Cuban interferon alpha-2b. Thirty years as an effective and safe drug

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RESEARCH

ABSTRACT

Since 1986, the Center for Genetic Engineering and Biotechnology of Havana, Cuba, has produced the recombinant human interferon alpha-2b, marketed as Heberon® Alfa R. The therapeutic use of this product has accumulated a high number of researches carried out in the country's public health network. For the analysis of its safety profile, 28 years of reports of adverse events were reviewed in 5806 individuals, both children and adults, coming from 147 clinical trials or healthy assistances using the product. This review also contains a safety comparison between lyophilized and liquid formulations. In addition, an analysis of the connection between the occurrence of adverse events and the demographic characteristics of the patients, an analysis of immunogenicity and another on the variation of the thyroid function associated to the use of Heberon® Alfa R were included. Finally, a general analysis of the product's efficacy based on the number of treated patients and the clinical results obtained are presented. Adverse events were reported in 4864 subjects (84 %). The main adverse events were those corresponding to the flu-like syndrome, with higher frequency in male white patients. Hypothyroidism and immunogenicity behaved lower than similar products in the international pharmaceutical market. Approximately 60 % of the treated patients obtained a relevant therapeutic response and a liquid formulation offered a better benefit/ risk ratio. The extensive clinical information evaluated recognizes a Heberon® Alfa R as a safe and effective drug, 30 years after its first production.

Keywords: IFN alpha, Heberon® Alfa R, adverse events, efficacy, Cuba

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RESUMEN

Interferón alfa-2b cubano. Treinta años como un medicamento eficaz y seguro. La producción por primera vez en 1986 del interferón alfa-2b humano recombinante cubano, comercializado como Heberon® Alfa R, dio inicio a la introducción y comercialización de diversos tipos y formulaciones de interferones por el Centro de Ingeniería Genética y Biotecnología, de La Habana, Cuba. El uso terapéutico de este producto fue evaluado en un gran número de investigaciones clínicas realizadas en el Sistema Nacional de Salud de Cuba. Para el análisis del perfil de seguridad del Heberon® Alfa R fueron revisados 28 años de reportes de eventos adversos en 5806 individuos entre niños y adultos, procedentes de un compendio de 147 investigaciones clínicas o usos asistenciales con el producto. Este trabajo contiene, además, una comparación de la seguridad entre las formulaciones liofilizada y líquida; Incluye también un análisis de la relación de ocurrencia de eventos adversos y las características demográficas de los pacientes; un análisis de inmunogenicidad y otro sobre la variación en la función tiroidea asociada al uso del Heberon® Alfa R. Por último, se muestra un análisis general de la eficacia del producto, a partir del número de pacientes tratados y los resultados clínicos obtenidos. Los principales eventos adversos observados fueron aquellos correspondientes con el síndrome pseudogripal, con mayor frecuencia en los pacientes masculinos blancos. El hipotiroidismo y la inmunogenicidad se comportaron inferiores respecto a los productos similares en el mercado farmacéutico internacional. Aproximadamente el 60 % de los pacientes tratados obtuvo una respuesta terapéutica relevante y la formulación líquida ofreció la mejor relación riesgo beneficio. La amplia información clínica evaluada reconoce al Heberon® Alfa R cubano como un medicamento efectivo y seguro, transcurridos 30 años desde su primera producción.

Palabras clave: IFN alfa, Heberon® Alfa R, eventos adversos, eficacia, Cuba

*I*ntroduction

Interferons (IFN) are heterogeneous families of proteins considered as one of the most active biological substances. The discovery in 1957 was a milestone in knowledge of the cytokines capable of interfering with the viral replication [1]. They are present in all animal species and their multifunctional character allows them to influence several cell processes, either as a component of the immune system during the defensive response in the presence of a virus [2], parasites [3] and certain tumors [4], or by inhibition or modulation of cell differentiation and proliferation [5]. Other actions include the antiproliferative activity on the splitting cells [6] and the immunomodulating activity which regulates the production of other cytokines, the activation of the macrophages, natural killer cells and T cells cytotoxicity, the increase of the expression of histocompatibility leukocyte antigens and activities similar to certain hormones [7]. The most recently described actions referred to their antifibrotic [8], neuromodulator [9] and antiangiogenic [10] properties.

