

CLINICAL STUDIES

Viusid, a nutritional supplement, in combination with interferon α -2b and ribavirin in patients with chronic hepatitis CEduardo Vilar Gomez¹, Bienvenido Gra Oramas², Enrique Arus Soler¹, Raimundo Llanio Navarro³ and Caridad Ruenes Domech¹

1 Department of Hepatology, National Institute of Gastroenterology, Havana, Cuba

2 Department of Pathology, National Institute of Gastroenterology, Havana, Cuba

3 Department of Gastroenterology, National Institute of Gastroenterology, Havana, Cuba

Keywords

antiviral therapy – chronic hepatitis C – complementary therapy – nutritional supplement – randomized controlled trial

Correspondence

Eduardo Vilar Gomez, Department of Hepatology, National Institute of Gastroenterology, Havana, Cuba, 23rd Avenue, 503, Vedado, Havana, Cuba.
 Tel: +53 7 8325067
 Fax: +53 7 8333 253
 e-mail: vilar@infomed.sld.cu

Received 7 March 2006

accepted 3 November 2006

DOI:10.1111/j.1478-3223.2006.01411.x

Abstract

Background: The pathogenesis of chronic hepatitis C (CHC) is associated to severe oxidative stress that leads to necro-inflammation and progression of fibrosis. Previous trials suggested that antioxidative therapy may have a beneficial effect. We evaluated the efficacy and safety of Viusid in combination with interferon α -2b (IFN α -2b) and ribavirin in patients with CHC. **Methods:** We randomly assigned 100 patients, between October 2002 and December 2004, in two arms: IFN α -2b (5 MU on alternate days), ribavirin at a dose of 13 mg/kg daily and Viusid (three sachets daily) vs. IFN α -2b (5 MU on alternate days) and ribavirin at a dose of 13 mg/kg daily. Subjects were treated for 48 weeks and then followed for an additional 24 weeks. The primary end point was the histologic response (reduction of at least two points without fibrosis worsening in the total score on the Histological Activity Index). **Results:** A significantly high proportion of patients who received combined therapy plus Viusid had a histologic response better than those patients who received IFN α -2b and ribavirin (57% vs. 37%, $P=0.03$). The patients with virologic response achieved the highest percentages of histologic response, irrespective of assigned treatment. Among non-responders, the highest reduction in the mean change from baseline score for necro-inflammatory activity (NA) and fibrosis (F) was reported in patients treated with Viusid [NA, -1.50 (Viusid), -1.20 (without Viusid); F, -0.31 (Viusid), 0.00 (without Viusid)]. Sustained normalization of serum alanine aminotransferase concentration was highest in the Viusid group compared with standard therapy (67% vs. 41%, $P=0.009$). The overall safety profile was similar in both groups, but interestingly, the anemia was less intense in the group with Viusid ($P=0.04$). **Conclusions:** Our results suggest that triple therapy with Viusid, IFN α -2b and ribavirin was well tolerated and may have a beneficial effect on histologic and biochemical variables. The intensity of anemia is reduced in patients treated with Viusid.

The current international standard of care treatment for chronic hepatitis C (CHC), peginterferon (PEG-IFN) α -2b in combination with ribavirin, has proven highly effective, achieving sustained viral eradication in approximately 54–61% of patients (1–3). However, treatment is expensive, it does not completely eradicate hepatitis C virus (HCV) infection; it is not suitable for all patients and it is associated with a significant morbidity. Therefore, there is an obvious need for the continuous development of new antiviral agents for HCV-infected patients. Although the primary aim of therapy in chronic hepatitis C is long-

lasting viral eradication, the secondary end points of reducing liver inflammation and preventing fibrosis progression are also important. The combination with antiviral, anti-inflammatory (antioxidant) and anti-fibrotic agents could increase the beneficial effect of the treatment, particularly for patients who fail to respond to antiviral therapy. Viusid, a nutritional supplement, has chemical ingredients (ascorbic acid, zinc and glycyrrhizic acid) with recognized immunomodulatory and antioxidant properties (4–8). The different compounds of the supplement are activated through a molecular activation principle that strongly increases

their biological activity without modifying their structure. With this method, the molecules are treated under a determined electric field, during a time span calculated beforehand, and under certain specific conditions for each kind of molecule (4). The chemical composition is summarized in Table 1.

Glycyrrhizin (GL), the most important molecule of the supplement, is known to have various immunomodulating, antiviral and biological response-modifier activities (9). GL is used in patients with hepatitis to reduce the activity of liver inflammation and the viral load, particularly in patients with chronic hepatitis C (10, 11).

The administration of antioxidant and immunomodulatory drugs for different disorders, including CHC, has generated a lot of interest recently; however, heterogeneous or controversial results on virologic, biochemical and histologic responses were observed between treatment regimens in any of the trials (12–17).

The adjuvant benefits of an antioxidant cocktail added to the short-acting IFN and ribavirin combination for patients with CHC have not been evaluated in clinical trials. Therefore, a randomized, controlled and pilot study was conducted to evaluate whether the addition of Viusid to the short-acting IFN and ribavirin therapy would be associated with better histologic improvement compared with the dual interferon and ribavirin therapy in naive and previously treated patients with CHC.

Methods

Selection of patients

Male and female patients ≥ 18 years of age, who had a positive test for antiHCV antibody, HCV RNA detectable in serum by PCR, alanine aminotransferase (ALT) persistently elevated > 1.5 times upper limit of normal (ULN) at least on two occasions, liver biopsy consistent with chronic hepatitis and IFN-naïve patients or patients who were non-responders to previous treatment with IFN, were included in the study.

Table 1. Ingredients of Viusid

Malic acid	0.666 g
Glycyrrhizic acid	0.033 g
Glucosamine	0.666 g
Arginine	0.666 g
Glycine	0.333 g
Calcium pantothenate	0.002 g
Ascorbic acid	0.020 g
Folic acid	66 mcg
Cyanocobalamine	0.3 mcg
Zinc sulfate	0.005 g
Pyrodoxal	0.6 mg

Criteria for exclusion included a coexisting serious medical or psychiatric condition, presence of other liver diseases, decompensated cirrhosis, HIV and HBV infection, previous organ transplantation, autoimmune diseases, severe retinopathy, serum α -fetoprotein concentration above 50 ng/ml and ultrasound without evidence of hepatocellular carcinoma within 3 months before the screening, a neutrophil count below 1500 ml^{-3} , a platelet count below $75\,000 \text{ ml}^{-3}$, hemoglobin concentration below 12 g/dl for women and 13 g/dl for men, a creatinine concentration > 1.5 times the upper limit of normal or unwillingness to practice contraception.

