

## Pilot study of interferon gamma for chronic hepatitis C<sup>☆</sup>

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**Background/Aims:** Currently, there are no effective therapies available for patients with chronic hepatitis C who have failed to respond to optimal interferon alfa-based regimens. The aims of this pilot study were to assess the antiviral activity and safety of interferon gamma in chronic hepatitis C.

**Methods:** Patients with chronic hepatitis C, genotype 1, who had not responded to or who had relapsed after therapy with interferon alfa and ribavirin were enrolled in a trial of interferon gamma 1b given in doses of 100, 200 or 400 µg subcutaneously three times weekly for 4 weeks. Frequent blood samples were obtained for HCV RNA levels.

**Results:** Fourteen patients were enrolled. Geometric mean HCV RNA levels remained unchanged. Serum aminotransferase levels also did not change, while there were significant decreases in neutrophil counts (–41% from baseline) and hematocrit (–5%). Low grade fever and malaise were common with the first injection of interferon gamma, but no serious side effects were encountered.

**Conclusions:** Although relatively well tolerated, interferon gamma in doses of 100–400 µg thrice weekly had no effect on HCV RNA levels in patients with chronic hepatitis C who had failed to achieve a sustained response to interferon alfa-based therapies.

Published by Elsevier B.V. on behalf of European Association for the Study of the Liver.

**Keywords:** Antiviral therapy; Cytokines; Interferon alfa; Interferon gamma; Viral kinetics; Bone marrow suppression

### 1. Introduction

Chronic hepatitis C affects more than 170 million people worldwide and is one of the most important causes of cirrhosis and hepatocellular carcinoma [1–3]. There have been marked improvements in the efficacy of antiviral therapy of hepatitis C since the first demonstration of the

utility of interferon alfa in 1986 [4]. However, even the optimal current regimen—the combination of peginterferon alfa and ribavirin for 24–48 weeks—yields sustained response rates of only 50–60% [5]. Furthermore, therapy is poorly tolerated and expensive, and many patients with chronic hepatitis C have contraindications to therapy. New therapeutic approaches for non-responders are urgently needed.

The interferons are a family of proteins with multiple actions including antiproliferative, antiviral and immunomodulatory activities. The interferons play a major role in the innate immune response to viral infections [6]. The expression of these cytokines is induced by exogenous stimuli such as viral infections or foreign antigens. Interferons then bind to cellular receptors and elicit a broad set of genes via complex intracellular signaling cascades [7]. Proteins encoded by these interferon response genes have direct antiviral properties by different mechanisms of action: activation of intracellular RNAases (2',5'

Received 4 January 2005; received in revised form 13 February 2005; accepted 14 February 2005; available online 26 April 2005

<sup>☆</sup> The authors who have taken part in this study have declared that they do not have a relationship with the manufacturers of the drugs involved either in the past or present and did not receive funding from the manufacturers to carry out their research.

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Abbreviations: HCV, hepatitis C virus; PCR, polymerase chain reaction.

oligoadenylate synthetase); inhibition of viral protein synthesis (double-stranded RNA-activated protein kinase) and blocking transport of viral ribonucleoproteins to the nucleus (MxA) [8,9].

Interferon alfa and beta (type I interferons) are thought to have predominantly antiproliferative and antiviral effects, whereas interferon gamma (type II interferon) is considered largely an immune modulator and mediator of T cell action [6]. Using the replicon system to study HCV replication, however, interferon gamma has been shown to have potent antiviral activity against HCV that appears to be equivalent or greater than interferon alfa or beta [10,11]. Because the actions of interferon gamma are mediated through different cell surface receptors than are used by interferon alfa, intracellular resistance may not be shared by the two interferons. For these reasons, interferon gamma is an attractive candidate treatment for chronic hepatitis C.

This pilot study was designed to assess the antiviral activity of interferon gamma as a single agent against HCV in patients with chronic hepatitis C who had failed to respond to a previous course of therapy with interferon alfa.

