria: serum alanine aminotransferase (ALT) levels persistently greater than 1.5 times the normal value during the follow up period, presence of serum HCV RNA, and had undergone a liver biopsy in the 24 months before entering the study. Patients were excluded if they had: decompensated cirrhosis; cirrhosis with signs of portal hypertension—that is, oesophageal varices; serum human immunodeficiency virus or hepatitis B surface antigen

Abbreviations: HCV, hepatitis C virus; ALT, alanine aminotransferase; MU, million units.

Table 1 Demographic, virological, biochemical, and histological characteristics of the 114 non-responders according to the group assigned for re-treatment

	Group A (IFN+riba)	Group B (IFN+riba)	Group C (IFN+riba+ama)
No of patients	44	46	24
Sex (male)	29 (66%)	31 (67%)	16 (68%)
Age (y) (median (range))	51 (32–59)	51 (30–60)	50 (30–59)
ALT (UI/l) (median (range))	103 (60–240)	98 (62–308)	105 (64–284)
HCV RNA (eq/ml×10^6) (median (range))	2.8 (0.6–21.5)	3.0 (0.7–18.4)	3.2 (0.8–28.6)
HCV genotype 1	31 (70%)	33 (72%)	17 (71%)
HAI (median (range))	5.6 (4–10)	5.3 (4–9)	5.4 (4.10)
Cirrhosis	11 (25%)	12 (26%)	8 (33%)
Previous interferon treatment			
Dose (MU) (median (range))	287 (252–432)	284 (264–504)	286 (260–432)

IFN, interferon; riba , ribavirin; HCV, hepatitis C virus; ALT, alanine aminotransferase; HAI, histological activity index.

positivity; serum markers of autoimmunity with or without associated disease; alcohol intake; serum haemoglobin concentration less than 12 g/dl for women and 13 g/dl for men; white cell count of less than 3000 mm^3; platelet count less than 100 000 mm^3; haemoglobinopathy or haemolytic anaemia; renal, cardiovascular, or pulmonary diseases, diabetes mellitus, or psychiatric disorders. Before entering this study and during the study, serum HCV RNA was measured by polymerase chain reaction (second generation Amplicor HCV; Roche Diagnostic Systems, Basel, Switzerland) and quantitation performed using bDNA assay (Quantiplex 2.0; Chiron Corporation, Emeryville, California, USA). HCV genotype was determined by line probe assay (InnoLiPA; Innogenetics, Zwijndrecht, Belgium). Histological liver damage was evaluated according to Desmet and colleagues.[11] Liver disease was graded according to the histological activity index proposed by Knodell and colleagues[12] and staging according to the Scheuer score.[13] Blood counts and other biochemical tests were performed using standard methodology.

Study design, therapy, and monitoring

The study was a prospective open randomised trial. A total of 114 patients were enrolled. The study protocol was in accordance with the ethical guidelines of the Declaration of Helsinki and all patients agreed to the study and provided written informed consent.

Initially, the study was planned to evaluate the long term effect of interferon induction plus ribavirin in chronic hepatitis C non-responsive to interferon and patients were randomised to receive therapeutic regimen A (interferon plus ribavirin) or B (interferon induction plus ribavirin). In 1998, on the basis of preliminary results reported in the literature (Brillanti *et al*. *Hepatology* 1997:**26**:367A), we thought it would be of great interest to add a third arm (regimen C) to the trial with the aim of also evaluating the effect of a triple therapeutic regimen (interferon induction plus ribavirin plus amantadine). At the time regimen C was added, 42 patients were already included and randomised between groups A and B. For this reason, the 114 patients included were randomised to receive either therapeutic regimen A, B, or C in a final 2:2:1 ratio. Patients included in regimen A received subcutaneous interferon alpha 2b (Intron A; Schering-Plough, Segrate (Milan), Italy) at a dose of 3 MU three times a week, and oral ribavirin (Schering-Plough) at a daily dose of 1000 mg; patients enrolled in regimen B received subcutaneous interferon alfa 2b, at a dose of 3 MU daily for the first four weeks and subsequently three times a week, and ribavirin as in regimen A; and patients randomised to regimen C received interferon and ribavirin as in regimen B, plus oral amantadine hydrochloride (Mantadan; Boehringer Ingelheim, Florence, Italy) 200 mg/day administered in two doses of 100 mg.

