Treatment of chronic hepatitis C in patients unresponsive to interferon
Interest of re-treatment combining interferon induction therapy and ribavirin (a multicenter pilot study)

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SUMMARY

Aim — About 45% of patients with chronic hepatitis C are unresponsive to the present reference treatment combining pegylated interferon plus ribavirin; before pegylated interferon was available, the non-response rate was around 60%. This open multicenter pilot study, initiated before pegylated interferon became available, was designed to evaluate, in patients unresponsive to interferon monotherapy, the rate of biological and virological response and side-effects of the ribavirin- alpha 2b interferon combination.

Methods. The combination protocol was ribavirin (1 to 1.2 g/d) plus alpha 2b interferon at induction doses (9 MU/d the first week; 4.5 MU/d the eleven following weeks; 3 MU/2 days the 36 following weeks).

Results. Among the 27 included patients, 17 (63%) were viremia-negative (PCR) after 12 weeks of treatment, 9 (33%) were complete responders (undetectable viremia and normal transaminases) at the end of treatment (48 weeks) and of follow-up (72 weeks). Patients with non-1, non-4 genotypes who derived full benefit from this therapeutic strategy (6/7 (86%) were complete responders: 4/5 with genotype 3 and 2/2 with genotype 5). Quality-of-life was impaired during treatment, especially during the first 12 weeks of high-dose interferon therapy.

Conclusion. While waiting for new therapeutic possibilities, these good results suggest interferon induction at the beginning of treatment remains a valid option.

RÉSUMÉ

Traitement de l’hépatite chronique virale C chez des malades non répondeurs à l’interféron : intérêt d’une induction par interféron quotidien en association à la ribavirine (étude pilote multicentrique)
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Environ 45 % des malades atteints d’hépatite chronique virale C ne répondent pas au traitement de référence actuel qui associe l’interféron pegylé et la ribavirine. Avant la disponibilité de la forme pegylée de l’interféron le taux de non-réponse avoisinait les 60 %.

L’intérêt de cette étude pilote, ouverte, multicentrique, initiée avant la disponibilité de l’interféron pegylé, était d’évaluer, chez des malades non répondeurs à l’interféron en monothérapie, le taux de réponse biologique et virologique ainsi que la tolérance d’un schéma thérapeutique associant ribavirine (1 à 1,2 g/j) et interféron alpha 2b en induction (9 MU/j la première semaine ; 4,5 MU/j les 11 semaines suivantes ; 3 MU/2j les 36 semaines suivantes).

Parmi les 27 malades inclus, 17 (63 %) ont eu une virémie indéetectable par PCR qualitative après 12 semaines de traitement, 9 (33 %) ont été répondeurs complets (virémie indéetectable et transaminases normales) à la fin du traitement (S48) et du suivi (S72). Les malades de génotype non-1, non-4 ont été ceux qui ont le plus bénéficié de cette stratégie thérapeutique (6/7 (86 %) étaient répondeurs complets : 4/5 génotypes 3 et 2/2 génotypes 5). La qualité de vie a été altérée pendant le traitement, particulièrement pendant les 12 premières semaines de posologie maximale d’interféron.

En attendant de nouvelles possibilités thérapeutiques, ces bons résultats incitent à réévaluer l’intérêt d’une induction par posologie renforcée d’interféron en tout début de traitement.

Introduction

Chronic hepatitis C virus (HCV) infection is one of the most widespread chronic liver diseases. More than 300 million persons are affected worldwide. Chronic HCV infection can lead to cirrhosis then hepatocellular carcinoma and is the leading indication for liver transplantation in Europe. Despite major advances in antiviral treatments, the problem raised by patients who fail to respond to these treatments remains unsolved: 85% of those given primary treatment with interferon (IFN) monotherapy and 60% of those given standard alpha-IFN-ribavirin dual therapy are non-responders. Since the introduction of combined pegylated interferon (PEG-IFN)-ribavirin the rate of non-response remains at 45%, particularly for genotype 1 [1].

