Interferon plus ribavirin and interferon alone in preventing hepatocellular carcinoma: A prospective study on patients with HCV related cirrhosis

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INTRODUCTION

A number of studies have reported that treatment of HCV related cirrhosis might have a preventive effect on hepatocellular carcinoma development. This has been recently confirmed by a meta-analysis concluding that “Interferon (IFN) prevents or delays the development of hepatocellular carcinoma (HCC) in patients with HCV-related cirrhosis, the magnitude of the overall effect is low and the benefit may be partly due to spurious associations. The preventive effect seems more evident among sustained responders to IFN”. However, with old interferon schedules, sustained responders did not exceed 10-15% of treated patients.

The response rate to IFN has changed since the introduction of ribavirin, the induction protocols and, finally, the pegylated interferons. Data on the preventive effect on HCC of more powerful therapeutic schemes are lacking.

Our study was started in 1997 with the purpose of assessing the efficacy of interferon (given with an induction protocol) plus ribavirin (the gold standard treatment at that time) in the prevention of HCC development.

MATERIALS AND METHODS

Patients

A total of one hundred and one consecutive patients (62 males and 39 females, mean age 55.1±1.4 years) with HCV related liver cirrhosis diagnosed by liver biopsy plus compatible biochemical parameters and ultrasonographic signs of portal hypertension were enrolled in the study. The baseline histologic activity of the liver was assessed and the patients were stratified according to sex and AgNOR-PI (cut-off = 2.5). Forty-one patients (27 males, 14 females) were only followed up after the end of an yearly treatment with IFN-alpha2b (old treatment control group = OTCG). Sixty naive patients were stratified according to sex and AgNOR-PI and then randomized in two groups: 30 were treated with IFN-alpha2b + ribavirin (treatment group = TG), the remaining were not treated (control group = CG). Nonresponders (NR) or relapers in the TG received further IFN/ribavirin treatments after a 6 mo withdrawal.

RESULTS: AgNOR-PI was significantly lowered by IFN (P<0.001). HCC incidence was higher in patients with AgNOR-PI>2.5 (26% vs 3%, P<0.01). Two NR in the OTCG, none in the TG and 9 patients in the CG developed HCC during follow-up. The Kaplan-Mayer survival curves showed statistically significant differences both between OTCG and CG (P<0.004) and between TG and CG (P<0.003).

CONCLUSION: IFN/ribavirin treatment associated with retreatment courses of NR seems to produce the best results in terms of HCC prevention. AgNOR-PI is a useful marker of possible HCC development.

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Abstract

AIM: To determine the role of interferon (IFN) with or without ribavirin in preventing or delaying hepatocellular carcinoma (HCC) development in patients with hepatitis C virus (HCV) related cirrhosis. Data on the preventive effect of IFN plus ribavirin treatment are lacking.

METHODS: A total of 101 patients (62 males and 39 females, mean age 55.1±1.4 years) with histologically proven HCV related liver cirrhosis plus compatible biochemical and ultrasonography were enrolled in the study. Biochemistry and ultrasonography were performed every 6 mo. Ultrasound guided liver biopsy was performed on all detected focal lesions. Follow-up lasted for 5 years. Cellular proliferation, evaluated by measuring Ag-NOR proteins in hepatocytes nuclei, was expressed as AgNOR-Proliferative index (AgNOR-PI) (cut-off = 2.5). Forty-one patients (27 males, 14 females) were only followed up after the end of an yearly treatment with IFN-alpha2b (old treatment control group = OTCG). Sixty naive patients were stratified according to sex and AgNOR-PI and then randomized in two groups: 30 were treated with IFN-alpha2b + ribavirin (treatment group = TG), the remaining were not treated (control group = CG). Nonresponders (NR) or relapers in the TG received further IFN/ribavirin treatments after a 6 mo withdrawal.

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Liver biopsy

1 yr

Follow-up without therapy: 5 yr

End FU

41 pts

IFN*

No therapy

OTCG

*6MU ad×12 mo

Liver biopsy

Follow-up: 5 yr

60 naive pts, 6 mo follow-up, randomised by sex and AgNor-PI

30 IFN+ribavirin; further courses for NR and relapsers 6 mo after withdrawal

30

No therapy

TG

CG

*IFN-α2b dosage: 6 MU/d for a month followed by 3 MU/d for 11 mo
Ribavirin dosage: 1 g/d

OTCG = old treatment control group    TG = treatment group      CG = control group

Figure 1 Study design.

