RESEARCH

An open, non-controlled clinical study to assess the efficacy and safety of the recombinant human alpha 2b pegylated interferon PEG-Heberon® plus ribavirin for the treatment of chronic hepatitis C virus infection in Cuban patients

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ABSTRACT

The pegylated forms of recombinant human interferon-alpha 2 have substantially improved the sustained virologic response (SVR) and are in the current guidelines for the treatment of chronic hepatitis due to the hepatitis C virus (HCV), but their high costs limit their availability. The aim of this study was to evaluate the efficacy and safety of PEG-Heberon® (40-kDa pegylated interferon alpha-2b), a formulation developed by the Center for Genetic Engineering and Biotechnology. An open, uncontrolled clinical trial was conducted at the National Gastroenterology Institute of Havana, in 147 patients that were chronically infected with the hepatitis C virus; 55 % were females of 50.1 ± 10.8 years of age, 94.6 % were of genotype 1 with high viral loads, and 54.4 % were mostly non-responders to previous treatments. Response-guided therapy was applied according to the genotype and viral load with Peg-Heberon® and ribavirin. Viral serum titers of the hepatitis C virus were used to evaluate the response to treatment. The intention to treat statistical analysis was carried out and logistic regression analyses were performed to identify independent predictors of SVR. A 46.3% of naive patients attained SVR versus 21.3 % in non-responders to previous treatments. The rapid virological response achieved on the fourth week of the treatment was the main independent predictor of SVR. A total of 98 % of 920 adverse events were mild to moderate, while systemic adverse events were more frequent (68.4 %). Hematological alterations were found in 26.8 % of the patients. PEG-Heberon® is safe, well tolerated and effective, and therefore, a good option for the treatment of HCV chronically-infected patients.

Keywords: hepatitis C virus, chronic hepatitis C, pegylated interferon, interferon alpha 2b, ribavirin, clinical trial

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RESUMEN

Estudio clínico abierto, no controlado para la evaluación de la seguridad y eficacia del interferón alfa 2b pegilado PEG-Heberon® más ribavirina para el tratamiento de la infección crónica por el virus de la hepatitis C en pacientes cubanos. Las formas pegiladas de interferón alfa-2 humano recombinante, han mejorado considerablemente la respuesta virológica sostenida (RVS) y aún permanecen en las guías de tratamiento de la hepatitis crónica por virus de hepatitis C (VHC), pero su elevado costo limita su disponibilidad. El objetivo del estudio fue evaluar la eficacia y seguridad de PEG-Heberon® (interferón alfa-2b pegilado, 40kDa) formulación desarrollada por el Centro Nacional de Ingeniería Genética y Biotecnología. Se realizó un Ensayo Clínico, abierto, no controlado enel Instituto de Gastroenterología, en 147 pacientes infectados crónicos por virus de hepatitis C, 55 % del sexo femenino, 50.1 ± 10.8 años de edad promedio, 94.6 % del genotipo 1, elevadas cargas virales y 54.4 % no respondedores a tratamientos previos. Se aplicó PEG-Heberon® y ribavirina en régimen personalizado según genotipo y carga viral. La viremia se cuantificó para evaluar la respuesta al tratamiento. Se realizó análisis estadístico por intención de tratar y regresión logística para identificar las variables predictoras de respuesta virológica sostenida (RVS). La SVR en los pacientes vírgenes fue del 46.3 % contra 21.3 % en los no respondedores a terapias previas. La respuesta virológica resultó ser el predictor independiente de SVR más consistente del estudio, alcanzada en la cuarta semana de tratamiento. El 98 % de los 920 eventos adversos fueron leves o moderados, con predominio sistémico (68.4 %). El 26.8% de los pacientes presentaron alteraciones hematológicas. En general, el tratamiento de la infección crónica por VHC con PEG-Heberon® es seguro, bien tolerado y eficaz como opción terapéutica para este tipo de paciente.

Palabras clave: virus de la hepatitis C, hepatitis C crónica, interferón pegilado , interferón alfa 2b, ribavirina, estudio clínico

*I*ntroduction

In Cuba, viral hepatitis is among the 35 main causes of death. The incidence of acute hepatitis due to the hepatitis C virus (HCV) has been reported in the years 2012 and 2013 to be at 1.2 and 1.0 per 100 000 population, respectively [1]. In previous years the morbidity rates due to HCV have been lower (between 0.1 and 0.9), with the exception of the years 1996 and 1997, reporting the highest number of cases (1.4 and 1.5,

respectively). Persons of 25 to 64 years of age are more highly affected [2], with hepatitis due to the HCV as one of the main causes of liver cirrhosis, which is one of the ten main causes of death in Cuba [1].