IFN alpha is recognized as a treatment for wide range of diseases. The main indication is the infection by the hepatitis B [11] and C [12] viruses, followed by different manifestations of human papilloma virus [13] and Herpes Simplex virus [14]. Other well established indications are onco-hematologic diseases [15], as well as skin [16], bladder [17] and kidney [18] carcinomas. Likewise, its effectiveness is recognized in childhood's hemangiomas [19] and solid tumors such as melanoma [20] and ovaries [21].

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The Center of Biological Research (CIB), in Havana, and the Center for Genetic Engineering and Biotechnology (CIGB) set up a scientific and productive complex with IFN experiences accumulated since 1981. The production of the natural leukocyte IFN alpha from blood donors, first, and since 1986 the recombinant IFN alpha-2b, made possible to conduct therapeutic researches following the Clinical Good Practices regulations established in Cuba, using hospital institutions throughout the country. Therefore, the information has been obtained guarantees the efficacy and safety of products [22].

From 1981 to 2014, IFN alpha from CIGB was used in more than 8000 individuals among Cuba, Pakistan, Iran, Ukraine, Brazil and many other countries. Six therapeutic effects have been evaluated and 14 medical specialties have applied the product as treatment for 69 diseases. It has been administered by subcutaneous, intramuscular, intralesional, intrathecal, intravenous and intraperitoneal parenteral routes; and locally applied in the forms of cream, gel and eye drops [22].

Cuban IFN alpha is marketed as lyophilized and liquid (LQ) Heberon® Alfa R, both with similar pharmacokinetics and pharmacodynamics profiles to Intron A® [23, 24]. Its biological properties have been meticulously studied in several cell lines [25-29] and clinically demonstrated since the classic uses [30-34] up to other such as: asymptomatic carriers of the human immunodeficiency virus [35], hepatic cirrhosis [36], Peyronie's disease [37], multiple sclerosis [38], schizophrenia [39] and epidemic neuropathy [40].

In 2010, PEG-Heberon® was introduced by CIGB, an IFN alpha-2b molecule conjugated to two-branched 40 kDa polyethylene glycol molecules, showing increased bioavailability and pharmacokinetics in rabbits [41] and further tested in a bioequivalence trial in healthy human subjects [42]. More recently, a phase I trial with the formulation was reported containing IFNs alpha and gamma in synergistic proportions [43].

IFN alpha has been associated with substantial toxicity in relation to the neurologic, cutaneous, musculoskeletal, gastrointestinal, cardiovascular, renal, hepatic, and hematologic systems. However, the main manifestation is the flu-like syndrome, represented by headache, fever, chills, asthenias, myalgia and arthralgia. The incidence and severity of the adverse events are clearly dose-related; some occur more frequently and are more severe with longer duration of the therapy. Most of them are both predictable and completely reversible with interruption of therapy [44].

In 2016, a safety and efficacy analysis of Heberon ® Alfa R was carried out, with data from its clinical use after 30 years of existence as a leading product of the Cuban biotechnology.

Materials and methods

According to the evaluation and characterization of Heberon® Alfa R safety profile 5806 subjects with evidence of the product administration within 28 years (1986-2014) were considered. The range dosage included from 3 to 30 million international units (MIU) weekly in therapeutic regimens since one week to five years of extension (Table 1).

The 5806 individuals were subdivided in 5332 adults (92 %) and 474 children (8 %). The information was collected from 147 clinical reports, conducted 128 (87 %) in adults and 19 (13 %) in children. The trials were Phase I studies in healthy volunteers, Phase II exploratory, Phase III confirmatory and Phase IV clinical studies of national extension in Cuba; also, several reports of treated cases were used. Fifty-two different therapeutic indications were covered by 13 medical specialties.