Study design and organization

This pilot, open-label, parallel-dose and randomized trial was conducted at only one center, (National Institute of Gastroenterology, Havana, Cuba) between October 2002 and December 2004. The study was conducted in compliance with the Declaration of Helsinki and approved by the ethics committee and the institutional review board of the center. All patients provided written informed consent. Consecutive patients who met the eligibility criteria were enrolled in this study. A simple randomization was performed and random assignments were made according to a computer-generated scheme generated by Applied Logic Center (Havana). Randomization numbers were sequentially allocated in the order in which patients were enrolled. The investigator and patients were blinded to randomization. Patients who met all eligibility criteria were randomized (ratio 1:1) to receive: Viusid, three oral sachets (50 g) daily; IFN α -2b (Heberon, Biotechnology Genetic Engineering Centre, Havana, Cuba) at a dose of 5 MU on alternate days plus oral ribavirin at weight-based regimen ($13 \text{ mg} \times \text{kg}$ of weight daily; $n = 49$) or IFN α -2b plus oral ribavirin at the same doses and duration as those of the experimental group ($n = 51$). Subjects were treated for 48 weeks and then followed for an additional 24 weeks.

Laboratory tests were performed for the assessment of serum HCV-RNA levels, ALT concentrations, viral genotyping and histologic evaluation of biopsy specimens at periodic intervals throughout the study. During the treatment, patients were assessed as outpatients at weeks 2, 4 and then every 4 weeks for the duration of treatment and the treatment-free follow-up. Biochemical and hematological testing were done with the same frequency. Serum HCV-RNA (Amplicor HCV Monitor, version 2.0; limit of detection, 50 IU/ml; Roche Molecular Systems, Basel, Switzerland) was measured

before treatment, during the treatment at weeks 4, 12, 24 and 48, and 24 weeks after treatment.

HCV genotyping was performed by genotype-specific synthetic peptides derived from the NS4 amino acid sequence using an enzyme-linked immunosorbent assay (ELISA) to detect the presence of type specific antibodies (HC02, Abbott Murex Laboratories, Abbott Park, IL, USA). Histologic evaluation was done before the treatment and 24 weeks after treatment. Biopsy specimens were examined without blinding before randomization, and evaluated in parallel at week 72 by a pathologist who was unaware of the patients' treatment assignment, response or timing of the biopsy relative to treatment. Early stopping rules based on genotype and HCV-RNA levels were not applied in the study. Subjects were treated for 48 weeks irrespective of HCV-RNA levels after 12 and 24 weeks of treatment. Patients were withdrawn from the study if they missed four consecutive weeks of the treatment, or if an investigator was concerned about safety. The analyses were entirely carried out in a single blinded manner at all levels (virology, histology, ALT and side effects).

Assessment of efficacy

Efficacy measures (end point)

Efficacy analysis included all randomized patients who received at least one dose of study medication. The primary end point was the histologic response.

Histologic response was defined as a reduction of at least two points without fibrosis worsening in the modified Histological Activity Index (HAI) of Ishak score (18) as compared with the pretreatment score. Score for this index can range from 0– to 24, with inflammation graded from 0 (none) to 18 (severe) and fibrosis graded from 0 (none) to 6 (cirrhosis). Patients with pretreatment and posttreatment liver-biopsy specimens were independently evaluated for necro-inflammatory activity and fibrosis. Both were ranked as improved, unchanged and worse.

The secondary end points were sustained virologic response, defined as undetectable serum HCV-RNA levels 24 weeks after the treatment was concluded, and sustained biochemical response, defined as normalization of ALT levels at the end of the follow-up period. Patients without follow-up data were considered as non-responders.

Safety analysis

The adverse events, biochemical and hematological measurements, clinical measurements and vital signs were considered as measures of safety.

All the possible adverse events that were reported with the use of IFN, ribavirin and Viusid were queried (54 items [frequent and uncommon adverse events]). The investigators recorded adverse events on a standard form and rated the severity as mild, moderate, severe or potentially life-threatening according to the modified World Health Organization (WHO) guidelines. Moreover, the investigators attempted to assign causal status of the adverse event in any way. During the treatment, patients were assessed as outpatients at week 1, 2, 4, 6, 8 and 12, and then every 4 weeks for the duration of the treatment, and also 4, 12 and 24 weeks after the end of the therapy. All randomized patients who received at least one dose of medication were included in safety analysis. Dose modifications of IFN α -2b to IFN α -3 or 1.5 MU were permitted in patients who experienced clinically significant adverse events or laboratory abnormalities. Therapy was permanently discontinued for life-threatening, and the subject was monitored during the 24-week follow-up or every 2 weeks thereafter, until the event was resolved or the patient's condition stabilized. All grade 3 adverse events except flu-like symptoms were controlled by dose reduction; dose had increased if the adverse event improved to grade 1 or no longer occurred. The doses of ribavirin were reduced to 800 mg when the hemoglobin concentration was decreased below 9 g/dl, or discontinued in patients with hemoglobin levels less than 8 g/dl. The use of erythropoietin was prohibited.

Statistical analysis

Descriptive statistics for patients were reported. Continuous variables were summarized as means \pm SD. Efficacy measurements included all patients who were randomized and received at least one dose of study medication. The study was designed to have a statistical power of 80% to detect an absolute difference of 28% in the rates of histologic response (67% in the group with Viusid vs. 39% in the standard combination group) at a 5% level of significance, and given the enrolment of 51 and 49 patients in each group. Except for changes from the baseline in histologic findings, all efficacy end points were evaluated by intention to treat analysis. No interim analysis was performed. The primary end point was the comparison of histologic response between each treatment group. The analysis of the histologic end point included all patients with assessable baseline biopsy specimens. Patients with missing posttreatment biopsy data were counted as histologic non-responders. The differences between histologic response rates were tested with a Fisher's exact test on a significance level of $\alpha = 0.05$. A change