This pilot study was conducted before PEG-IFN became available. The prior protocol, using alpha-IFN (3 MU 3 times a week) had been found to be inadequate for most patients with HCV infection. During the chronic phase of the infection, viral replication reaches a characteristic plateau with a 3-hr half-life of free HCV virions and a daily production of 10^{12} viral particles. For many patients, the decline in viral load induced 24 hours after a unique injection of 3 MU is insufficient. A higher dose would pro...
voke a greater fall. Furthermore, intermittent administration of IFN is associated with a rebound in viral replication on day 2, reducing the slope of the viral elimination curve [2]. The aim of our study designed in 1998 was to test, in patients non-responsive to IFN monotherapy, the capacity of combination IFN-ribavirin using daily injections of IFN in the first 12 weeks and induction with high dose IFN the first week, to induce virological response, evaluated at the end of the 12 weeks of high-dose treatment. The advent of pegylated IFN has eliminated the need for studies on daily injections of IFN, but until anti-helicase and anti-protease agents currently under investigation become available, induction treatment using a higher dose of IFN [3, 4] still appears to be a valid option.

### Patients and methods

#### Patient selection

Eligible patients were adults aged 18-55 years who were unresponsive to monotherapy using alpha-IFN. Non-response was defined as the absence of normal serum ALAT levels and absence of viral C RNA clearance at the end of treatment using 3 to 6 MU, 3 times a week for at least 3 months. These patients had not received alpha-IFN (3MU 3 times a week) for more than 18 months. This treatment had to be interrupted for at least 6 months. Active chronic hepatitis was defined as elevated ALAT at least twice the upper limit of normal (ULN). Chronic active hepatitis was defined by the following criteria observed during the 6 months preceding inclusion: presence of anti-HCV antibodies (ELISA 3, Ortho Diagnostic), presence of HCV-RNA determined by qualitative polymerase chain reaction (PCR) [AmpliC HCV 2.0 Roche Diagnostics, detection threshold 50 IU/mL], METAVIR score [5] A1 and F1 for liver biopsy performed between two treatments and not more than 24 months before inclusion. Viral genotype was determined by INNOLIPA (Innogenetics). Viremia was measured qualitatively in each center during patient screening, then at weeks 12, 48, 52, and 72. Serum samples drawn weekly during the first month then at weeks 6, 8, 12, and every 4 weeks to the end of treatment as well as 1, 3 and 6 months after treatment end were preserved (−80°C) by each center. These samples were assayed by quantitative PCR (AmpliC Monitor HCV 2.0, Roche Diagnostics, detection threshold 600 IU/mL) at the Orleans center to determine HCV-RNA.

Minimal values required for inclusion were: hemoglobin 12 g/dL for women and 13 g/dL for men, white cell count 3 X 10^9/L, neutrophil count 1.5 X 10^9/L, platelet count 100 X 10^9/L. Normal levels were required for serum bilirubin, serum albumin, serum creatinine, prothrombin time, blood urea nitrogen, and blood glucose.

Patients were not eligible for inclusion in the event of: pregnancy desired in next 18 months, hepatitis B virus (HBV) co-infection, human immunodeficiency virus (HIV) co-infection, uncontrolled cirrhosis, alpha-fetoprotein level > 50 ng/mL or focal liver lesion identified at ultrasonography, history of severe heart disease, diabetes, psychiatric disorder, epilepsy, respiratory insufficiency, hepatitis disorder, autoimmune disease, hyperuricemia, immunosuppressor or hepatotoxic treatment, active or recent drug abuse (less than 6 months abstinence), alcohol consumption > 20 g/dl, liver disease unrelated to HCV. All patients who accepted the treatment protocol were included in each center. Inclusions were discontinued as soon as the main objective was reached.

#### Study design and organization

Five French centers participated in this open multicentric prospective pilot study which was approved by the Tours ethics committee and the Paris-Ouest ethics committee. Eligible patients were given a combination treatment with ribavirin and interferon Pegylated Interferon (PEG-IFN alpha-2b 9 MU/d the first week; 4.5 MU/d for the next 11 weeks; then 3 MU every 48 hours for 36 weeks). Patients were seen at consultation at weeks 1, 2, 3, 4, 6, 8, and 12 after treatment onset. They are then at the end of the 49th week, in which time treatment was discontinued. Routine biological and hematological tests were monitored. Therapeutic doses were adapted to the hematological and chemistry results.