Duration of treatment was 12 months and a one year follow up period was planned. During therapy, blood counts and serum transaminases were measured at 2–4 week intervals. Any side effects were carefully monitored. Serum HCV RNA was determined after one, six, and 12 months of treatment, and after 12 months of follow up.

The end point of the study was sustained response at the end of the follow up period evaluated by both normalisation of serum ALT levels and disappearance of serum HCV RNA (sustained biochemical and virological response).

Statistical analysis was based on an intention to treat. Statistical comparisons were made using Fisher's exact test and the χ^2 test. The Student's *t* test was used for parametric analysis. The Mann-Whitney U test was used to compare levels of HCV RNA. All statistical analyses were done using SPSS for Windows. A p value of less than 5% was considered to be significant.

RESULTS

A total of 114 consecutive patients who were non-responders to a previous course of interferon were enrolled in the study, of whom 44 received regimen A (interferon three times weekly plus ribavirin), 46 regimen B (interferon induction plus ribavirin), and 24 regimen C (interferon induction plus ribavirin plus amantadine). The characteristics of the three groups are shown in table 1. Seventy six (67%) of the patients enrolled were male and 31 (27%) had cirrhosis. The three groups were similar with regard to demographics, biochemical, histological, and virological characteristics as well as to the previous dose of interferon received (table 1). In particular, 21% of patients had received 3 MU three times a week, 51% had received 5 MU, and 28% had received 6 MU. However, for approximately half of the patients who had received 3 MU three times a week, after four months of treatment the dose had been increased to 5 or 6 MU three times a week for an additional 2–3 months. Mean duration of the previous course of interferon was six months (range 4–8). Duration of the first course of interferon was similar in the three groups.

As expected for an interferon non-responder population, of the 114 patients enrolled, 81 (71%) were infected with HCV genotype 1 (1b, 75 (92.6%) and 1a, 6 (7.4%)). This proportion was higher than that generally observed in the naive population in our geographical area.[14] Twenty three patients (20%) were infected with HCV genotype 2a/c and 10 (9%) with genotype 3a.

The end of treatment response and the sustained response at the end of the follow up periods for the three groups are shown in table 2. At the end of treatment the complete response rate (biochemical and virological) was significantly higher for group C than that observed for groups A and B. In fact, group C showed a response rate of 68% whereas groups A and B showed response rates of 25% and 29%, respectively ($p<0.005$) (table 2). Of interest, 10 (41.6%) patients in group C (triple treatment) achieved normalisation of ALT values and

Table 2 Response rates at the end of treatment and sustained response after one year of follow up

Response rate	Group A (IFN+riba) (n=44)	Group B (IFNi+riba) (n=46)	Group C (IFNi+riba+ama) (n=24)	Group C minus group A and B rate (95% CI)	p Value*
End of treatment (overall)	11 (25%)	13 (29%)	16 (67%)	40% (19–61%)	0.001
HCV genotype 1	26% (6/31)	30% (10/33)	53% (9/17)	28% (3–53%)	0.054
HCV genotype non-1	23% (5/13)	23% (3/13)	100% (7/7)	70% (29–111%)	0.004
Chronic hepatitis	33% (11/33)	38% (13/34)	69% (11/16)	33% (6–60%)	0.034
Cirrhosis	0% (0/11)	0% (0/12)	62.5% (5/8)	62% (33–92%)	0.001
One year follow up (overall)	1 (2%)	2 (4%)	6 (25%)	22% (10–34%)	0.002
HCV genotype 1	—	3% (1/33)	12% (2/17)		
HCV genotype non-1	8% (1/13)	8% (1/13)	57% (4/7)		
Chronic hepatitis	3% (1/33)	6% (2/34)	31% (5/16)		
Cirrhosis	—	—	12.5% (1/8)		