Significantly, the combined therapy that includes pegylated interferon plus ribavirin has been the standard treatment in patients with chronic HCV infection for more than a decade [3]. Many patients, however, still do not respond to this therapy or develop adverse effects that prevent its use. New drugs have been recently introduced as direct antiviral agents, formed by protease inhibitors and polymerase inhibitors, which when added to the standard treatment, show better efficacy, tolerance and shorter therapy duration [4]. But the costs of these drugs are extremely high and limit their use in developing countries [5]. The same happens for pegylated interferons, which are widely used in the world, including PegIntron® (Peginterferon alfa 2b) and Pegasys® (Peginterferon alpha 2a), marketed by the large pharmaceutical companies, Merck Sharp & Dohme Corp and Roche, respectively. Their high prices are at the range of 800 to 900 dollars a vial, making them unaffordable for low income countries [6]. This was the cause to seek for affordable alternatives for pegylated interferon production in Cuba, due to the high costs of these products in the world market.

In 2005, the Formulations Development Department of the Center for Genetic Engineering and Biotechnology published a paper on the domestic production of the pegylated interferon PEG-Heberon® (recombinant human interferon alpha-2b conjugated with Polyethylene glycol, 40 kDa) that had a significantly higher half-life in the blood in comparison to the non-pegylated interferon molecule, without the presence of significant toxicity [7]. The pharmacokinetic, pharmacodynamic and biological safety results obtained, showed that the product PEG-Heberon® is 'biologically similar' to Pegasys® and its use was suggested in the same cases as any other recombinant pegylated interferon alpha 2 product existing in the market, without any additional risk for the patients and with similar therapeutic outcomes [8].

The growing trend of hepatitis due to the HCV incidence in Cuba in the last 15 years has produced many studies to optimize its diagnosis and treatment [9-11]. As soon as PEG-Heberon® was produced, and with the high demand of these type of product as a therapeutic option in this disease, a study was designed to introduce it in routine clinical practice and to produce more useful information on its efficacy and safety.

Materials and methods

Study design

An open, non-controlled clinical trial was conducted by the Gastroenterology Institute (Havana, Cuba), in the period from May 2011 to October 2014.

Patients

Patients of both sexes of 19 to 72 years of age were included. They had shown positive HCV antibodies and a detectable viremia (RNA HCV) in the plasma with the characteristic liver damage of chronic HCV hepatitis, according to the histological results and non-invasive criteria of liver cirrhosis, documented in their clinical record.

The excluded patients were those having a history of: previous anti-viral treatments with pegylated interferon and ribavirin, auto-immune hepatitis; decompensated liver cirrhosis; severe cardiopathy, as well as unstable or uncontrolled cardiopathy within the last six months; pregnancy; breast feeding; men with pregnant spouses; those with a history of hypersensitivity to alpha interferon and/or components of the PEG-Heberon® formulation or to ribavirin and/or any component of the capsule; any active or uncontrolled case of depression, or any other psychiatric disorder; auto-immune processes where the conditions include the thyroids, and those with hemoglobin parameters below 12 g/dL in men and 11 g/dL in women were also excluded.

The main data of the patients involved in the study are shown in table 1.

Ethics

The protocol of this study was approved by the ethics committee of the institution and the Cuban regulatory agency (Center for the State Control of Drugs, Havana, Cuba). The informed consent was obtained from the patients before they were included in the study. The trial was carried out according to the Helsinki Declaration, adopted by the World Medical Association in 1964 and to its systematic revisions up until 2008 [12].

Study treatment

A personalized treatment regime was applied according to genotype and the result of the virological assessment on week 12. The maximum extent of the treatment was of 48 weeks for genotypes 1, 4, 5, 6, and of 24 weeks for genotypes 2 and 3. In all cases we carried out a 24-week post-treatment follow-up.

Table 1. General characteristics of the Cuban patients with chronic infection by the HCV and treated with PEG-Heberon® plus ribavirin, at the Gastroentyerology Institute, Havana, from 2010 to 2014*

Characteristic	Values
Patients	147
Sex	
Female	81 (55 %)
Male	66 (45 %)
Age (mean ± SD)	50.1 ± 10.8
Body mass index (mean ± SD)	26.2 ± 4.6
Clinical stage	
Chronic hepatitis	130 (88.4 %)
Liver cirrhosis	17 (11.6 %)
Genotype	
1	139 (94.6 %)
2	4 (2.7 %)
3	3 (2.0 %)
6	1 (0.7 %)
Basal viral load (IU/mL)	
Min	15
Max	16 300 000
Median	782 000
Previous treatment	
Treated	80 (54.4 %)
Naive	67 (45.6 %)

* Data extracted from the Data Recording Form.
SD: standard deviation.

1. Ministerio de Salud Pública de Cuba. Anuario Estadístico de Salud 2013. Direccion de Registros Medicos y Estadisticas de Salud. La Habana: MINSAP. 2014 [cited 10 de enero 2015]. Available from: www.sld.cu/sitios/dne/

2. Series de Tiempo de Morbilidad. Cuba 1970 - 2005. Biblioteca virtual de salud. 2005 [cited 10 de enero 2015]. Available from: http://www.sld.cu/sitios/dne/

3. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet. 2001;358(9286):958-65.