## 2. Patients and methods

### 2.1. Selection of patients

Enrollment criteria were: (1) age above 18 years, (2) serum alanine (ALT) or aspartate aminotransferase (AST) activities above the normal range, (3) presence of anti-HCV in serum, (4) presence of HCV RNA in serum in levels above 10,000 IU/ml, (5) HCV genotype 1 and (6) previous therapy with an adequate course of interferon alfa and ribavirin without a sustained virological response, 6-months or more before enrollment. An adequate course of therapy was defined as at least 24 weeks of interferon alfa in starting doses of 3 million units thrice weekly and ribavirin in starting doses of at least 1000 mg daily. Exclusion criteria were: (1) decompensated liver disease; (2) ALT levels higher than 1000 IU/l; (3) pregnancy or inability to practice adequate contraception; (4) pre-existing, moderate or severe bone marrow compromise; anemia (hematocrit < 34%), neutropenia (< 1000 polymorphonuclear cells/mm<sup>3</sup>) or thrombocytopenia (< 70,000 cells/mm<sup>3</sup>); (5) evidence of another form of liver disease in addition to hepatitis C; (6) significant systemic or major illnesses other than liver disease; (7) organ transplantation; (8) serious psychiatric disease or depression; and (9) evidence of hepatocellular carcinoma by imaging study done at the time of enrollment. Informed consent was obtained from each patient included in the study and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

### 2.2. Clinical study design

Patients underwent an initial evaluation that included a medical history and physical examination, a comprehensive battery of blood tests, and a quantitative determination of serum HCV RNA by polymerase chain reaction (PCR) using the Cobas Amplicor™ HCV Monitor Test, v2.0 (Roche Molecular Systems, Inc., Pleasanton, CA). Samples with results above the linear range of the assay were retested after dilution. All enrolled patients had a previous liver biopsy. Patients had at least three HCV RNA determinations in the 2-month period before starting therapy.

Patients were stratified according to previous response (responders or relapsers to interferon alfa) and randomized to receive 100 or 200 µg of interferon gamma-1b (Actimmune®, kindly provided by InterMune, Inc., Brisbane, CA) by subcutaneous injection three times per week for 4 weeks. After analysis of results from the initial 11 patients, the clinical research protocol was modified and the remaining patients received a dose of 400 µg

thrice weekly for 4 weeks. Blood samples for measuring HCV RNA levels were obtained before, at 6, 12, 18, 24 and 48 h after the first injection, and at weeks 1, 2, 3, 4, 6 and 8 afterwards. Compliance was determined from patient diaries. Visual analogue scales were used to monitor symptoms at every visit.

The primary endpoint of this pilot study was a decrease of HCV RNA levels by one log or more during treatment as detected by quantitative PCR. Samples were taken frequently after the first injection of interferon gamma to allow for study of initial viral kinetics.

### 2.3. Statistics

Continuous variables were compared with Student's *t*-test and categorical variables compared with Fischer's exact test or  $\chi^2$ -test as appropriate. All reported *P* values were two-sided and values of  $\leq 0.05$  were considered significant.

## 3. Results

### 3.1. Baseline patient characteristics

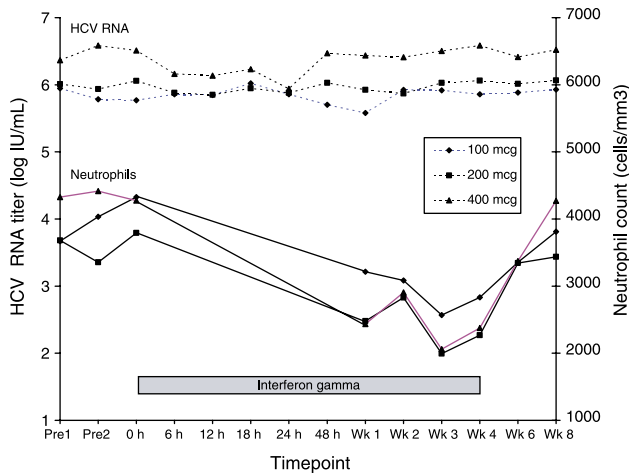
Fourteen patients were enrolled including 9 men and 5 women (Table 1), ages 41–59 years. Three patients were African Americans and 11 were Caucasians. Ten patients were non-responders to a previous course of interferon alfa and ribavirin (remaining HCV RNA positive during therapy), and four were relapsers (becoming HCV RNA negative during therapy but with reappearance of HCV RNA afterwards). Liver biopsies done within the previous 3 years showed active liver disease in all patients (inflammatory histology activity index scores ranging from 4 to 10) and some degree of fibrosis in 11 patients. Only one patient had cirrhosis. Six patients were assigned to receive 100 µg, five 200 µg and three 400 µg of interferon gamma thrice weekly.