If at the end of the 12-week induction phase ALAT levels were > ULN and serum HCV-RNA remained detectable by PCR, treatment could be interrupted and the patient was seen every 3 months for one year.

### Efficacy and tolerance

The main outcome criterion was virological response measured at 12 weeks (W12, end of the induction period) by qualitative PCR. Undetectable HCV-RNA at W12 was considered to be the expected favorable response.

The second outcome criterion was complete response defined by the association of undetectable viremia by qualitative PCR and normal ALAT level. Patients were tested for complete response at W48 and 6 months later. Sustained complete response distinguished between responders and relapsers who were viremia-positive again between 48 and 72 weeks.

Secondary outcome criteria included negative viremia at W4, kinetics of the virological response assessed by quantitative PCR, influence of genotype, initial viral load, type of response to first treatment by IFN alone (initial nonresponse or relapse).

Tolerance was determined on the basis of undesirable effects and quality-of-life, measured on a visual analog scale, assessed at each consultation.

#### Statistical analysis

An independent clinical research group from the Faculty of Medicine at Tours performed the statistical analysis.

The study was designed according to the triangular test method [6] using the Phase II software developed by Bellissant et al. [7]. The highest response rate considered to be sufficient to decide not to progress to phase III (p0 = 0.2) was set at 0.2. The rate considered to be pertinent to detect clinical benefit (p0 = 0.5) was set at 0.5. The alpha and beta risks were accepted at 5%. The number of subjects to include in each step of the sequential trial (N) was set at 5. The triangular test thus was a sequential statistical analysis performed every 5 patients (i.e. when the W12 results of 5 new patients were available). The triangular test estimated two statistical values, Z and V (figure 1), which schematically represent the rate of response and the number of inclusions respectively. These values were represented by a point on a plane (V on the x-axis and Z on the y-axis) whose position was compared with the pre-established boundaries defining the rules of trial discontinuation and conclusion of the trial, considered to demonstrate efficacy if the point was above the upper boundary and inefficacy if the point was below the lower boundary.

### Results

#### Patient characteristics (table I)

In this study, the statistical analysis led to the conclusion that the treatment was effective after results were available for the first 10 patients, confirming that the rate of virological response was
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Table I. – Patient characteristics at treatment onset according to virological response at week 12 and sustained response at week 72.

<table>
<thead>
<tr>
<th>Total (N = 27)</th>
<th>Responders at W12 (N = 17)</th>
<th>Non-responders at W12 (N = 10)</th>
<th>Sustained responders at W72 (N = 9)</th>
<th>Sustained non-responders at W72 (N = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex-ratio F/M</td>
<td>7/20</td>
<td>4/13*</td>
<td>3/7*</td>
<td>4/5*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.4 ± 8.3</td>
<td>40.4 ± 9.1*</td>
<td>45.7 ± 5.4*</td>
<td>41.1 ± 6.8*</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>74.6 ± 15.4</td>
<td>75 ± 17.2*</td>
<td>73.8 ± 12.6*</td>
<td>68.2 ± 16.2*</td>
</tr>
<tr>
<td>Pretherapeutic status (relapsers/non-responders)</td>
<td>2/25</td>
<td>2/15*</td>
<td>0/10*</td>
<td>2/7*</td>
</tr>
<tr>
<td>Pretherapeutic viremia (IU/mL)</td>
<td>664190 ± 196766</td>
<td>698600 ± 197695</td>
<td>578167 ± 18164*</td>
<td>741429 ± 192614*</td>
</tr>
<tr>
<td>METAVIR and fibrosis score F3-F4/F1-F2</td>
<td>5/22</td>
<td>3/4</td>
<td>2/8*</td>
<td>3/6*</td>
</tr>
</tbody>
</table>

* not significant.