4. Diseases AAftSoL, America IDSo. Recommendations for testing, managing, and treating hepatitis C. Alexandria, VA, AASLD and IDSA. 2014 [cited 2014 July 10]. Available from http://www.hcvguidelines.org/full-report-view

5. Jayasekera CR, Barry M, Roberts LR, Nguyen MH. Treating hepatitis C in lower-income countries. New England Journal of Medicine. 2014;370(20):1869-71.

6. Roche. Pegylated interferons. 2010 [cited 2015 Jan 20]. Available from: http://www.goodrx.com/pegintron

7. Ramon J, Saez V, Baez R, Aldana R, Hardy E. PEGylated interferon-α2b: a branched 40K polyethylene glycol derivative. Pharmaceutical Res. 2005;22(8):1375-87.

8. García-García I, González-Delgado CA, Valenzuela-Silva CM, Díaz-Machado A, Cruz-Díaz M, Nodarse-Cuní H, et al. Pharmacokinetic and pharmacodynamic comparison of two. BMC pharmacology. 2010;10(1):15.

9. Delgado G. Situación de la hepatitis C en Cuba. Reporte técnico de vigilancia Unidad de Análisis y Tendencias de Salud. 1999;4(1):1-6.

The detection of viremia on week 12 defined the interruption of the treatment. Different medications were used in a linear schedule with the administration of a weekly subcutaneous dose of PEG-Heberon® from one milliliter vials of the injectable solution at a dose of 180 µg, combined with Ribavirin (Novatec Laboratories, Havana, Cuba), and in capsules of 200 mg for oral administration adjusted to the weight of the patient, ranging from 1000 mg (< 75 kg body weight) to 1200 mg (≥ 75 kg). No blinding was used for any of the two products.

Safety assessment

The treatment was carried out in an ambulatory regime with clinical, biochemical and virologic supervision in the 12 consultations (every four weeks and up to week 72). Patients were questioned and checkups were made in relation to the tolerability and adverse events shown with the administration of the drugs. The results were noted down in the Data Recording Form. The empty ribavirin flasks and empty PEG-Heberon® vials were collected as additional proof of their administration, as well as all products that were not used for any reason.

A reduction of over 2 g of hemoglobin required a reduction of the dose of ribavirin to 800 mg/day. The decline of the hemoglobin below 10g/L was treated with a gradual reduction of the ribavirin at the rate of 200 mg/day and values below 80 g/L were also given recombinant Erythropoietin (EPOCIM®) at a rate of 40 000 IU weekly. Neutropenia below 1.0×10^9/L was treated with the granulocyte stimulating factor (Hebervital) given at 300 mcg weekly, without modifying the anti-viral schedule; in the case of neutropenia at a range of 0.5-1.0 $\times 10^9$/L the dose of PEG-Heberon was reduced to 135 mcg/week. The hemoglobin values below 6.5 g/L and neutrophils below 0.5×10^9/L were the indicators used to immediately and definitively interrupt the treatment and withdraw from the protocol.

Efficacy assessment

Rapid virological responders (RVR) were those that had their HCV RNA undetectable or detectable levels with a 2-log or higher reduction in the initial viral load on week 4; the early virological responders (EVR) or late virologic responders (LVR) were those having undetectable HCV RNA on weeks 12 and 24, respectively. The final virologic response (FVR) and the sustained virologic response (SVR) were considered when the HCV RNA was undetectable on weeks 48 and 72 respectively. The responders on week 48 that showed detectable amounts of HCV RNA on week 72 were considered relapses.

Naive patients were those that had never received anti-viral treatment, and we considered as re-treated patients those that had had at least one previous anti-viral treatment (except for pegylated interferon and ribavirin). According to the possible genotypes, these were: 1, 2, 3, 4, 5 and 6. The basal viral loads were classified as follows: low when the record showed up to 600 000 IU/mL, and high with more than 600 000 IU/mL. Breakthrough of the virologic response was considered when the RNA of the HCV was below the detection limit (15 IU/mL) but it increased to values of over 100 IU/ml or ≥ 1 log during therapy. The individual therapeutic success was the SVR.

Laboratory analyses and liver synthesis markers

The determinations were made using reverse transcription polymerase chain reaction (RT-PCR) for the qualitative and quantitative determination of the viral load, using the commercially available kits. The qualitative variant was used before starting the treatment for diagnostic screening and at the end of the treatment and during the follow-up, as a response criterion. This determination was made using the COBAS Amplicor® test for the hepatitis C virus, v2.0 (Roche). The quantitative variant was used before starting the treatment and on weeks 4, 12 and 24 as a prognostic response factor. In this case we used the COBAS AmpliPre/COBAS TaqMan HCV test (Roche). The lowest detection limit was 15 IU/mL. The virus genotyping test was HCV Linear Array (Roche) with the COBAS Amplicor HCV v2.0 test. Blood samples taken were also used for the qualitative evaluation before the treatment.