Table 1. Distribution of patients and Heberon® Alfa R therapeutic schemes

Doses (MIU)	Frequency (times/week)	Patients per treatment: Up to 24 weeks	%	Up to 48 weeks	%	More than 48 weeks	%	Total	%
1	5	2	0.07	0	0	0	0	2	0.03
1	1	0	0	0	0	159	20.5	159	2.7
2	5	119	4.2	0	0	0	0	119	2.0
3	3	664	23.5	2005	90.7	400	51.5	3069	53.0
3	2	219	7.8	62	2.8	190	24.5	471	8.1
3	1	40	1.4	0	0	0	0	40	0.7
5	3	171	6.1	0	0	0	0	171	3.0
6	5	16	0.6	0	0	0	0	16	0.3
6	3	483	17.1	44	2.0	0	0	527	9.0
6	1	9	0.3	0	0	0	0	9	0.2
6	2	1	0.04	0	0	27	3.5	28	0.5
9	3	70	2.5	0	0	0	0	70	1.2
9	2	22	0.8	0	0	0	0	22	0.4
10	5	66	2.3	0	0	0	0	66	1.1
10	3	276	9.8	85	3.8	0	0	361	6.2
10	2	138	4.9	0	0	0	0	138	2.4
10	1	447	15.9	0	0	0	0	447	7.7
20	2	47	1.7	0	0	0	0	47	0.8
20	1	30	1.1	14	0.6	0	0	44	0.8
	Total	2820	48.6	2210	38.0	776	13.4	5806	100

MIU: Million of International Units.

It was considered an adverse event any unfavorable medical incident presented in individuals to whom there was administrated the pharmaceutical product and who necessarily did not have a causality relation with the treatment. There was also considered any involuntary unfavorable sign (including an abnormal laboratory find), symptom or disease temporarily present, related or not with IFN alpha.

Adverse events were classified by intensity, according to the following grading scale: mild (if they did not require treatment), moderate (if they implied the administration of a specific therapy) and severe (if a modification of the original therapeutic scheme was necessary). Besides, they were also classified by consequence as: serious adverse event whatever untoward medical occurrence that at any dose resulted in death, required admission or prolongation of hospital stay, or persistent significant disability or life threatening [45].

For the nomenclature of adverse events, we used the terminology established by the World Health Organization (WHO) for the codification of the clinical information with respect to medicinal treatments (WHO Adverse Reaction Terminology, WHO-ART). This methodology is based on the classification according to a defined list of the different systems and organs of the human body [46]. The frequency of adverse events was classified according to the criteria of the Council of International Organizations of Medical Sciences (CIOSM), based on the number of individuals affected [47].

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We studied the presence of anti-IFN alpha antibodies in serum samples collected from patients and processed in biological laboratories in the CIGB. A simple immunoenzymatic assay (ELISA) 'sandwich' type (IFN alpha-2b/sample/protein A–peroxidase conjugate) was used with specificity confirmation, developed and validated by the CIGB [48]. A second bioassay was implemented in cells, to assess the ability of antibodies to neutralize the antiviral activity of IFN [49]. Both steps were in line with the patterns recommended by WHO and the International Society for IFN and Cytokine Research [50].

Thyroid function was evaluated every three months by clinical laboratories in the hospitals using ELISA systems and commercial kits for the determination of thyroid hormones T3, T4 and TSH.

Descriptive statistics were used to present the characteristics of the patient population studied. Dichotomous qualitative variables were used and frequency tables were designed for categorical data. Contingency tables and Pearson's Chi square test (χ^2) was used with a 95 % confidence level (5 % significance level) in order to determine the relation of dependence or independence between the type of Heberon® Alfa R formulation and the occurrence of adverse events.

Results

Up to 147 clinical reports were analyzed for safety and efficacy profile of Heberon® Alfa R and 103 (70 %) were conducted with the lyophilized formulation and 38 (26 %) used the liquid formulation. The other 6 (6 %) was conducted with eye drops or gel preparations.

The population studied was heterogeneous in terms of dose, frequency and duration of treatment with Heberon® Alfa R (Table 1). From 5806 individual analyzed, 4401 (76 %) received daily doses or cyclic administration (3 times per week on alternate days), while 1405 (14 %) were treated with intermittent (once or twice per week). Long-term regimens were applied in 2575 (44 %) patients and consisted in 9 to 30 million of international units (MIU) weekly during 48 or more weeks of treatment.

Adverse events were reported in 4864 subjects (84 %). There were compiled 18 234 reports of adverse events, with 211 different types of manifestations, where 185 (88 %) were detected by physical examination, while 26 (12 %) corresponded to alterations in the normal levels of the hematologic, biochemical and endocrine parameters evaluated by the clinical laboratory.