Significantly greater than 20%, the reference threshold (estimated rate 70%; 95% CI 33.0-92.0) (P = 0.003) (figure 1). This analysis was obtained after 27 patients had been included. Inclusions were thus interrupted at this time and the final analysis concerns 27 patients. A 28th patient wanted to withdraw consent before starting the therapeutic phase. This patient was thus not treated and was not retained for analysis.

The 27 patients were included in the following centers: Limoges (N = 7), Tours (N = 7), Orleans (N = 6), Clermont-Ferrand (N = 5), Poitiers (N = 2) from June 1999 to March 2000. There were 7 women and 20 men, mean age 42.4 ± 8.3 years, mean body weight 74.6 ± 15.4 kg. Twenty-five patients were unresponsive IFN alone and 2 were relapsers (table I). The duration of viral infection was estimated at 20.1 ± 7.6 years and was considered to result from blood transfusion before 1991 (N = 10 patients, 37%), former intravenous drug abuse (N = 8, 30%), a nosocomial source (N = 5, 19%), or a non-medical event increasing vulnerability (N = 2, 7%). In two patients (7%), the cause of HCV infection could not be determined.

The viral genotype was 1a in 5 patients (19%), 1b in 12 (44%), 3a in 5 (19%), 4 in 2 (7%), and 5 in 2 (7%). One patient was infected by genotypes 1b and 4. The METAVIR score was 1.6 ± 0.6, and the fibrosis score was 1.7 ± 0.9.

Treatment response (table II)

At W12, viral RNA was undetectable by qualitative PCR in 17 patients (63%). Complete response was observed at W48 in nine patients (33%). This result was confirmed in all these patients at W72, six months after interruption of treatment.

At W4, viremia, assessed by qualitative PCR performed on the frozen serum samples, was negative in 7 patients (26%). Six of these 7 patients were sustained responders. The seventh patient was lost to follow-up after 8 weeks of treatment and was considered to be a non-responder.

Among the factors affecting sustained response, genotype appeared to be determinant. Four of the 5 patients infected by genotype 3 and 2 of the patients infected by genotype 5 were sustained responders, i.e. 6 of the 7 patients infected with non-1, non-4 genotypes (86%). Conversely, only 3 out of 20 patients (15%) with genotype 1 and/or genotype 4 were responders at W72. None of the patients with genotype 4 were sustained responders.

It was noted that sustained responders who were infected by genotypes 3 or 5 had already responded at W4. Nine patients infected by genotype 1 were non-responders at W4 and were viremia-negative at W12. Three of them were sustained responders.

Tolerance

Among the 27 patients in the study, 15 (56%) completed the entire 48-week treatment protocol. The 9 sustained responders were among these 15.
Four patients (15%) interrupted treatment because of severe adverse effects: one at W1 due to depression, 1 at W9 for maculopapular exanthema, 1 at W12 for severe asthenia, 1 at W24 after 20 weeks of reduced dosage for oral lichen.

The quality-of-life analysis (figure 2) clearly demonstrated a sharp decline in the score during the induction period, the fall was greater than 2.5 points on average. The greatest fall corresponded to the highest IFN dose and the period of daily injections. There was a discrete improvement when the injections were less frequent. The curve nevertheless exhibited a downward slope towards the end of treatment reflecting patient tiring. The pretherapeutic level was achieved again between 3 and 6 months after the end of the antiviral treatment.

Discussion

The main objective of this trial, designed in light of the scientific knowledge at the time of its conception in 1998, was thus reached after the virological results of the first 12 weeks were obtained for the second group of 5 patients. At this time, 27 consecutive patients had been included. The virological results observed in these 27 patients after 12 weeks of induction therapy (HCV-RNA undetectable by PCR in 63% of patients) confirmed the good results obtained in the first 10 patients included (70% negative viremia).

Obtaining 33% complete response at 48 weeks of combination therapy is a good result for patients who did not respond to standard alpha IFN monotherapy and who had a high viremia, particularly so since the majority of patients (18/27) were infected with genotype 1 and none had genotype 2. Nearly three-quarters of patients in this study (20/27, 74%) had genotypes 1 and/or 4. This result cannot however be extrapolated to all non-responders. For this trial, we selected young patients (18-55 years) with no significant co-morbid conditions.