Counts of red blood cells, white blood cells and platelets, the hemoglobin and hematocrit were measured with the hematological analyzer ABX Micros 60 (ABX Diagnostics, Montpellier, France). Bilirubin (µMol/L), gamma Glutamyl transferase (IU/L), alanine aminotranferase (IU/L), aspartate aminotransferase (IU/L), alkaline phosphatase (IU/L), albumin (g/L), total proteins (g/L), glycemia (mmol/L), creatinine (mmol/L), triglycerides (mmol/L) and total cholesterol (mmol/L) were determined in the clinical laboratory of the Gastroenterology Institute using the routine validated methods (automated clinical chemistry Hitachi 902, Roche).

Statistical analysis

The statistical package SPSS, version 21 (IBM software, SPSS Statistics, 2012) was used to carry out the statistical analysis. Data were analyzed as the mean ± standard deviation (SD), the median, the minimum and maximum values for the quantitative variables, and absolute numbers and percentages were used for qualitative variables. The associations were made through contingency tables and the statistical calculations were made through Mantel-Haenszel (chi square and its significance level). All the analyses considered a significance level of 5 %. Predictive variables were dichotomized according to sex: male/female, age: older/younger than 40 years old, body mass index: higher/less than 25 kg/m^2, diagnostic: liver cirrhosis/chronic hepatitis, previous anti-viral treatments: re-treated/naive, rapid and early virologic responses: absent/present, viral genotypes: 1 and 6/2 and 3 and the basic viral loads higher/lower than 600 000 UI. To identify the predictive variables, their relation to the sustained virological response (dependent variable) was analyzed; a binary univariate and multivariate regression analysis was used; risk was assessed as relative risk (RR), with a CI of 95 %. For the multivariate analyses we selected those variables that besides being significant in the univariate analysis, had odds ratios (OR) above 1.2. The efficacy of the intervention was assessed through the 'intention to treat' analysis.

10. Infante Velázquez M, Arús Soler E, Fernández Naranjo A, Grá Oramas B. Hallazgos clínicos, bioquímicos y morfológicos en 103 pacientes con anticuerpos contra el virus de la hepatitis C. Rev Cubana Med. 1998;37(2):66-71.

11. Sabina Molina D, García Valdés F, Asconegui Moya Á, Martínez López O. Características epidemiológicas de la hepatitis C en donantes de sangre. Rev Cubana Hig Epidemiol. 2002;40(3):279-93.

12. World Medical Association. Declaration of Helsinki. Ethical principles for medical research involving human subjects. 2008 [cited 2013 Jan 16]. Available from: http://www.wma.net/e/policy/b3.htm

Results

Patients enrolled

A total of 272 patients with positive HCV antibody response were tested for the detection of viremia using PCR; we found that 208 had detectable viral loads, and of these, 147 patients were included in the trial. Women prevailed in the sample, with an average of 50 years of age; more than 85 % of the cases had the diagnostic of chronic hepatitis. The viral genotype prevailing was 1; the median of the basal viral load was of over 600 000 IU/ml and more than 50 % of the cases had received previous anti-viral treatments. Most of them had received recombinant interferon alpha 2b and ribavirin within 5 years prior to the start of this project. Most of the patients were from the western part of the country (88.4 %), mainly from Havana, Pinar del Río, Mayabeque and Artemisa provinces. The mean of the body mass index (BMI) was of over 25 kg/m^2, showing that the patients were slightly obese.

Virological response

The patients that were naive to a previous treatment showed much more favorable results than those retreated. The SVR in naive patients was of 46.3 %, which was higher than the retreated patients that only reached 21.3 %, (p = 0.002). However, in the first group the relapse was higher 22.3 % vs. 15 %. The overall SVR of the study was of 32.7 % (48 patients) (Figure).

The best responses to the treatment were obtained in patients infected with HCV genotypes 2 and 3; all of them showed a response at the end of the treatment, however, two of them lost the response at the end of the follow-up for a SVR of 71.4 %. In genotype 1 (prevailing in the study), the FVR was of 49.6 % while the SVR was of 32 %. The patient with genotype 6 was considered a non-responder as of week 12 of the treatment. Of the 87 patients with viral loads of over 600 000 IU/ml, only 37 (42.5 %) achieved a FVR, while those with low viral loads had higher responses and 66.7% responded. Similar behavior was observed with the SVR of 23 % vs. 43.7 % respectively.

The patients that were naive to a previous treatment and also belonged to genotype 1, a total of 60 patients, showed a FVR of 68.3 %, while the SVR was of 46.6 %.