Table 2 shows thirty-three adverse events presented in more than 1 % of the patients evaluated; in which the main ones corresponded to flu-like syndrome, represented by fever, headache, myalgia, chills, arthralgia and asthenia, reported by more than a thousand patients (≥ 20 %). Among hematologic events, the most frequent was anemia in approximately 15 %, while a smaller number of individuals were affected by thrombocytopenia and neutropenia. In the classification by WHO-ART, the highest incidences corresponded to six general disorders, followed by four gastrointestinal and four psychiatric disorders.

Frequency classification by CIOSM is presented in Table 3, showing that 84 % of adverse events were unrelated to Heberon® Alfa R; most of them were considered as rare and another part infrequent ones. There were 29 events reported in two subjects and 60 were single. Each event occurred in less than half of patients.

Table 2. Distribution of patients treated with Heberon® Alfa R by adverse events classified according to WHO-ART *

No.	Adverse events	N (patients)	%	System-organ classes for disorders classification
1	Fever	2877	49.6	General
2	Headache	2094	36.0	Central & peripheral nervous system
3	Myalgia	1852	32.0	Muscle-skeletal system
4	Arthralgia	1467	25.0	Muscle-skeletal system
5	Chills	1436	24.7	General
6	Asthenia	1155	20.0	General
7	Anemia	846	14.6	Red blood cell
8	Anorexia	571	9.8	Psychiatric
9	Leucopenia	552	9.5	White cell and reticuloendothelial system
10	Malaise	447	7.7	General
11	Nausea	314	5.4	Gastro-intestinal system
12	Fatigue	275	4.7	Metabolic and nutritional
13	Weight loss	271	4.7	General
14	Vomiting	246	4.2	Gastro-intestinal system
15	Irritability	230	4.0	Psychiatric
16	Hair loss	227	3.9	Skin and appendages
17	Depression	221	3.8	Psychiatric
18	Thrombocytopenia	189	3.3	Platelet, bleeding & clotting
19	Insomnia	186	3.2	Psychiatric
20	Injection site pain	153	2.6	Application site
21	Diarrheas	148	2.5	Gastro-intestinal system
22	Dizziness	148	2.5	Central & peripheral nervous system
23	Burning at the injection site	144	2.5	Application site
24	Dry mouth	131	2.3	Gastro-intestinal system
25	Tremors	117	2.0	Central & peripheral nervous system
26	Pruritus	101	1.7	Skin and appendages
27	Body pain	104	1.8	Muscle-skeletal system
28	Neutropenia	92	1.6	White cell and reticuloendothelial system
29	Erythema at the injection site	92	1.6	Application site
30	Abdominal pain	85	1.5	General
31	Somnolence	86	1.5	Psychiatric
32	Skin rash	82	1.4	Skin and appendages
33	Hyper-menstrual bleeding	71	1.2	Female reproductive system

* Adverse events with more than 1 % of affected patients. WHO-ART: World Health Organization - Adverse Reaction Terminology.

Data from 541 patients (111 children and 430 adults) included in three trials with administration of Heberon® Alfa R; during 48 or more weeks, are shown in Table 4. This subpopulation had 7886 reports of adverse events, 79 % of them were classified as mild intensity, mainly during the first administrations. Less than 1 % was reported with severe intensity, the majority were hematologic manifestations as anemia, leukopenia or neutropenia in patients with chronic hepatitis C. No adverse events were classified as serious.

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Table 3. Adverse events distributions of patients treated with Heberon® Alfa R according to scale CIOSM

Adverse events (%)	Affected patients	Classification	CIOSM scale for the amount of affected patients
7 (3 %)	≥ 581	Very frequent	CIOSM ≥ 1/10
26 (12 %)	58-580	Frequent	1/100 ≤ CIOSM < 1/10
51 (24 %)	6-57	Infrequent	1/1000 ≤ CIOSM < 1/100
127 (60 %)	< 6	Rare	1/10 000 ≤ CIOSM < 1/100

CIOSM: Council of International Organizations of Medical Sciences.

The relation between demographic characteristics and the incidence of adverse events was analyzed in 1660 patients. A statistically significant relation by chi-squared was found for sex ($p < 0.010$), skin color ($p < 0.000$) and for intrathecal, intralesional, intramuscular and subcutaneous routes of administration ($p < 0.000$). The higher frequency of adverse events was observed in male white patients with a fever as unique events related.