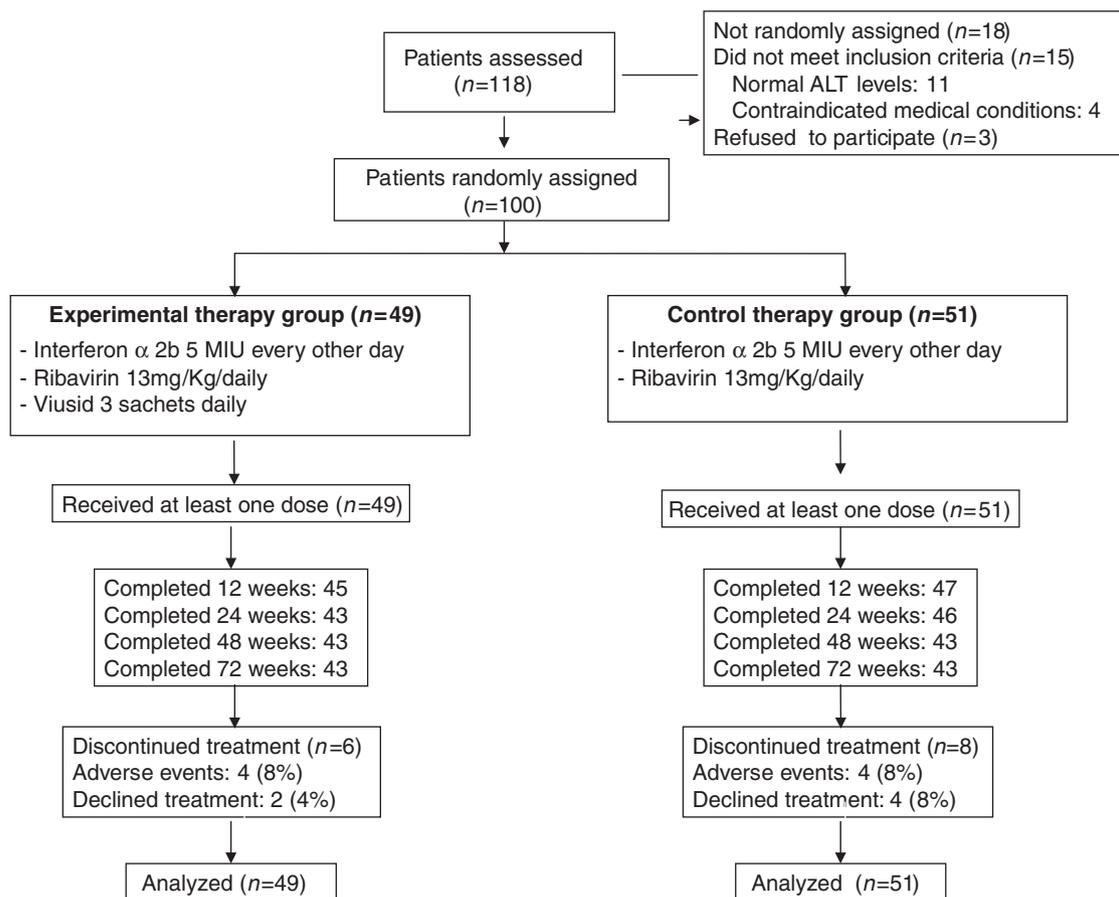


Fig. 1. Flow of participants through the study.

from baseline in the total HAI score was analyzed only in patients who had undergone both pretreatment and posttreatment liver biopsy. A paired Student's *t*-test was used to assess the significance of change in scores within each treatment group.

Fisher's exact test was used to compare virologic, biochemical and histologic responses between each group of treatment. A paired Student's *t* test was used to assess the significance of change in aminotransferase, hemoglobin and ribavirin mean concentrations between each group of treatment. Statistical analyses were done using SPSS Inc. for Windows, release 10, Chicago, IL. All confidence intervals, significance tests and resulting *P* values were two sided, with an α level of 0.05.

Patients with missing postbaseline virologic and biochemical data were considered as non-responders.

Results

Characteristics of the patients at baseline

Enrolment began in March 2002 and the study was completed in October 2004. A total of 118 patients

were screened and 100 were enrolled, randomly assigned and treated (Fig. 1). Baseline demographics and disease characteristics were comparable across the two groups (Table 2). Most patients were infected with HCV genotype 1.

Of the 100 patients enrolled, 86 completed the treatment. The end of assessment period was completed by 43 patients in each group of treatments. None of the patients was lost during the follow-up period. Patients who discontinued drug therapy were assessed upto week 72. The main reasons for withdrawal were hepatic failure, refusal of treatment or laboratory abnormalities. None of the patients received cointerventions during the trial that could have affected the outcomes.

Histologic response (primary end point)

The primary analysis was based on 100 patients with assessable baseline liver biopsy specimens (intention-to-treat analysis). A total of 75 patients (74%) had assessable pretreatment and posttreatment liver biopsy specimens (37 and 38 patients in control and Viusid

Table 2. Baseline characteristics of the patients

Characteristics	IFN α -2b	
	Ribavirin (<i>n</i> = 51)	Ribavirin/ Viusid (<i>n</i> = 49)
Age (years), mean \pm SD*	48.8 \pm 10.7	48.2 \pm 11.5
Sex no. (%)		
Male	22 (43)	21 (43)
Female	29 (57)	28 (57)
ALT (U/l), mean \pm SD	88.3 \pm 18.3	78.7 \pm 11
Body mass index (mean \pm SD)	21 \pm 2.99	20.6 \pm 2.98
Prior HCV medications – no. (%)		
Naive	39 (76)	36 (73)
Non-responders to interferon	12 (24)	13 (27)
HCV genotype – no. (%)		
1	43 (84)	46 (94)
2/3	4 (8)	–
Unknown	4 (8)	3 (6)
Viral load – no. (%)		
> 800 000 IU	36 (70)	34 (69)
< 800 000 IU	12 (24)	14 (29)
Unknown	3 (6)	1 (2)
Histologic diagnosis		
Ishak score (mean \pm SD)		
Total	6.88 \pm 3.54	6.28 \pm 3.87
Necro-inflammatory activity	4.04 \pm 1.52	3.65 \pm 1.85
Fibrosis	2.84 \pm 2.64	2.63 \pm 2.60
Brindging fibrosis – no. (%)	3 (6)	2 (4)
Cirrhosis – no. (%)	19 (37)	16 (33)

*All values expressed with a plus/minus sign are means \pm SD.

IFN α -2b, interferon α -2b.

group respectively). The histologic improvement, defined as a reduction in at least two points of HAI without fibrosis worsening, was observed in 57% (28/49) of the patients assigned to the Viusid group in comparison with 37% (19/51) of the patients in the control group ($P=0.036$), with an absolute difference of 19.9% and a 95% confidence interval for the difference of 1 – 39 [odds ratio, 2.25 (95% confidence interval (CI): 1.01–5.01); relative risk, 1.53 (95% CI: 1.00–2.36)]. A secondary analysis was based only on paired-liver biopsies. The analyzed groups were stratified according to virologic and biochemical responses (responders and non-responders), and histologic response (HAI, necro-inflammatory activity and fibrosis). HAI, necro-inflammatory and fibrosis score improvements were also clinically important in the overall group assigned to receive Viusid in combination with standard therapy. HAI score improved in 28 of 38 patients treated with Viusid compared with 19 of 37 patients treated with combination therapy alone (95% CI: 1–44%; $P=0.038$) [odds ratio, 2.65 (95% CI: 1.01–6.98); relative risk 1.43, (95% CI: 0.99–2.07)].

Moreover, the proportion of patients with necro-inflammatory activity (NA) and fibrosis (F) improvements, although it was not statistically significant, was clinically higher in the group of patients receiving Viusid (NA, 28 of 38 patients [74%]; F, 11 of 38 patients [29%]) in comparison with the patients receiving IFN and ribavirin (NA, 22 of 37 patients [59%]; F, 7 of 37 patients [19%]).

The patients with sustained virologic response (SVR) achieved higher percentages of histologic response, irrespective of the treatment regimen they received. Among non-responders, 47% of the patients receiving Viusid had some evidence of HAI improvement in comparison with 32% of the patients treated with IFN and ribavirin. At the same time, improvement in NA and fibrosis was observed more frequently in non-responder patients receiving Viusid in combination with IFN and ribavirin. In addition, a higher reduction in the mean change from baseline score for NA and fibrosis was reported in non-responder patients treated with Viusid in comparison with those treated with standard therapy [NA, –1.50 (Viusid), –1.20 (without Viusid); fibrosis, –0.31 (Viusid), 0.00 (without Viusid)]. Table 3 summarizes the histologic response in patients with paired liver biopsies.