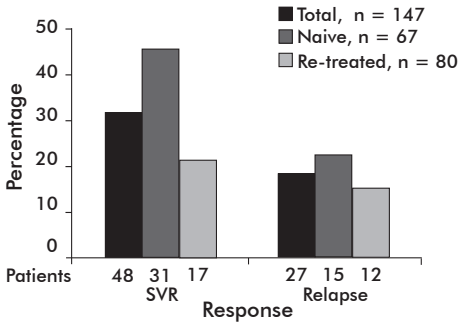

Figure. Sustained virological response (SVR) and relapses of Cuban patients with chronic infection by the HCV after treatment with PEG-Heberon® plus ribavirin, at the Gastroentyerology Institute, Havana, from 2010 to 2014. Retreated patients were those previously treated with standard interferon.

Of the 48 patients reaching a SVR, 36 (75 %) had reached it as of week 4 of the treatment (39.7 % of genotype 1 and 100 % of genotypes 2 and 3) and they were considered to be RVR and 12 (25 %) patients reached the response at week 12, and are considered to be EVR. Only 8 patients were shown to be LVR, of which 2 were able to reach FVR and had relapses at the end of the follow-up.

The logistic regression analysis included several factors acknowledged as predictors of the response to treatment. Within the univariate analysis the main predictors are: patients that are naive to previous antiviral treatments, rapid virologic response and basal viral loads of less than 600 000 IU. The multivariate study included all factors identified as single independent predictors of a favorable response to the treatment through the virologic response obtained on week 4 of the treatment (Table 2).

Adverse events

Out of the 147 patients starting the treatment, 51 (34.6 %) did not finish it for three main reasons: no response, adverse events and voluntary dropouts. The withdrawal from the treatment because of no response was found in 35 patients (23.8 %), of which 32 left on week 12 and three patients had virological rupture (breakthrough). The withdrawals because of adverse events were found in five patients (3.4 %); three of them were severe events endangering their lives or having sequels of disabilities, and two patients had had repeated moderate adverse events. There were eleven dropouts (7.5 %), basically because of personal problems that affected their assistance to the follow-up appointments.

Table 2. Logistic regression analysis of the predictive factors of response to antiviral therapy with PEG-Heberon® plus ribavirin, in Cuban patients with chronic infection by the HCV in an open, non-controlled clinical study conducted at the Gastroentyerology Institute, Havana, from 2010 to 2014

Reference variables	Univariate analysis			
	OR	CI (95 %)		P
		Lowest	Highest	
Sex: Female	0.4	0.2	1.0	0.050
Age: < 40 years old	2.4	1.0	5.8	0.038
BMI: < 25 kg/m^2	2.1	1.0	4.3	0.030
Diagnostic: chronic hepatitis	4.1	0.9	18.7	0.051
Previous treatment: Naive	3.1	1.5	6.5	0.001
Rapid virological response	8.8	4.0	19.6	< 0.001
Early virological response	0.6	0.2	1.3	0.252
Genotype: 2 and 3	5.4	1.0	28.9	0.030
Basal viral load: <600 000 IU/mL	2.9	1.4	5.9	0.012
Reference variables	**Multivariate analysis**			
	OR	CI (95 %)		P
		Lowest	Highest	
Previous treatment: Naive	2.1	0.9	4.9	0.068
Rapid virological response	7.2	3.1	16.4	< 0.001
Basal viral load: <600 000 IU/mL	1.8	0.8	4.2	0.133

OD: Odds ratio; CI: Confidence interval; BMI: Body mass index.

Treatment administration was safe and well tolerated. There were no adverse events recorded in nine patients, and the rest had at least one adverse event at some time during the treatment. Most of the events were observed in women (55.8 %) although there were no significant differences compared to men. In general, 920 adverse events were reported, of which 96.63 % (889 events) were mild or moderate. There were 28 adverse events that were considered to be serious, of which 21 (75 %) were hematological (Table 3).

The life endangering adverse events were found in three patients. One was a 60 year old woman with compensated liver cirrhosis and a history of controlled arterial hypertension, who had acute lung edema on the twelfth week of the treatment. The second case was a 42 year old woman with chronic hepatitis and a history of kidney lithiasis who, on the 28th week of the treatment showed a right kidney abscess with severe generalized sepsis; a nephrectomy was made and she evolved favorably. The last case was a 38 year old man with chronic hepatitis who started having suicidal psychiatric disorders as of 4 weeks of the treatment.

Of the 920 reported events, the systemic adverse events were the most frequent ones (68.4 %), where the highest frequencies were of asthenia, myalgia, headache and arthralgia. The local events had the lowest frequency (4.8 %), where pain and stinging at the injection site were the most common. Hematologic adverse events were 26.8 % of all reports, with leukopenia being the most relevant one (Table 4). Hebervital® (granulocyte-colony stimulating factor, Center for Genetic Engineering and Biotechnology, Havana, Cuba) was required in 21 patients (14.2 %) having hematological adverse events with neutropenia of less than 1.0×10^9, while the cases of anemia were of less than 80 g/L, and were observed in 10 patients (6.8 %) who were treated with EPOCIM® (human alpha erythropoietin, Center of Molecular Immunology, Havana, Cuba).