*Group C versus groups A and B.
IFN, interferon; IFNi, interferon induction; riba , ribavirin; ama, amantadine; HCV, hepatitis C virus.

serum HCV RNA clearance within the first month of treatment whereas for the two other groups the response was obtained between the third and eight month of treatment. In addition, the response rate for patients with HCV genotype 1 infection was higher, although not significantly ($p<0.08$), for those who received the triple regimen (group C) than for those who received the double combinations (groups A and B). Moreover, cirrhotic patients who received the triple combination showed a similar response rate to that observed for chronic hepatitis patients whereas none of the cirrhotic patients who received regimen A or B cleared their serum HCV RNA (table 2).

By month 6 of follow up, relapse had occurred in 73% (8/11) of the end of treatment responder patients in group A, in 69% (9/13) of group B, and in 50% (8/16) of patients who had received the triple therapy (data not shown). Therefore, at that point the response rates for groups A, B, and C were 7%, 9%, and 38%, respectively (data not shown).

At one year of follow up, a sustained response was observed for six patients (25%) treated with the triple therapy (group C), for one patient (2%) treated with regimen A, and for two patients (4%) in group B ($p<0.002$) (table 2). Of the six responders in group C, four were infected with HCV genotype 2a/c and two with type 1b. The responders in groups A and B were infected with genotype 1b (one patient) and 2a/c (two patients). In patients in group C infected with HCV genotype 2a/c and 1, sustained response rates of 67% (4/6) and 12% (2/17), respectively, were achieved. Five of these six patients with a sustained response had chronic hepatitis and one had cirrhosis. Overall, the sustained response rate in group C was 31% (5/16) in chronic hepatitis and 12.5% (1/8) in cirrhotics. No significant difference was found in levels of HCV RNA between sustained responders and non-responders. It is of particular interest that all patients who achieved a sustained response showed ALT normalisation and HCV RNA clearance within the first month of treatment.

Therapy was associated with the typical side effects of interferon and ribavirin. Amantadine did not increase the frequency or severity of side effects (table 3). In the amantadine group, no episode of severe depression was observed. No life threatening events were observed during treatment. Therapy was discontinued before the end of treatment in 23 patients (20%): 11 (22.7%) in group A, 10 (21.7%) in group B, and two (8%) in group C, the most frequent cause being mental depression, followed by a severe flu-like syndrome and gastric intolerance. Severe anaemia occurred in one patient. A reduction in the dose of ribavirin due to haemolytic anaemia was necessary in 16 (14%) patients, with a similar prevalence in the three groups. It should be noted that the decision to discontinue the treatment by the two patients in group C was a personal decision in the absence of significant side effects. The decision was made between the first and second months of treatment and at that time both patients had normal ALT values and serum HCV RNA was negative.

Table 3 Side effects in groups A, B, and C

	Groups A and B (n=90)	Group C (n=24)
Flu-like syndrome	75 (72%)	17 (70%)
Marked depression	7 (8%)	—
Moderate depression	5 (5.5%)	2 (8%)
Anxiety and irritability	9 (10%)	3 (12.5%)
Gastric discomfort	21 (23%)	6 (25%)
Anaemia	19 (21%)	5 (20.8%)
Itch	12 (13.3%)	3 (12.5%)
Thyroiditis	1 (1.1%)	—
Weight loss	5 (5.5%)	1 (4%)