One of the reasons for these good results was probably the daily injections of IFN during the first 12 weeks of treatment. On the other hand, a multicentric trial [8] conducted in patients who failed to respond to the first IFN treatment combining ribavirin and IFN given daily for the first 6 months at 3 MU/injection, was unable to demonstrate any benefit in comparison with a 3 times a week protocol at 6 MU/injection given for the same first 6 months. The second arm of this study used a higher dose. Currently, daily injections of IFN are replaced by weekly injections of PEG-INF [1, 9] which enable a more stable antiviral effect. One could speculate that the period of IFN injections given daily was too short for the 6 patients in our study infected by genotype 1 and for the 2 patients with genotype 4, who were responders at W12 but later relapsed. These patients could have benefited from a longer duration of daily injections of IFN.

The higher dose of induction IFN is another possible explanation for these encouraging results. The 9 MU/d given during the first week and the 4.5 MU/d during the next 11 weeks had a marked antiviral effect: 26% undetectable viremia after the first 4 weeks at the highest dose, 63% after the 12 weeks of the induction phase. The superiority of the 5 MU dose compared with 3 MU administered 3 times a week for 12 weeks in dual therapy has already been demonstrated [10] in treatment-naïve subjects. The usefulness of the 10 MU/d induction dose of alpha 2a IFN administered for the first two weeks of combination therapy treatment-naïve patients with genotype 1 has also been established [11]. The first phase of viral decline (day 1), like the second (day 2 to day 21) appears to be influenced by the initial dose of IFN. The third phase of viral decline (up to the 3rd week) appears to depend mainly on ribavirin [12]. Non-response to dual therapy, generally observed in patients with genotype 1, is related to viral resistance, as is shown by the insufficient decline in the slope of the viral curve which does not reach eradication at week 48 [13]. Considering these more recent data on viral kinetics, it has been suggested that induction with PEG-INF can be used with a higher initial dose or with 2 weekly injections at the beginning of treatment [14].

In our patients, the main problem with induction was tolerance. Treatment had to be interrupted in 15% of the patients for severe adverse effects. This problem was not encountered by Ferenci et al. [11] who studied treatment-naïve patients. Symptoms of depression can sometimes be controlled by psychological support or treatment, but the skin and mucosal effects which accounted for 50% of the severe adverse events in this series, cannot at the present time be predicted and are difficult to treat. Patients interrupt treatment because of these effects which constitute a limitation to this therapeutic strategy.

We did not have any cases of relapse in our patients during the follow-up. This illustrates the exceptional nature of relapse demonstrated by qualitative PCR, which now has a very low detection threshold. Zeuzem et al. [15] proposed that sustained response should be evaluated at 12 weeks and not at 24 weeks after the end of treatment [15]. Using this approach, our results would not have changed.

Evaluating outcome as we did at 12 weeks appears to be pertinent. All sustained responders were viremia-negative at 12 weeks while at 4 weeks two-thirds were viremia-negative. None of the patients with detectable HCV-RNA beyond week 12 were sustained responders. This suggests that treatment should be discontinued in non-responders at 12 weeks. This could limit the secondary effects and treatment cost in non-responders who do not have histologically severe lesions warranting continued suppressive treatment as proposed by the consensus conferences [16, 17]. Proposing treatment withdrawal in the event of negative virological response at 12 weeks would make this retreatment scheme more acceptable to patients. PCR provides pertinent information and remains the gold standard for assessing HCV-RNA (negative viremia or 2-log drop off) [16, 17]. Studies are currently under way to validate measurement of the HCV capsid antigen in this indication [18].

Suppressive treatment is not aimed at achieving viral eradication. Since at the present time there is no other treatment alternative and while waiting for anti-HCV antiprotease and anihilicase agents to become available for routine use, induction treatment by pegylated interferon in combination with ribavirin should be evaluated in motivated subjects who have failed to respond to classical regimens and who are free of co-morbid conditions which could favor adverse effects. Our results favor...
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re-treatment of patients infected with non-1 non-4 genotypes even if the presence of genotype 1 was not an absolute criterion of non-response in this study.

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REFERENCES