Table 5 shows the causality assessment. No remote adverse events were identified, and the highest frequency corresponded to the events that were probable, with 662 events (71.9 %), followed by the possible events with 152 events (16.6 %) and the very probable ones with 106 events (11.5 %).

Table 3. Classification of adverse events recorded among Cuban patients with chronic infection by the HCV after treatment with PEG-Heberon® plus ribavirin, at the Gastroentyerology Institute, Havana, from 2010 to 2014

Adverse events (intensity)	Incidence	Frequency (%)
Mild	557	60.5
Moderate	332	36.0
Serious	28	3.0
Severe	3	0.3
Total	920	100

Table 4. The most frequent adverse events recorded among Cuban patients with chronic infection by the HCV after treatment with PEG-Heberon® plus ribavirin, at the Gastroentyerology Institute, Havana, from 2010 to 2014*

Adverse events (intensity)	Incidence	Frequency (%)
Clinical events		
Asthenia	87	59.1
Myalgia	49	33.3
Headache	47	31.9
Arthralgia	47	31.9
Stinging and pain at the injection site	45	30.6
Fever	41	27.8
Hematological events		
Leukopenia $< 5.0 \times 10^9$/L	82	55.7
Hemoglobin reduction < 12 g/dL men/ < 11 g/dL women	73	49.6
< 8.0 g/dL	10	6.8
Thrombocytopenia $< 150 \times 10^9$/L	69	46.9
Neutropenia		
$< 2.0 \times 10^9$/L	63	42.8
$< 1.0 \times 10^9$/L	21	14.2

*Only the adverse events with an incidence higher than 25 % are shown.

Table 5. Causality of adverse events recorded among Cuban patients with chronic infection by the HCV after treatment with PEG-Heberon® plus ribavirin, at the Gastroentyerology Institute, Havana, from 2010 to 2014

Adverse events (intensity)	Incidence	Frequency (%)
Possible	152	16.6
Probable	662	71.9
Very probable	106	11.5
Total	920	100

*D*iscussion

The clinical efficacy of the different forms of pegylated Interferons (alpha 2a and alpha 2b) have been explored in comparative studies published in the last decade [13]. Most of them have been carried out in chronic HCV patients who were naive to previous antiviral treatments. The trial named IDEAL (Individualized Dosing Efficacy versus Flat Dosing to Assess Optimal Pegylated Interferon Therapy) by McHutchison *et al*. [14] was one of the most comprehensive studies up to now with 3070 patients that were naive to the treatment with genotype 1, and showed similar SVR for both types of pegylated interferons (38 % for pegylated interferon alpha 2b and 40.9 % for the alpha 2a variant). The SVR reported in other studies are variable and may be as low as 24 % or as high as 68.7 % [15, 16].

The SVR obtained in this study with the use of PEG-Heberon®, in naive patients, has been successful, since it is similar to reports of other authors and it complied with the expected standards of the product. A similar situation was observed in relation to relapses, since it is expected in 20 to 32 % of the cases [14].

For non-responders to the standard interferon treatment under monotherapy, the re-treatment with pegylated interferon and ribavirin offered 20 to 40 % SVR, while for those having the combined treatment of standard interferon and ribavirin it reached less than 10 % [17-19]. The results obtained are considered to be satisfactory since they surpass 20 %, although most of the patients treated had a history of the combined treatment using standard interferon and ribavirin.

Comparisons cannot be established in this study because this is the first time pegylated interferon was tested in Cuba in a large population of chronic hepatitis C patients. It must be underlined that this

13. Foster GR. Pegylated interferons for the treatment of chronic hepatitis C. Drugs. 2010;70(2):147-65.

14. McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, *et al*. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med. 2009;361(6):580-93.

15. Backus LI, Boothroyd DB, Phillips BR, Mole LA. Predictors of response of US veterans to treatment for the hepatitis C virus. Hepatology. 2007;46(1):37-47.

16. Ascione A, De Luca M, Tartaglione MT, Lampasi F, Di Costanzo GG, Lanza AG, *et al*. Peginterferon alfa-2a plus ribavirin is more effective than peginterferon alfa-2b plus ribavirin for treating chronic hepatitis C virus infection. Gastroenterology. 2010;138(1):116-22.

17. Taliani G, Gemignani G, Ferrari C, Aceti A, Bartolozzi D, Blanc PL, *et al*. Pegylated interferon alfa-2b plus ribavirin in the retreatment of interferon-ribavirin nonresponder patients. Gastroenterology. 2006;130(4):1098-106.

18. Shiffman ML, Di Bisceglie AM, Lindsay KL, Morishima C, Wright EC, Everson GT, *et al*. Peginterferon Alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment 1, 2, 3. Gastroenterology. 2004;126(4):1015-23.

report shows superiority, compared to previous therapies performed in Cuba, particularly with the use of the combination of non-pegylated interferon alpha and ribavirin. One of the earliest studies was carried out in a randomized double blind and placebo controlled trial by Galban et al. [20], who included only 47 patients reaching 65 % negativization of the viral RNA. The phase IV clinical trial carried out at the Gastroenterology Institute by Sánchez Rodríguez *et al*. [21], published in 2010, showed that SVR was of 32.9 % in naive patients, which was lower than that of this study (> 45 %).