Similar to the virologic response, biochemical response was associated with evident histologic improvement, irrespective of the assigned treatment regimen. Among patients who did not achieve a sustained biochemical response, there was more frequent improvement of IHA, NA and fibrosis in the group treated with Viusid in comparison with control group [IHA score, –1.75 (Viusid), –0.66 (without Viusid); NA, –1.62 (Viusid), –1.16 (without Viusid); fibrosis, –0.20 (Viusid), 0.50 (without Viusid)].

Virologic response (secondary end point)

The SVR rate was comparable in the two groups: patients treated with Viusid (49% [24/49]) in association with IFN and ribavirin, and patients with standard combination therapy (43% [22/51]) (95% CI: –14 to 25%; $P=0.68$). The virologic response is summarized in Table 2. Among the subjects who had no detectable viral RNA for the first time more than 24 weeks after the start of the treatment, four patients assigned to receive Viusid achieved end treatment virologic response as compared with only one patient treated with IFN and ribavirin. Undetectable or 2-log, or greater drop in serum HCV-RNA, was observed at week 12 in 73% (36/49) of patients assigned to receive Viusid in comparison with 65% (33/51) of the subjects who received IFN and ribavirin alone ($P=0.21$).

Table 3. Rates of histologic response and changes in necro-inflammatory activity and fibrosis scores from baseline to week 72

Variable: all subjects with paired biopsies	IFN α -2b		Ribavirin/viusid ($n = 38$)	
	Ribavirin ($n = 37$)		Ribavirin/viusid ($n = 38$)	
	Number and (%) of improved patients	Mean change in score	Number and (%) of improved patients	Mean change in score
Total score*				
All patients	19 (51)	- 2.25	28 (74)	- 1.95
Virologic responders	16 (73)	- 3.41	23 (96)	- 2.60
Virologic non-responders	9 (31)	- 1.20	12 (48)	- 1.50
Biochemical responders	15 (71)	- 3.65	23 (70)	- 2.28
Biochemical non-responders	8 (26)	- 0.66	10 (62)	- 1.75
Necro-inflammatory activity†				
All patients	22 (59)	- 2.25	28 (74)	- 1.95
Virologic responders	18 (81)	- 3.41	22 (91)	- 2.60
Virologic non-responders	10 (45)	- 1.20	14 (58)	- 1.50
Biochemical responders	16 (76)	- 2.92	24 (72)	- 1.87
Biochemical non-responders	10 (33)	- 1.16	10 (62)	- 1.62
Fibrosis‡				
All patients	7 (19)	- 0.27	11 (29)	- 0.44
Virologic responders	6 (27)	- 0.58	8 (33)	- 0.90
Virologic non-responders	3 (10)	0.00	6 (24)	- 0.31
Biochemical responders	6 (29)	- 0.55	9 (27)	- 0.70
Biochemical non-responders	1 (4)	0.50	5 (31)	- 0.20

*Histologic response was defined as a ≥ 2 points improvement in the HAI score without worsening of fibrosis.

†Necro-inflammatory activity improvement was defined as a ≥ 2 points reduction.

‡Fibrosis improvement was defined as a reduction in at least 1 point.

There was no virologic benefit of the combination with Viusid for patients with genotype 1, high viral load and marked fibrosis (cirrhosis or bridging fibrosis). The virologic response is summarized in Table 4.

Biochemical response (secondary end point)

At the end of treatment (week 48), the proportion of patients with normalized ALT levels was 77% (38/49) in the Viusid group in comparison with 58% (30/51) in the control group (95% CI: 1 – 37%; $P = 0.036$) [odds ratio, 2.42 (95% CI: 1.01–5.79); relative risk, 1.32 (95% CI: 1.00–1.74)]. The proportion of subjects with normal ALT levels at the end of the follow-up period was significantly higher for the Viusid group [67% (33/49)] than for the control group [41% (21/51)] (95% CI: 7 – 45%; $P = 0.009$) [odds ratio, 2.95 (95% CI: 1.30–6.67); relative risk, 1.64 (95% CI: 1.12–2.40)]. In patients without a virologic response during treatment, the percentage of normalization of ALT levels at the end of the treatment was significantly higher in the Viusid group (10/24 [42%]) compared with the patients treated with standard therapy (2/22 [9%]; $P = 0.018$) [odds ratio, 7.14 (95% CI: 1.35–37.75); relative risk, 4.58 (95% CI: 1.13–18.65)]. The biochemical response is summarized in Table 4. Median ALT levels were consistently lower during the

treatment and follow-up period in subjects treated with Viusid compared with patients treated with IFN and ribavirin.

The patterns of ALT levels throughout the study are shown in Fig. 2. Strength association was observed between ALT levels and the histologic response during the treatment, up to the end of the follow-up period.

In the Viusid group, 25 of 28 patients (89%) with histologic improvement had reached normal values of ALT by week 12, comparable with 15 of 19 patients (79%) receiving standard treatment. At the end of the follow-up period, all patients with histologic improvement and independently of the treatment assignment had normal levels of ALT.

Combined response

The combined rates of sustained biochemical and virologic responses were 43% (21 of 49 patients) in the group treated with Viusid in comparison with 31% (16 of 51 patients) in the group treated with IFN and ribavirin (95% CI: - 7 to 30%; $P = 0.16$).

Safety

The incidence of discontinuation of treatment was low in the two groups. Two deaths were observed in the

Table 4. Rates of virologic, biochemical and histologic response*

Variable	IFN α -2b, no. (%) [95% CI]†		P-value
	Ribavirin (n = 51)	Ribavirin/Viusid (n = 49)	
Virologic response			
Week 4	12 (24) [13–38]	14 (29) [17–43]	0.36
Week 12	32 (63) [48–76]	36 (73) [59–85]	0.17
Week 24	30 (59) [44–72]	28 (57) [42–71]	0.64
Week 48	28 (55) [40–69]	32 (65) [50–78]	0.19
Week 72	22 (43) [29–58]	24 (49) [34–64]	0.35
Biochemical response			
Week 12	30 (59) [44–72]	38 (78) [63–88]	0.036
Week 24	32 (63) [48–76]	40 (82) [68–91]	0.029
Week 48	30 (58) [44–72]	38 (77) [63–82]	0.036
Week 72	21 (41) [28–56]	33 (67) [52–80]	0.009
Combined response			
Week 72	16 (31) [19–46]	21 (43) [29–58]	0.16
Histologic response			
Week 72	19 (37) [24–51]	28 (57) [42–71]	0.036

*The assessment of efficacy was carried out by intention-to-treat principle. Virologic response was defined as undetectable HCV-RNA (< 50 IU/ml). Biochemical response was defined as normalization of ALT levels below the upper limit of normal (49 IU/l). Combined response was defined as combination of undetectable HCV-RNA plus normalization of ALT. Histologic response was defined as reduction of at least two points, without concurrent worsening of fibrosis, in the modified Histological Activity Index score according to criteria of Ishak et al. (8). Patients without paired biopsy samples were classified as having non-response.

†CI denotes confidence interval (exact 95% confidence interval for binomial probabilities).