DISCUSSION

In the past, interferon was the only drug with any effect in the treatment of chronic hepatitis C but approximately 50% of patients did not respond to this treatment. Re-treatment with interferon at a higher dose was ineffective in these patients,[15] and the efficacy of a combination of interferon with ribavirin after six months of follow up was also found to be disappointing, with a sustained response rate of 5–15%.[15–17] A meta-analysis[18] of trials on this combination (interferon with ribavirin) for non-responders to interferon showed that a sustained virological response may be achieved in approximately 13% of patients and that a higher response rate was associated with HCV genotype non-1 and a long duration of treatment (48 weeks or longer). Recently, two new studies have been published on the combination of interferon plus ribavirin in the treatment of patients with chronic hepatitis C who had not responded to interferon alone.[19 20] Saracco and colleagues[19] using 3 MU three times per week for 48 weeks reported a sustained response rate of 17% whereas Di Bisceglie and colleagues[20] using the same schedule showed a higher sustained response rate (30%). In addition to these conflicting data in terms of efficacy, a high number of patients (12.5% and 21%, respectively) discontinued the interferon-ribavirin combination. In both studies[19 20] approximately 20% of patients reported side effects.

The six month follow up results of our study on the effects of the interferon-ribavirin combination in the treatment of interferon non-responders were in agreement with the results of the meta-analysis,[18] but not with the results of the last two studies quoted.[19 20] In our study, using 3 MU of interferon, with or without induction, plus ribavirin for one year in a cohort of 90 non-responders, we observed a response rate of 27% at the end of treatment; after six months of follow up, an observation

period similar to the studies included in the meta-analysis[18] and to the last two studies,[19 20] 8% of patients treated maintained their response. However, after one year of follow up only 3% had a sustained response. Our data highlight several new issues concerning combination treatment in interferon non-responders. Firstly, in contrast with reports on interferon treatment of naive patients, interferon non-responsive patients seem to be a "particular" group in which a high relapse rate after interferon-ribavirin re-treatment may occur, even after six months of follow up; thus long term follow up is necessary to evaluate the real therapeutic potential. In our study relapse rates were higher than those reported in previous studies. A possible explanation may be the criteria we used for selection of patients. We included only "true" interferon non-responders whereas previous studies have also included breakthroughs and patients with normal transaminase levels. It has been reported that prior non-responders with breakthroughs have a greater chance of having a sustained response to re-treatment than non-responders without breakthroughs.[21 22] Furthermore, the data demonstrate that the combination of α-interferon at a dose of 3 MU plus ribavirin for patients who have been non-responders to interferon alone has a negligible long term sustained response. Saracco and colleagues[19] reported that with a higher dose of interferon (5–6 MU) in a selected population, such as young patients infected with a genotype other than 1, it is possible that a better result may be obtained even after a long follow up. At present there are no data to support this hypothesis. Indeed, two other studies using a high dose of interferon (5–6 MU) plus ribavirin have shown a sustained response rate of 5–10%.[15 16] However, a high prevalence of side effects was observed.

Recently, Brillanti and colleagues[10] reported promising results on the use of a combination of interferon, ribavirin, and amantadine for one year in the treatment of patients who were non-responders to interferon alone. They showed that at the end of the sixth month of follow up, 48% of patients had achieved a sustained response. Our data confirm the improved efficacy of triple therapy in the treatment of interferon non-responders. After six months of follow up, 38% of patients in our study showed a sustained response. However, there are differences between our study and that of Brillanti *et al* in terms of the population studied and therapeutic protocol. There was a higher prevalence of patients infected with HCV genotype 1 in our study (71% *v* 57.5%, respectively). In addition, our treatment schedule consisted of one month of daily administration (induction) of interferon at 3 MU followed by 3 MU three times a week for an additional 11 months, whereas Brillanti *et al* used a treatment schedule of 5 MU three times a week for 12 months. The follow up period was also different (12 *v* 6 months). Our results therefore extend the data on triple therapy. In fact, our data appear to indicate that the triple regimen may even be effective for non-responders with a high percentage of HCV genotype 1. Furthermore, our treatment schedule seems to have achieved similar results to those obtained by Brillanti and colleagues[10] but by using a lower dose of interferon. Hence it seems that by using an induction period, it is possible to reduce the dose of interferon without jeopardising the final result. If this is true, the advantages are obvious in terms of cost reductions and side effects. Moreover, our study showed that this triple regimen may afford a long term (one year) sustained response for 25% of interferon non-responders.