The general characteristics of the study group are disadvantageous for reaching the best results of the antiviral treatment with the application of the PEG-Heberon®. The existence of multiple unfavorable predictive factors, both of the host and of the infecting virus, was the main challenge of the study [3]. In spite of these conditions, there are no contraindications for the antiviral therapy [22]. The results obtained in this trial confirm the virological efficacy of the PEG-Heberon® and ribavirin therapy in the treatment of the chronic infection of the HCV.

Of all the predictive factors assessed in the study, the value of the RVR stands out. This response has been reported as having a high predictive value regardless of the genotype and type of treatment. Few patients reach responses as early as those here reported, of 15 to 20 % for genotype 1 and 66 % for genotypes 2 and 3 [23, 24]. The greatest drawback of this indicator is its poor negative predictive value; for this reason, the absence of RVR is not an indication for the interruption of the treatment. Studies show the usefulness of the RVR to shorten the treatment period from 48 to 24 weeks in genotype 1 patients [25, 26]. In this study, the high percentage of patients reaching RVR was surprising and was much higher than that expected. The appearance of protease and polymerase inhibitors have redirected the antiviral therapies in relation to antiviral drug combinations and shortening the length of the treatment. It has been, however, demonstrated that the triple therapy of pegylated interferon, ribavirin and boceprevir is not better than the combined pegylated interferon and ribavirin treatment in patients with genotype 1 HCV and low viral loads [27]. The shorter the time needed for viral clearance, the higher the probability of achieving a sustained response; the therapies must be therefore individualized [28, 29].

The dropouts from the treatment because of a lack of response, was slightly lower than expected. The interruptions due to dropouts have been reported to be of about 25 % [3]. The definitive interruptions of the treatment because of adverse events were observed in less than 5 % of the patients, which is lower than that found by Fried *et al*. [30] who detected a range of 10 % to 14 %, and McHutchison *et al*. [14] who found a range of 9.6 to 13 %. Although in this study almost all the patients showed adverse events, those dropping out because of this reason were actually very few. This shows that the treatment was well tolerated and there was a good patient adherence; at the same time, the researchers showed a strict compliance to the good clinical practices demanded in the initial protocol for the application of the antiviral treatment.

The most frequent adverse events were the systemic events, similar to those reported by other authors. Those of the highest frequency were flu-like expressions, characterized by the combination of headaches, myalgia, arthralgia and fever. Asthenia is an unspecific symptom that may be attributed to the viral chronic hepatitis itself, and that could worsen with the antiviral therapy [3, 14, 30].

In general, the hematological adverse events were observed with a higher frequency than in other studies, due to the fact that the reference ranges taken to assess these events were higher, in order to address the presence of anemia, leukopenia and neutropenia. When the stratified analysis according to the severity of the event was made, it was found that the severe declines of the hemoglobin and neutrophils, requiring co-adjuvant treatment, were observed at a lower proportion. This corresponds to most of the trial reports made with similar products, so that neutropenia ranges from 2 to 27 % and anemia is of 25 to 30 % [14, 31]. The availability and use of Hebervital® and EPOCIM® allowed for a better adherence to the treatment. Although the co-adjuvant treatment has not been shown in general to improve the SVR, it is a solution for the side effects that appear with the antiviral therapy, even when the cost of the therapy increases; it clearly improves tolerance and adherence to the treatment [32-35].

In relation to the causality of the results obtained, in general, these have to do with the controlled clinical trials, where the probable side effects are reported to be the most frequent ones; this is related to the adherence of the treatment. Before the marketing stage no unforeseen events were observed [36].

The leading trend is that of establishing therapies that do not contain interferon for the hepatitis C treatment. The high price, however, of the direct action antiviral drugs even in developed countries, makes it unaffordable to carry out a government program for the treatment of this disease. In spite of the high efficacy of these drugs, the approval of the regulatory authorities is not enough to ensure the implementation of their use. In countries with limited economic resources such as Cuba, the application of direct action antiviral drugs would face the obstacle of the country having to establish or maintain its financial conditions for the continuous purchase of the drug in the world market.

In contrast, the combination of pegylated interferon and ribavirin has undergone the struggle against other antiviral drugs and its efficacy has remained invariable with acceptable therapeutic safety. Hence, in contrast with the unaffordable prices of the current antiviral drugs, pegylated interferon has been decreasing its price in the world market, which together with having soon expiring patents, may help many patients that would finally have this treatment alternative, after years of waiting and the progression of their liver disease.