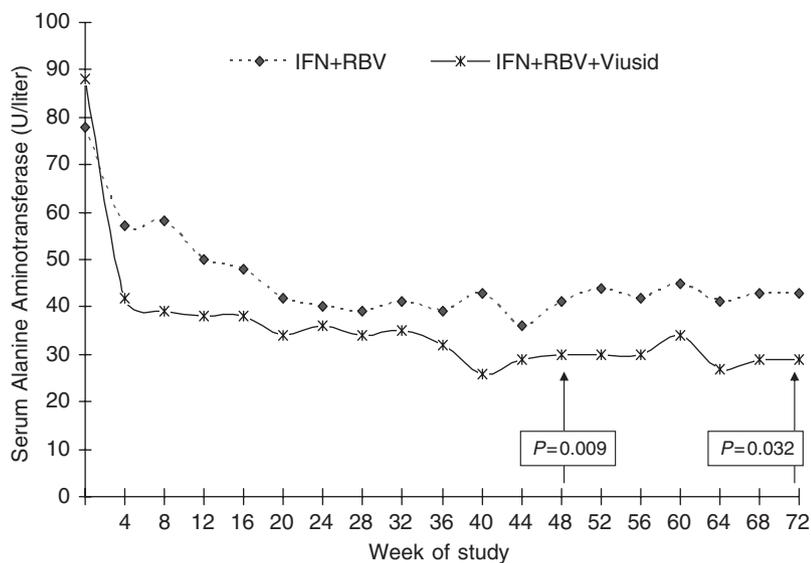


Fig. 2. Mean serum alanine aminotransferase levels during the 72-week study period.

group with IFN and ribavirin. Hepatic failure was reported as the cause of the deaths. They occurred in patients with previously compensated cirrhosis, 18 and 20 weeks after the end of treatment, respectively. Modification of ribavirin dose was necessary because of anemia in 15 of 51 patients (29%) assigned to IFN and ribavirin as compared with six of 49 (12%) of those assigned to the Viusid group ($P=0.030$) [odds ratio, 2.99 (95% CI: 1.05–8.49); relative risk, 2.40 (95% CI: 1.01–5.69)]. The mean ribavirin dose at the

start of HCV therapy was similar in both study groups (1173 ± 68.1 mg/day in the conventional group and 1173 ± 69.4 mg/day in the Viusid group). In the Viusid group, the mean ribavirin dose had not changed significantly from baseline (1145 ± 124 mg/day) to 12 weeks of the treatment, as compared with the group treated without Viusid (1073 ± 159 mg/day, $P=0.020$). No discontinuation of ribavirin was reported.

Anemia was most frequent in the first 12 weeks of the treatment. A sudden drop in hemoglobin

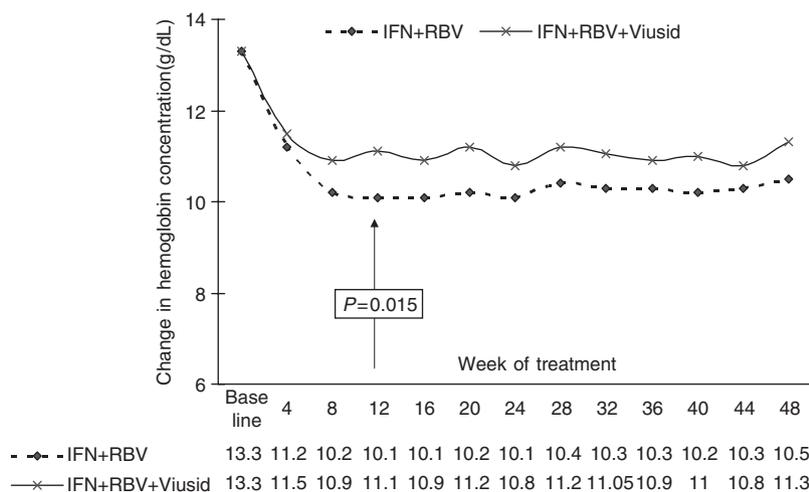


Fig. 3. Mean change in hemoglobin concentration during the 48-week treatment period.

concentration of more than 4 g/dl was reported in the first 12 weeks of the treatment in 23 of 51 patients assigned to IFN and ribavirin (44%) in comparison with 13 of 49 patients assigned to Viusid treatment (26%) [odds ratio, 2.27 (95% CI: 1.01–5.27); relative risk, 1.70 (95% CI: 1.00–2.96); $P=0.046$]. Hemoglobin concentration decreased to <10 g/dl in 43% and 29% of patients treated with IFN and ribavirin and the combination with Viusid, respectively ($P=0.036$); and to <8.5 g/dl in 27% of patients without Viusid and 14% of patients with Viusid ($P=0.08$). At baseline, mean hemoglobin concentration was similar in the two groups (13.3 g/dl). However, mean hemoglobin concentration throughout the 48 weeks of treatment was higher in the group receiving Viusid (Fig. 3).

In the majority of the patients, the total platelet count remained above the normal value. No patient in either group had a platelet count of less than $100\,000\text{ ml}^{-3}$ during the treatment, and in none of the patients, was treatment discontinued because of thrombocytopenia. The proportion of patients with a reduction of neutrophil count was similar in both groups (10%). None of the patients required dose reduction secondary to neutropenia. Neutrophil, as well as platelet counts and the hemoglobin concentrations returned to baseline levels shortly after treatment was stopped. No patient discontinued treatment because of anemia, thrombocytopenia or neutropenia.

The rates of adverse events were similar in both groups. Table 5 summarizes the adverse events that occurred during the first 12 weeks of the treatment.

The most common adverse events were typical of those previously reported for IFN α - and ribavirin, including fever, asthenia, myalgia, irritability, depression, insomnia and anorexia. Nausea was reported in

49% of patients receiving Viusid and in 39% of those receiving IFN and ribavirin. Anorexia and dizziness were reported more frequently in the group assigned to receive standard therapy as compared with the group receiving Viusid. Serious adverse events were infrequent. One serious infection (bacterial endocarditis) was reported in the group with Viusid. Hepatic decompensation was reported in two previously compensated cirrhotic patients of each group during the treatment period.

Discussion

Alone or combined with ribavirin, liver histology is a surrogate end point of the long-term efficacy of IFN treatment. Histologic response is clearly related to antiviral response (1–3, 19–24). Several recent large multicenter RCTs (1–3) have demonstrated that pegylated IFN, in combination with ribavirin, produces high percentages of virologic response and subsequently high percentages of histologic improvement; however, in those patients without virologic response, the histologic improvement is limited. In our study, we defined histologic response as reduction of at least two points in the HAI, without concurrent worsening of fibrosis. However, in secondary analysis, we defined histologic response as a decrease of at least one point in fibrosis score (staging) relative to the baseline biopsy score, or a decrease of at least two points in the activity score (grading). These definitions were based on recommendations appearing in the most recent consensus statements of the EASLD and the NIH (25, 26). In the current study we found that Viusid, in combination with IFN and ribavirin, significantly reduces HAI score compared with standard

Table 5. Incidence of discontinuation of treatment, dose modification and adverse events*