The study was carried out according to the Helsinki protocol and all patients gave their written informed consent.

Ultrascanographies, blood cell count, α1-fetoprotein, γGT, transaminases, PT, total protein and their fractions were performed every 3 mo in all patients. Additional tests were performed in patients under active treatment: blood cell count every 10 d for two months and then monthly; transaminases, urea, creatinine and uric acid were tested monthly. When focal lesions were detected by ultrasound (US), US-guided liver biopsy was performed.

Protocol treatments

The 41 patients in OTCG were treated with IFNα2-2b 6 MU/d for a month followed by 3 MU/d for 11 mo. The 30 patients in TG received the following α-2b schedule: 6 MU/d for a mo then 3 MU/d for 11 mo plus ribavirin 1 g/d for 12 mo. IFN and ribavirin dose reductions were made according to the biochemistry and tolerance of each patient. However, a total dose equal to or greater than 540 MU and 400 mg of ribavirin per day were considered suitable. Nonresponders and/or relapsers received further IFN treatment courses after a 6-mo withdrawal.

Liver histology and AgNOR-PI determination

Ultrasound guided liver biopsies were fixed in 40 g/L formaldehyde solution for 6 h and embedded in paraffin wax. Four 4 µm thick sections were cut from routinely processed paraffin blocks. Hematoxylin-eosin, silver impregnation, Pearl’s staining were performed to define the severity of parenchymal, portal and periporal inflammation and the stage of disease by evaluating fibrosis and the presence of stainable iron into the liver. Histology was evaluated by two blinded independent observers according to Scheuer score.

The AgNOR staining was performed on routine sections of liver tissue on poly-lysine pretreated slides after immersion in xylene and ethanol. After progressive re-hydration sections were covered with plastic resistant to high temperature, put in pressured ovens (120 °C for 10 min, at 37 °C). Then, sections were stained by silver impregnation in a gelatine solution (formic acid 10 mL/L and silver nitrate 500 g/L, 100:2 v/v) according to Ploton[20], for 13 (43%) (9 genotype 2 and 4 genotype 1). None of the re-treated patients showed a sustained response.

A significant reduction in AgNOR-PI was observed after IFN-treatment (Figure 2).

A significant difference in HCC development was observed according to AgNOR-PI: 9 out of 35 (26%) with basal AgNOR-PI >2.5% vs 2 out of 66 (3%) with basal AgNOR-PI <2.5% (P<0.01) (Figure 3).

Virological response

Six months after IFN withdrawal 8 out of 19 responders (24.5%) achieved sustained response (6 with genotype 2 or 3 and 2 with genotype 1) in the OTCG. Twenty one out of 30 patients in TG (70%) achieved a virological response that was sustained in 13 (43%) (9 genotype 2 and 4 genotype 1). None of the re-treated patients showed a sustained response.

Statistical analysis

Results were expressed as mean±SE. The statistical analysis was carried out according to the intention to treat analysis. Wilcoxon test was used when appropriate and the Kaplan-Mayer model was applied to the evaluation of survival probability.

RESULTS

Biochemistry

Demographic and biochemical characteristics of the patients at enrollment are shown in Table 1. The three groups were comparable for age, sex, biochemical parameters, genotype distribution and AgNOR-PI.

Table 1 Baseline patient characteristics

|                | OTCG   | TG     | CG     | P<   |
|----------------|--------|--------|--------|------|
| M:F ratio      | 27:14  | 17:13  | 18:12  | NS   |
| Age (yr)       | 55.3±1.8 | 54.6±2.1 | 57.2±2.0 | NS   |
| AST (U/L)      | 67.1±6.6 | 61.9±7.2 | 79.4±8.4 | NS   |
| ALT (U/L)      | 92.5±10.8 | 79.8±8.7 | 91.8±9.1 | NS   |
| γGT (U/L)      | 56.5±7.2 | 52.1±7.3 | 62.9±9.4 | NS   |
| Albumin (g/dL) | 4.2±0.07 | 4.2±0.07 | 4.1±0.06 | NS   |
| α1feto (ng/mL) | 6.8±1.25 | 8.4±2.36 | 6.3±1.0 | NS   |
| HCV1b (%)      | 63%    | 67%    | 65%    | NS   |
| AgNOR-PI (%)   | 20.1±2.35 | 19.6±2.84 | 18.2±2.6 | NS   |

OTCG = old treatment control group; TG = treatment group, CG = control group.