Our data indicate that the triple regimen offers clear advantages compared with combination therapy in the treatment of interferon non-responders. In fact, higher rates for both the initial response and sustained response were observed in patients who received the triple regimen (table 2). Addition of amantadine to the combination of interferon induction-ribavirin seemed to strengthen the antiviral effect, inducing rapid (within the first month) serum HCV RNA clearance in a high percentage of cases (44%). Of particular interest is the fact that all sustained responders cleared serum HCV RNA within the first month of treatment, although not all patients who eliminated HCV RNA at that time achieved a sustained response. This could be used as a criterion to identify patients with the highest possibility of achieving a sustained response. In addition, patients with HCV genotype non-1 and without cirrhosis also seem to have a greater chance of achieving a sustained response.

At a recent meeting, Teuber and colleagues[23] reported data on the treatment of primary interferon non-responsive patients with chronic hepatitis C. Only a slightly higher sustained response rate was observed for patients receiving the triple treatment compared with those treated with interferon-ribavirin alone (23.4% *v* 17.1%, respectively). We can only infer that the selection of patients, short follow up period (six months), and the different amantadine preparation from that used in our and Brillanti's study (amantadine sulphate compared with amantadine hydrochloride, respectively) may have affected the efficacy of the treatment.

The triple regimen was well tolerated and no further side effects were observed compared with the interferon-ribavirin combination. The rate of treatment discontinuation in patients who received the triple regimen because of side effects was lower than expected—that is, approximately 20%, on the basis that they received a similar dose of interferon and ribavirin, even if amantadine had no side effects. In particular, in the group who received amantadine, we did not observe severe depression, which was the major adverse event necessitating discontinuation of treatment in the group who received the interferon-ribavirin combination. This important point may have several explanation. Firstly, in agreement with the findings of Di Bisceglie and colleagues,[20] there may have been psychological issues at work as many patients who dropped out realised that they were not having a virological response. Secondly, the antidepressive effect of amantadine may have improved the malaise associated with interferon.[24–26] Thus better compliance with treatment obtained with amantadine further supports the use of the triple regimen in interferon non-responders.

Our results and those of Brillanti and colleagues[10] raise an important question. How will the new group of relapsers (previous non-responders) react to further antiviral treatment? In general, re-treatment of relapsers results in sustained virological response rates approaching 70%.[3]

We do not know in what way amantadine acts synergistically with interferon and ribavirin in chronic hepatitis C patients who were non-responders to interferon alone. In vitro studies have shown that amantadine is unable to inhibit HCV protease, helicase, ATPase, or polymerase.[27] Thus factors other than inhibition of viral replication may be implicated. An in vitro study has reported that amantadine may act against HCV through an immune mediated mechanism.[28] Further support for this is the demonstration that amantadine can induce production of interleukins.[29] It is possible that modification of the host immune system induced by amantadine may enhance the effect of interferon and ribavirin against HCV.

In conclusion, our study demonstrated that the interferon-ribavirin combination has poor efficacy in the short term (six months) and negligible efficacy in the long term (one year) in primary interferon non-responsive patients with chronic hepatitis C. In contrast, the triple regimen of interferon induction, ribavirin, and amantadine may be an effective treatment for these patients. A large trial is necessary to confirm these promising results. At present, a valid alternative to a daily interferon regimen may be the use of peginterferon administered once a week as it maintains constant serum levels. Recently, it has been shown that peginterferon has a greater therapeutic effect than α-interferon.[30] Peginterferon in combination with ribavirin and amantadine could also improve the therapeutic response in chronic hepatitis C

patients who are non-responders to interferon alone. Future trials should assess the efficacy of the triple regimen in interferon-ribavirin non-responders.

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