The existence for over a decade of pegylated interferon and ribavirin as the alternative of choice for the hepatitis C treatment, has made it possible to accumulate a significant amounts of clinical information generated in hundreds of clinical trials, which in any of the circumstances and setting assessed, have

19. Jacobson IM, Gonzalez SA, Ahmed F, Lebovics E, Min AD, Bodenheimer HC, *et al*. A randomized trial of pegylated interferon α-2b plus ribavirin in the retreatment of chronic hepatitis C. Am J Gastroenterol. 2005;100(11):2453-62.

20. Galbán E VE. Empleo de terapia combinada IFN alfa + Ribavirina en hepatitis C crónica. GEN. 2000;54(3):169-86.

21. Sánchez Rodríguez Y, Arús Soler E, Grá Oramas B, López Saura P. Nodarse Cuní H. Interferón alfa 2b recombinante más ribavirina en el tratamiento de la hepatitis crónica C. Rev Cubana Med. 2010;49(2):11-21.

22. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology. 2009;49(4):1335-74.

23. Ferenci P, Fried MW, Shiffman ML, Smith CI, Marinos G, Gonçales FL, *et al*. Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alfa-2a (40KD)/ribavirin. J Hepatol. 2005;43(3):425-33.

24. Shiffman ML, Suter F, Bacon BR, Nelson D, Harley H, Solá R, *et al*. Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. N Engl J Med. 2007;357(2):124-34.

25. Jensen DM, Morgan TR, Marcellin P, Pockros PJ, Reddy KR, Hadziyannis SJ, *et al*. Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon α-2a (40 kd)/ribavirin therapy. Hepatology. 2006;43(5):954-60.

26. Zeuzem S, Buti M, Ferenci P, Sperl J, Horsmans Y, Cianciara J, et al. Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia. J Hepatology. 2006;44(1):97-103.

27. Pearlman BL, Ehleben C. Hepatitis C genotype 1 virus with low viral load and rapid virologic response to peginterferon/ribavirin obviates a protease inhibitor. Hepatology. 2014;59(1):71-7.

28. Mauss S, Berg T, Rockstroh J, Sarrazin C, Wedemeyer H, editors. Short guide to hepatitis C. Berlin: Flying publisher; 2014.

29. Poordad F, Reddy KR, Martin P. Rapid virologic response: a new milestone in the management of chronic hepatitis C. Clinical Infect Dis. 2008;46(1):78-84.

30. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL, *et al*. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med. 2002;347(13):975-82.

31. Mangia A, Minerva N, Bacca D, Cozzolongo R, Ricci GL, Carretta V, *et al*. Individualized treatment duration for hepatitis C genotype 1 patients: a randomized controlled trial. Hepatology. 2008;47(1):43-50.

32. Shiffman ML, Salvatore J, Hubbard S, Price A, Sterling RK, Stravitz RT, *et al*. Treatment of chronic hepatitis C virus genotype 1 with peginterferon, ribavirin, and epoetin alpha. Hepatology. 2007;46(2):371-9.

33. Del Rio RA, Post AB, Singer ME. Cost-effectiveness of hematologic growth factors for anemia occurring during hepatitis C combination therapy. Hepatology. 2006;44(6):1598-606.

demonstrated that viral replication is controlled in the patient in a safe and effective manner [37, 38]. The risk–benefit ratio that considers this therapeutic option as valid is supported by the appropriate safety profile that ensures its clinical application. Although its application does have the occurrence of adverse events, these are considered to be controllable, reversible and having very little influence on the poor adherence of the patient to the treatment. It can therefore be concluded that this therapeutic alternative, from the pharmacoeconomics viewpoint, offers substantial advantages in comparison to the antiviral agents that were recently approved by regulatory authorities and are now in the world market.

In short, PEG-Heberon® demonstrated that it is safe and well tolerated, and it is an effective therapeutic option for the treatment of patients with chronic hepatitis due to HCV infection.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

34. Afdhal NH, Dieterich DT, Pockros PJ, Schiff ER, Shiffman ML, Sulkowski MS, et al. Epoetin alfa maintains ribavirin dose in HCV-infected patients: a prospective, double-blind, randomized controlled study. Gastroenterology. 2004;126(5):1302-11.

35. Shiffman ML. Optimizing the current therapy for chronic hepatitis C virus: peginterferon and ribavirin dosing and the utility of growth factors. Clinics Liver Dis. 2008;12(3):487-505.

36. Marcellin P, Rizzetto M. Response-guided therapy: optimizing treatment now and in the future. Antivir Ther. 2008;13 Suppl 1:1-2.

37. Awad T, Thorlund K, Hauser G, Stimac D, Mabrouk M, Gluud C. Peginterferon alpha-2a is associated with higher sustained virological response than peginterferon alfa-2b in chronic hepatitis C: Systematic review of randomized trials. Hepatology. 2010; 51(4):1176-84.

38. Chopra, S., Muir, A. J., Di Bisceglie, A. M., & Bloom, A. (2012). Treatment regimens for chronic hepatitis C virus genotype 1. 2012 [2015 Jul 31]. Available from: http://www.uptodate.com/contents/treatment-regimens-for-chronic-hepatitis-c-virus-genotype-1

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