### 3.2. HCV RNA levels

There was no significant overall change in geometric mean HCV RNA levels during treatment with interferon gamma and no patient had a decrease of more than 0.5 logs at any point during treatment. There were no significant differences in HCV RNA levels between previous non-responders and relapsers (data not shown) or among the three different doses used (Fig. 1).

**Table 1**  
Baseline patient characteristics

|  |             |
|--|-------------|
| Number   | 14          |
| Sex (male/female)  | 9/5         |
| Age (mean ± SD, years)                                     | 50.3 ± 5.2  |
| Race (White/African American)                              | 11/3        |
| Weight (mean ± SD, kg)                                     | 94.3 ± 20.2 |
| Body mass index (mean ± SD, kg/m <sup>2</sup> )            | 31.3 ± 6.4  |
| Interval from exposure (mean ± SD, years)                  | 25.3 ± 10.2 |
| Previous response to interferon (non-responders/relapsers) | 10/4        |
| Liver fibrosis (median Ishak score, 0–6)                   | 2           |
| Liver inflammation (median HAI score, 0–18)                | 8           |
| HCV genotype (1a/1b/1 not subtypeable)                     | 6/4/4       |



**Fig. 1.** HCV RNA levels during treatment by interferon gamma dose (dashed lines) and neutrophil counts (continuous lines) according to interferon gamma dose. The time axis is not to scale in order to show the early phase kinetics. There are no changes in HCV viral load, whereas there is a significant drop in neutrophil count (neutrophil counts from 6 to 48 h of treatment were interpolated). [This figure appears in colour on the web.]

### 3.3. Biochemical and hematological parameters

Aminotransferase levels did not change significantly during treatment (Table 2). There were significant decreases in white blood cell count (28%), neutrophils (41%), lymphocytes (11%) and hematocrit (5%) between baseline and week 4, but no patient developed neutropenia or anemia requiring dose modification (Grade III or IV toxicity). Strikingly, platelet counts did not change during or after treatment. In addition, there were no changes in serum bilirubin, albumin, blood urea nitrogen or creatinine levels (data not shown). Blood counts returned to pre-treatment levels within 4 weeks of stopping therapy.

### 3.4. Safety and side effects

Treatment was well-tolerated, and there were no severe adverse events. All patients completed the full 4 weeks of therapy without dose modification and no missed doses were recorded. Most patients complained of influenza-like symptoms of fatigue, nausea and low grade

fever 4–8 h after the initial injection. Oral temperatures did not rise above 38.5 °C. This reaction disappeared after the second or third injection in patients treated with the 100 and 200 µg doses. In contrast, the higher dose (400 µg) was associated with more frequent constitutional symptoms of fever, fatigue, and muscle aches, which lasted throughout the 4 weeks of treatment. Visual analogue scales were administered at each visit to assess fatigue and overall feelings of ‘well-being.’ Mean fatigue and well-being worsened minimally during therapy but returned to baseline after stopping treatment (data not shown). Body weight did not change.

## 4. Discussion

The antiviral effects of interferon gamma have been assessed in several small studies in chronic viral hepatitis, but mostly in chronic hepatitis B. In a study of hepatitis B, interferon gamma had similar effects to interferon alfa on serum levels of HBV DNA polymerase, but therapy for up to 6 months was poorly tolerated and did not lead to sustained remissions in disease [12]. Furthermore, when administered together, there was no evidence of synergy between interferon alfa and gamma. Several studies from other groups confirmed the lack of potent effects of interferon gamma in chronic hepatitis B [13–15]. There have been two published studies on the effects of interferon gamma in chronic hepatitis C. Investigators from Madrid, Spain, compared interferon alfa and gamma in 20 patients with chronic hepatitis C [16]. Therapy with interferon alfa led to decrease in serum aminotransferase levels, whereas interferon gamma had little effect. HCV RNA testing was not done, and only a single dose and regimen was evaluated. A second study from Japan used interferon alfa for 24 weeks followed by interferon gamma for 2 weeks, making it difficult to interpret whether interferon gamma had any antiviral activity above and beyond that of interferon alfa [17].