Variable no. (%)	IFN α -2b†	
	Ribavirin	Ribavirin/ viusid
Discontinuation		
Owing to adverse events‡	4 (8)	4 (8)
Owing to declined treatment	4 (8)	2 (4)
Dose modification		
Owing to adverse events	2 (4)	2 (4)
Owing to laboratory abnormality§		
Anemia	15 (29)	6 (12)
Neutropenia	5 (10)	5 (10)
Adverse events		
Influenza-like symptoms		
Fever	45 (88)	40 (82)
Asthenia	44 (86)	43 (85)
Myalgia	39 (76)	34 (69)
Fatigue	23 (45)	22 (45)
Headache	23 (45)	22 (45)
Psychiatric symptoms		
Irritability	33 (65)	32 (65)
Depression	31 (61)	29 (59)
Insomnia	26 (51)	29 (59)
Neurological symptoms		
Impaired concentration	22 (43)	19 (39)
Dizziness	22 (43)	14 (29)
Cramps	19 (37)	20 (41)
Gastrointestinal symptoms		
Anorexia	41 (80)	33 (67)
Nausea	20 (39)	24 (49)
Upper abdominal pain	15 (29)	13 (26)
Diarrhea	8 (16)	13 (22)
Dermatologic symptoms		
Dry skin	25 (49)	22 (45)
Pruritus	22 (43)	25 (51)
Alopecia	14 (27)	13 (26)

*Only symptoms that occurred in at least 20% of all patients were included. Patients may have had more than one adverse event.

†Interferon α -2b.

‡The death of two patients secondary to liver failure occurred in the control group.

§Other laboratory abnormalities consisted of thrombocytopenia, hypothyroidism and hyperthyroidism.

combination regimen. The absolute difference was 19.9% (95% CI: 1–39%) between the standard and experimental regimen. No clinically significant improvement was observed. The degree of histologic fibrosis is an important marker of the stage of diseases (27) because the natural history of hepatitis C involves the gradual progression of hepatic fibrosis that can eventually lead to cirrhosis. Treatment that could halt or diminish the progression of fibrosis would be theoretically beneficial (28). A favorable effect of antiviral therapy on hepatic histology, including fibro-

sis, has been demonstrated in most clinical trials. These studies have reported fibrosis improvement in 14–22% of patients treated with the different strategies of treatment with the short-acting and the pegylated IFN in monotherapy, or in combination with ribavirin, respectively (1, 19–24, 28–33). In the current study, we observed that the addition of Viusid to conventional treatment had a clinical impact on fibrosis reduction (29%) compared with the short-acting IFN and ribavirin combination, although these differences were not statistically significant. The main end point of treatment in non-responder patients is histologic improvement, in particular fibrosis. To date, a combination of PEG-IFN α -2b and ribavirin combination has been the best approach to halt or slow fibrosis progression in non-responders to antiviral treatment (28). Recently, Poynard et al. (29) have reported similar percentages of fibrosis improvement in patients without virologic response with the combination therapy with short-acting (17%) or pegylated IFN (18%) and ribavirin. In the current study we observed that Viusid, in addition to standard combination, provided a clinically significant fibrosis improvement in virologic (24% [Viusid] vs. 10% [standard combination]) and biochemical (36% [Viusid] vs. 4% [standard combination]) non-responder patients in comparison with standard treatment. Finally, an important mean reduction in the Ishak fibrosis score was noted in virologic and biochemical non-responder patients treated with Viusid, in comparison with unmodified or increased scores for patients treated with standard combination. Similarly, an insignificant median reduction (-0.1 [short-acting IFN and ribavirin]) or increase ($+0.2$ [PEG-IFN and ribavirin]) in the fibrosis score was observed in patients treated with short-acting or PEG-IFN and ribavirin (1, 19, 20, 22). The addition of Viusid to short-acting IFN and ribavirin therapy has demonstrated conclusively that notable improvement in term of fibrosis can be achieved in patients without virologic and biochemical responses and, to a lesser degree, in overall and virologic responder patients. However, these are only preliminary observations that should be carefully interpreted. Further studies may need to evaluate the histologic effect of Viusid on non-responder patients along with the antiviral treatment of patients with an advanced stage of fibrosis (F3 – F6). The mechanisms responsible for explaining the effect of Viusid on liver histology are unknown. Nevertheless, preliminary studies have observed that GL (the most important ingredient of Viusid) exerts anti-inflammatory actions on hepatic histology of patients with chronic hepatitis (34, 35).

GL has different anti-inflammatory properties [increased production of IL-10 (is a potent anti-inflammatory cytokine which inhibits the syntheses of many proinflammatory proteins)] (36), antiapoptotic effect (37), hepatocyte proliferation (38), and stabilization of hepatic cellular membranes (39). On the other hand, the immunomodulatory and antioxidant role of ascorbic acid and zinc in different diseases is well known (5–8). Nevertheless, the exact mechanism of liver injury and fibrosis in CHC is unclear. There is scientific evidence that oxidative stress might play a role in CHC liver damage. The evidence of oxidative stress as a pathogenetic mechanism is suggested by increased levels of lipid peroxidation products, high superoxide dismutase activity and increased glutathione-biosynthetic activity in serum of patients with CHC compared with healthy controls (40–45). Enhanced oxidative stress in the liver of patients with CHC (46) is believed to initiate a cascade leading to active fibrogenesis. Oxidative stress and reactive aldehydes induce activation of hepatic stellate cells. Activation of hepatic stellate cells is a critical step in liver fibrosis because activation markedly stimulates collagen production by these cells. Administration of antioxidants for different disorders, including CHC, has generated a lot of interest recently. The role of Viusid in the histologic improvement might be supported by the antioxidant properties of different compounds of the supplement; however, this is only a hypothesis, and further studies are needed to evaluate the potential mechanism of Viusid in patients with chronic hepatitis C.

The proportion of subjects who experienced a sustained normalization of ALT was higher among patients with SVR compared with subjects who experienced a relapse or showed no response. The overall group treated with Viusid showed the highest percentages of normalization of ALT in comparison with the group receiving IFN and ribavirin alone. The benefit of Viusid treatment on the normalization of ALT was also superior in patients with relapsed or no response to antiviral treatment. These results can be very encouraging as sustained biochemical response is associated with diminished hepatic inflammatory activity, and further progression of liver damage may be prevented. Several herbal medicinal products and supplements with potential virologic and biochemical effects in the treatment of CHC have been identified; however, according to a recent systematic review, no significant and sustained effects in virologic and biochemical response have been reported (47).

In general, the side effects observed in both groups of treatment were similar. The incidence of dizziness

and anorexia was lowest in patients treated with Viusid. Nausea was most apparent in patients receiving Viusid treatment. No new side effects were observed, but less frequent modifications of ribavirin dose were needed in patients receiving Viusid, particularly for anemia. The incidence of discontinuation by adverse events was similar in each group. The addition of Viusid to combination therapy improved HCV therapy-induced anemia. Patients who received Viusid demonstrated a mean increase in Hb concentration = 1 g/dl by week 12, in comparison with patients treated with IFN and ribavirin. Moreover, the severity of anemia during the first 12 weeks of the treatment was lesser in the Viusid group; because of the sudden drop in Hb concentration, ≥ 4 g/dl was avoided in higher percentages of patients. These results are very encouraging because ribavirin-related anemia in patients with CHC may alter adherence to treatment with impairment of virologic response (48–50). However, these results should be carefully interpreted and not be absolutely attributed to the addition of Viusid until new studies are developed. There are widely variable approaches to the management of anemia during combination therapy (51–53). Until now, recombinant human erythropoietin therapy has represented an alternative to ameliorate anemia and quality of life secondary to the administration of ribavirin; however, a recent study has reported that epoetin α is not cost-effective for genotype 1 HCV patients who have developed treatment-related anemia (54, 55).