AgNOR PI

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significant differences both between OTCG and CG (Figure 4). The Kaplan-Mayer survival model showed statistically
subject in TG while 9 (30%) patients in CG developed HCC
of follow-up after about 50 mo from interferon withdrawal. No
Two nonresponders in OTCG developed HCC during 5 years
HCC appearance
and between TG and CG (Figure 2).

Figure 2 AgNOR-PI in OTCG before and after 1 year of IFN
treatment.

Figure 3 Incidence of HCC according to AgNOR-PI.

Figure 4 Percentage of HCC according to response to IFN.

Figure 5 Survival probability evaluated by Kaplan-Mayer model.

**HCC appearance**

Two nonresponders in OTCG developed HCC during 5 years
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(Figure 4). The Kaplan-Mayer survival model showed statistically
significant differences both between OTCG and CG (P<0.004)
and between TG and CG (P<0.003) (Figure 5). The HCC annual
rate of incidence in the CG was 5%.

**DISCUSSION**
The present data add new evidence on the clinical efficacy of
IFN re-treatment of cirrhotic patients and show the usefulness
of AgNOR-PI. The ability of IFN to prevent HCC development
is evident both alone and in combination with ribavirin.

Previous observations in patients with chronic hepatitis C[21-24],
with or without cirrhosis, reported that re-treatment with IFN
was more effective than single courses in preventing HCC
appearance. However, in these studies[21-24], the vast majority
of patients had chronic hepatitis and no conclusions could be
drawn on cirrhotic patients. We extended those observations
to patients with HCV related cirrhosis. In accordance with a
previous study[11], a single course of IFN did not seem to be
protective toward HCC appearance in a long term follow-up. In
fact, in the two nonresponder patients of OTCG, HCC developed
after about 50 mo, suggesting that the protective effect of IFN
may vanish over time. This hypothesis is strengthened by the
observation that re-treatment of nonresponders in the TG
prevented HCC development during the 5 years of follow-up.

It is interesting to note that no statistically significant
difference was observed in survival between TG and OTCG.
This may suggest that the addition of ribavirin to IFN did not
add any significant benefit in cirrhotics. However, the number
of patients was probably not sufficient to appreciate any possible
difference coming from the higher rate of sustained response
obtained with the combination treatment. However, it remains
that the key to HCC prevention is treatment with interferon that
might be helpful even after a curative resection of HCC[23].

Our study also showed that AgNOR-PI was a useful marker
of hepatocyte regeneration which is able to predict a possible
evolution to HCC. Furthermore, the two patients who developed
HCC in the OTCG were those with the highest AgNOR-PI
without improvement after treatment. This underlines the
relevance of the index in the clinical setting, particularly in
nonresponders to IFN. In fact, it may restrict the need of a strict
surveillance only to those patients with a higher risk of
developing HCC.

Previous observations with different techniques[26-30] have
shown that high hepatocyte proliferation is associated with
HCC development. A recent paper evaluating nucleolar
hypertrophy in patients with HBV and HCV related cirrhosis
reported the index was significantly predictive of HCC
development only in patients with HBV related cirrhosis[16]. In
patients with HCV related cirrhosis the index was not significantly
related with HCC development, although a trend could be
appreciated. A much larger and more homogeneous population
of patients with HCV related cirrhosis (only Child A) in our
study could account for the different results between the two
studies.

In conclusion, the preventive effect of IFN on HCC
development in HCV related cirrhosis is confirmed. Furthermore,
a more efficacious treatment associated with re - treatment
courses of nonresponders seems to produce the best results in
term of HCC prevention. AgNOR-PI is a useful marker of
hepatocyte proliferation that identifies patients at higher risk
of developing HCC.

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