The effect of different cytokines combinations on HCV replication has been studied in the HCV replicon system in cell culture [18]. Both interferon alfa and gamma have antiviral activity against HCV

**Table 2**

**Biochemical, hematological and virological parameters at baseline, 4 weeks (end of interferon gamma treatment) and 8 weeks (follow-up)**

|  | Baseline (mean ± SD) | Week 4 (mean ± SD) | Week 8 (mean ± SD) | P       |
|--|----------------------|--------------------|--------------------|---------|
| ALT (IU/ml)                            | 76 ± 40              | 69 ± 23            | 72 ± 27            | NS      |
| AST (IU/ml)                            | 60 ± 31              | 60 ± 26            | 52 ± 14            | NS      |
| WBC (cells/mm <sup>3</sup> )           | 7432 ± 2672*         | 5294 ± 1698*       | 6790 ± 2080        | *0.0001 |
| Neutrophil (cells/mm <sup>3</sup> )    | 4232 ± 2090*         | 2532 ± 1289*       | 3676 ± 1455        | *0.0001 |
| Lymphocytes (cells/mm <sup>3</sup> )   | 2275 ± 790*          | 2018 ± 770*        | 2260 ± 884         | *0.005  |
| Hematocrit (vol%)                      | 44.3 ± 3.7*          | 42.2 ± 3.4*        | 43.8 ± 4.1         | *0.006  |
| Platelet count (plat/mm <sup>3</sup> ) | 253 ± 78             | 259 ± 85           | 254 ± 84           | NS      |
| HCV titer (log IU/ml)                  | 6.03 ± 0.74          | 6.09 ± 0.68        | 6.10 ± 0.71        | NS      |

and together show evidence of synergy [19]. The current clinical study demonstrated no evidence of antiviral activity of interferon gamma against HCV, but was not designed to assess possible synergy of the two cytokines. Interferon gamma has also been shown to have antifibrotic effects, but such activity was not the aim of the present study, and preliminary data from a phase II of interferon gamma have failed to show a significant effect in reversing fibrosis in cirrhotic patients with chronic hepatitis C [20].

One might argue that higher doses (800 or 1000  $\mu\text{g}$ ) or a more rigorous regimen (daily rather than thrice weekly) of interferon gamma should have been evaluated. Nevertheless, in this pilot study, the dose of interferon gamma was escalated to two- and four-times the conventional approved dose for chronic granulomatous disease. At a dose of 400  $\mu\text{g}$  thrice weekly, constitutional side effects became troublesome at the same time that there was no evidence of antiviral activity or improvement in serum ALT levels. Furthermore, decreases in neutrophils counts and hematocrit were significant and were similar to those that occur with effective doses of interferon alfa [21]. These findings indicate that even if higher doses were effective, they probably would not be tolerated clinically.

Viral kinetics has become a useful tool for assessing response to antiviral therapy in HIV infection and, more recently, in HCV infection [22,23]. Patients who have a sustained response to interferon alfa treatment usually show a rapid decrease in HCV RNA levels by 0.5–2 logs during the first 24–48 h ('first phase') and a slower ('second phase') decrease of 0.3–0.5 logs per week thereafter [24]. The first and second phase responses allow for early evaluation of the antiviral effects of therapy and are highly predictive of viral clearance. In this study, interferon gamma showed no evidence of either first or second phase effects on HCV RNA levels. During previous therapy with interferon alfa, none of the 14 enrolled patients had a sustained virological response, but four did become HCV RNA negative. These previous relapsers had no discernible decrease in RNA levels during 4 weeks of treatment with interferon gamma, suggesting that this form of interferon is unlikely to be effective even in naïve patients who might respond to interferon alfa.

In conclusion, the present study failed to show any significant antiviral effect of interferon gamma against HCV replication in patients with chronic hepatitis C who have previously failed to achieve a sustained response to interferon alfa therapy, despite showing biological effects as demonstrated by decreases in neutrophil and red blood cell counts. These results also indicate that *in vitro* activity seen in the HCV replicon model may not predict activity *in vivo* in patients. While the combination of gamma and alpha interferon may reveal synergistic antiviral effects between these two cytokines, the current results suggest that interferon gamma by itself is unlikely to have significant antiviral effects against the hepatitis C virus in humans.

## Acknowledgements

The authors thank the nursing staff of 8E and the NIDDK outpatient department for their support in managing patients in this study. The authors also thank Drs Edward Doo and Kittichai Promrat of the Liver Diseases Section, NIDDK, NIH; and Drs James Shih and Harvey Alter of the Department of Transfusion Medicine, Clinical Center, NIH for the help in conducting this study.

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