The mechanism of ribavirin-induced anemia has been recently described. The leading hypothesis is that the accumulation of ribavirin in the red cell leads to an overwhelming oxidative stress (56). The antioxidant action of Viusid by way of reduced glutathione (GSH) could be an important mechanism to ameliorate ribavirin-induced anemia. A recent study reported a stable and gradual increase of GSH concentrations in patients treated with Viusid during 12 weeks in comparison with baseline levels (57). The increase of GSH concentrations leads to an improvement in the antioxidant mechanism, resulting in less membrane oxidative damage. The erythrocyte is therefore less sensitive to oxidative stress.

The different results observed in our study should be carefully interpreted because of the small sample size and the open label design (it is not a placebo-controlled study). These were the main limitations of the study.

In summary, we have shown that the addition of Viusid to IFN and ribavirin is associated with improvement on hepatic histology and sustained normalization of ALT values, especially those patients who

do not develop a sustained virologic response. Our data suggest that Viusid may be a therapeutic option to ameliorate ribavirin-induced anemia, while optimal ribavirin doses are maintained. However, further studies are needed in order to evaluate the potential impact of Viusid in combination with PEG-IFN and ribavirin.

Acknowledgements

We are indebted to Dr. Scott L. Friedman, MD, of Mount Sinai School of Medicine, New York, for his critical review during the preparation of the manuscript.

Ethics approval: The study was approved by the Institutional Review Board and Ethics Committee of the National Institute of Gastroenterology, Havana, Cuba.

Sponsor details: Supported by Catalysis, Madrid, Spain.

Competing interest statement: Grant support was received from Catalysis Laboratories, Spain. Viusid and diagnostic kits for the detection of HCV-RNA were provided by Catalysis Laboratories. The study investigators had access to all study data, took responsibility for the accuracy of the analyses, and had full and independent authority over the preparation of the manuscript and decisions concerning publication.

Conflict of interest: none

References

- Manns MP, McHutchinson JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. *Lancet* 2001; **358**: 958–65.
- Fried MW, Shiffman ML, Reddy RK, et al. Peginterferon alfa-2a (40 KD) in combination with ribavirin: efficacy and safety results from a phase III, randomized, actively controlled multicenter study. *N Engl J Med* 2002; **347**: 975–82.
- Hadziyannis SJ, Sette H, Morgan TR, et al. Peginterferon- α 2a and ribavirin combination therapy in chronic hepatitis C. A randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140**: 346–55.
- Wintergerst ES, Maggini S, Hornig DH. Immune-enhancing role of vitamin C and zinc and effect on clinical conditions. *Ann Nutr Metab* 2006; **50**: 85–94.
- Duarte TL, Lunec J. Review: when is an antioxidant not an antioxidant? a review of novel actions and reactions of vitamin C. *Free Radic Res* 2005; **39**: 671–86.
- Cuevas LE, Koyanagi A. Zinc and infection: a review. *Ann Trop Paediatr* 2005; **25**: 149–60.
- Oteiza PI, Mackenzie GG. Zinc, oxidant-triggered cell signaling, and human health. *Mol Aspects Med* 2005; **26**: 245–55.
- Kang YJ, Zhou Z. Zinc prevention and treatment of alcoholic liver disease. *Mol Aspects Med* 2005; **26**: 391–404.
- Miyaji C, Miyakawa R, Watanabe H, et al. Mechanism underlying the activation of cytotoxic function mediated by hepatic lymphocytes following the administration of glycyrrhizin. *Int Immunopharmacol* 2002; **2**: 1079–86.
- van Rossum TG, Vulto AG, Hop WC, et al. Glycyrrhizin-induced reduction of ALT in European patients with chronic hepatitis C. *Am J Gastroenterol* 2001; **96**: 2432–7.
- Arase Y, Ikeda K, Murashima N, et al. The long term efficacy of glycyrrhizin in chronic hepatitis C patients. *Cancer* 1997; **79**: 1494–500.
- Look MP, Gerard A, Rao GS, et al. Interferon/antioxidant combination therapy for chronic hepatitis C – a controlled pilot trial. *Antiviral Res* 1999; **43**: 113–22.
- Ideo G, Bellobuono A, Tempini S, et al. Antioxidant drugs combined with alpha-interferon in chronic hepatitis C not responsive to alpha-interferon alone: a randomised, multicentre study. *Eur J Gastroenterol Hepatol* 1999; **11**: 1203–7.
- Grant PR, Black A, Garcia N, et al. Combination therapy with interferon-alpha plus N-acetyl cysteine for chronic hepatitis C: a placebo controlled double blind multicentre study. *J Med Virol* 2000; **61**: 439–42.
- Takagi H, Nagamine T, Abe T, et al. Zinc supplementation enhances the response to interferon therapy in patients with chronic hepatitis C. *J Viral Hepat* 2001; **8**: 367–71.
- Fujiyama S, Chikazawa H, Honda Y, et al. Treatment of chronic active hepatitis C with interferon alpha 2b alone and in combination with Stronger Neo Minophagen C. *Biother Jpn* 1998; **12**: 1495–513.
- Suzuki Y, Ikeda K, Saitoh S, et al. A prospective randomised administration of stronger neo minophagen C as an adjuvant therapy for chronic hepatitis type C treated with interferon. *Acta Hepatol Jpn* 1996; **37**: 363–7.
- Ishak KG, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; **22**: 696–9.
- Poynard T, Marcellin P, Lee SS, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. *Lancet* 1998; **352**: 1426–32.
- McHutchison JG, Gordon SC, Schiff ER, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *N Engl J Med* 1998; **339**: 1485–92.
- Heathcote EJ, Shiffman ML, Cooksley GE, et al. Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. *N Engl J Med* 2000; **343**: 1673–80.
- Lindsay KL, Trepo C, Heintges T, et al. A randomized, double-blind trial comparing pegylated interferon alfa-2b as initial treatment for chronic hepatitis C. *Hepatology* 2001; **34**: 395–403.
- Reddy KR, Wright TL, Pockros PJ, et al. Efficacy and safety of pegylated (40-kd) interferon alpha-2a compared with to interferon alpha-2a in noncirrhotic patients with chronic hepatitis C. *Hepatology* 2001; **33**: 433–8.

24. Zeuzem S, Feinman SV, Rasenack J, et al. Peginterferon alfa-2a in patients with chronic hepatitis C. *N Engl J Med* 2000; **343**: 1666–72.
25. Craxi A, Camma C, Giunta M. Definitions of response to antiviral therapy in chronic hepatitis C. *J Hepatol* 1999; **31**(Suppl. 1): 160–7.
26. Lindsay KL. Introduction to therapy of hepatitis C. *Hepatology* 2002; **36**(Suppl.): 114–20.
27. Poynard T, Bedossa P, Opolon P for the OBSVIRC, META-VIR, CLINIVIR and DOSVIRC groups. Natural history of the liver fibrosis progression in patients with chronic hepatitis C. *Lancet* 1997; **349**: 825–32.
28. Sobesky R, Mathurin P, Charlotte F, et al. Modeling the impact of interferon alfa treatment on liver fibrosis progression in chronic hepatitis C: a dynamic view. *Gastroenterology* 1999; **116**: 378–86.
29. Poynard T, McHutchinson J, Manns M, et al. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology* 2002; **122**: 1303–13.
30. Poynard T, McHutchinson J, Davis GL, et al. Impact of interferon alfa-2b and ribavirin on progression of liver fibrosis in patients with chronic hepatitis C. *Hepatology* 2000; **32**: 1131–7.
31. Shiratori Y, Imazeki F, Moriyama M, et al. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann Intern Med* 2000; **132**: 517–24.
32. Cammà C, Di Bona D, Schepis F, et al. Effect of peginterferon alfa-2a on liver histology in chronic hepatitis C: a meta-analysis of individual patient data. *Hepatology* 2004; **39**: 333–42.
33. Pockros PJ, Carithers R, PEGASYS International Study Group, et al. Efficacy and safety of two-dose regimens of peginterferon alpha-2a compared with interferon alpha-2a in chronic hepatitis C: a multicenter, randomized controlled trial. *Am J Gastroenterol* 2004; **99**: 1298–305.
34. Abe Y, Ueda T, Kato T, Kohli Y. Effectiveness of interferon, glycyrrhizin combination therapy in patients with chronic hepatitis C. *Nippon Rinsho* 1994; **52**: 1817–22.
35. Melhen A, Stern M, Shibolet O, et al. Treatment of chronic hepatitis C virus infection via antioxidants: results of phase I clinical trial. *J Clin Gastroenterol* 2005; **39**: 737–42.
36. Abe M, Akbar F, Hasebe A, et al. Glycyrrhizin enlaces interleukin-10 production by liver dendritic cells in mice with hepatitis. *J Gastroenterol* 2003; **38**: 962–7.
37. Yoshikawa M, Toyohara M, Ueda S, et al. Glycyrrhizin inhibits TNF induced, but no Fas-mediated, apoptosis in human hepatoblastoma line HepG2. *Biol Pharm Bull* 1999; **22**: 951–5.
38. Kimura M, Inoue H, Hirabayashi K, et al. Glycyrrhizin and some analogues induce growth of primary cultured adults rat hepatocytes via epidermal growth factor receptors. *Eur J Pharmacol* 2001; **431**: 151–61.
39. Shimoyama Y, Sakamoto R, Akaboshi T, et al. Characterization of secretory type IIA phospholipase A₂ (sPLA₂-IIA) as a glycyrrhizin (GL)-binding protein and the GL-induced inhibition of the CK-II-mediated stimulation of sPLA₂-IIA activity in vitro. *Biol Pharm Bull* 2001; **24**: 1004–8.
40. DeMaria N, Colantoni A, Fagioli S, et al. Association between reactive oxygen species and disease activity in chronic hepatitis C. *Free Rad Biol Med* 1996; **21**: 291–5.
41. Romero MJ, Bosch-Morell F, Romero B, et al. Serum malondialdehyde: possible use for the clinical management of chronic hepatitis C patients. *Free Rad Biol Med* 1998; **25**: 993–7.
42. Farinati F, Cardin R, DeMarie N, et al. Iron storage, lipid peroxidation and glutathione turnover in chronic anti-HCV positive hepatitis. *J Hepatol* 1995; **22**: 449–56.
43. Paradis V, Mathurin P, Kollinger M, et al. In situ detection of lipid peroxidation in chronic hepatitis C: correlation with pathological features. *J Clin Pathol* 1997; **50**: 401–6.
44. Boya P, Pena ADL, Belouqui O, et al. Antioxidant status and glutathione metabolism in peripheral blood mononuclear cells from patients with chronic hepatitis C. *J Hepatol* 1999; **31**: 808–14.
45. Larrea E, Belouqui O, Munoz-Navas MA, et al. Superoxide dismutase in patients with hepatitis C virus infection. *Free Rad Biol Med* 1998; **24**: 1235–41.
46. Houglum K, Venkatramani A, Lyche K, et al. A pilot study of the effects of d- α -tocopherol on hepatic stellate cell activation in chronic hepatitis C. *Gastroenterology* 1997; **113**: 1069–73.
47. Coon JT, Ernst E. Complementary and alternative therapies in the treatment of chronic hepatitis C. *J Hepatol* 2004; **40**: 491–500.
48. Bodenheimer HC Jr, Lindsay KL, Davis GL, Lewis JH, Thung SN, Seeff LB. Tolerance and efficacy of oral ribavirin treatment of chronic hepatitis C: a multicenter trial. *Hepatology* 1997; **26**: 473–7.
49. McHutchinson JG, Manns M, Patel K, et al. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; **123**: 1061–9.
50. Peck-Radosavljevic M, Wichlas M, Homoncik-Kraml M, et al. Rapid suppression of hematopoiesis by standard or pegylated interferon-alpha. *Gastroenterology* 2002; **123**: 141–51.
51. Brass CA. Do antioxidants ameliorate ribavirin related anemia in HCV patients? [abstracts]. *Gastroenterology* 1999; 1192–3.
52. Bacon BR and others. Antioxidant treatment does not substantially ameliorate the fall in hemoglobin in HCV patients treated with pegylated interferon alfa-2b and ribavirin. *Digestive Diseases Week*. May 15–20, 2004. New Orleans, LA. Abstract 1150.
53. Dieterich DT, Wasserman R, Brau N, et al. Once-weekly epoetin alfa improves anemia and facilitates maintenance of ribavirin dosing in hepatitis C virus-infected patients receiving ribavirin plus interferon alfa. *Am J Gastroenterol* 2003; **98**: 2491–9.

54. Afdhal NH, Dieterich DT, Pockros PJ, *et al.* Epoetin alfa maintains ribavirin dose in HCV-infected patients: a prospective, double-blind, randomized controlled study. *Gastroenterology* 2004; **126**: 1302–11.
55. Campbell MS, Sun JC, Lee BY, *et al.* Administration of erythropoietin for hepatitis C treatment-related anemia is not cost effective. Program and abstracts of the 56th Annual Meeting of the American Association for the Study of the Liver Diseases; November 11–15, 2005; San Francisco, CA. Abstract 379.
56. De Franceschi L, Fattovich G, Turrini F, *et al.* Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: role of membrane oxidative damage. *Hepatology* 2000; **31**: 997–1004.
57. Viusid information and clinical presentation.. Spain: Catalysis Hepatitis and AIDS Research, 2005. (Accessed December 12, 2005, at: <http://www.viusid.com>)