Thyroid function was evaluated by the clinical laboratory in 79 patients (Table 5) and detected 34 (43 %) with thyroid hormones induced by Heberon® Alfa R. Hypothyroidism found in 25 patients was the most common event and chronic hepatitis C the most associated disease.

The immunogenicity profile of the lyophilized formulation of Heberon® Alfa R was analyzed in 952 patients from 23 clinical investigations (Table 6). Antibodies with antiviral action neutralizing capacity were detected in 22 (2.3 %) individuals. This percentage was lower than 2.7 % reporting for the other IFN alpha-2b and also inferior than 25.7 % described for the IFN alpha-2a from the international market. A significant dependence was found between the type of product and the induction of antibodies when Heberon® Alfa R was compared against the Roferon A®.

The statistical analysis revealed that the type of Heberon® Alfa R preparation had a significant influence in their safety profile (Table 7). There were 85 different adverse events reported for the liquid formulation and 176 with the lyophilized formulation. Meanwhile the comparative analysis of the six most frequent clinical adverse events found highest significant incidences of fever, myalgia, arthralgia and chills associated with the lyophilized formulation while only headache and asthenia predominated with the liquid formulation (Table 7).

Comparison between Heberon® Alfa R and PEG-Heberon® (pegylated IFN alpha-2b produced by CIGB) similarly revealed that the occurrence of adverse events depended on the product administered (Table 8). The use of pegylated reduced from 211 to 80 the different types of adverse events reported and also evidenced a decrease in the percentage of patients reporting flu-like symptoms (Table 8).

Table 9 summarizes the efficacy of Heberon® Alfa R from 110 clinical trials performed in their different biological effects described. Nearly 60 % of the treated patients obtained a direct benefit from the use of Cuban IFN alpha as treatment for their disease; this includes disappearance of any clinical, histological or virological evidence.

Table 4. Adverse events classification by intensity in patients treated with Heberon® Alfa R

Disease	N	No adverse events (%)	Intensity (%)			
			Mild	Moderate	Severe	Total
RRP	162	62 (38.3 %)	143 (62.7 %)	75 (33.0 %)	10 (4.4 %)	228 (2.9 %)
CHB	33	5 (15.2 %)	86 (100 %)	0	0	86 (1.1 %)
CHC	346	18 (5.2 %)	5993 (79.1%)	1538 (20.3 %)	41 (0.5 %)	7572 (96.0 %)
Total	541	85 (15.7 %)	6222 (78.9 %)	1613 (20.5 %)	51 (0.65 %)	7886

RRP: Recurrent Respiratory Papillomatosis. CHB: Chronic Hepatitis B. CHC: Chronic Hepatitis C.

Table 5. Thyroid function evaluation in patients treated with Heberon® Alfa R

Disease	Patients	Hypothyroidism		Hyperthyroidism	
		Maintained	Induced	Maintained	Induced
CHC	54	14 (26 %)	21 (39 %)	4 (7 %)	7 (13 %)
MM	13	0	3 (23 %)	0	1 (7.7 %)
FM	11	0	1 (9 %)	0	1(9 %)
CML	1	0	0	0	0
Total	79	14 (18 %)	25 (32 %)	4 (5 %)	9 (11 %)
Total induced			34 (43 %)		

Maintained: Thyroid dysfunction existing before and during treatment. Induced: Treatment-induced thyroid dysfunction. CHC: Chronic hepatitis C. MM: Multiple myeloma. FM: Fungoid mycosis. CML: Chronic myeloid leukemia.

Table 6. Immunogenicity profile in patients treated with Heberon® Alfa R, compared to Roferon A® and Intron A®*

Interferon (trademark)	HSA content (mg/mL)	Evaluated patients	Anti-IFN neutralizing antibodies	%
IFN alfa 2a (Roferon A®)	5.0	548	141	25.7
IFN alfa 2b (Intron A®)	1.0	9911	268	2.7
IFN alfa 2b (Heberon® Alfa R)	1.5	952	22	2.3

*The three products were produced in *Escherichia coli* as a host organism and marketed as lyophilized formulations. Data for Roferon A® and Intron A® were from the Schering-Plough Research Institute, 2001. Heberon® Alfa R data was compared by the χ^2 test to that of Roferon A® ($p< 0.05$) and Intron A® ($p = 0.47$). HSA: human serum albumin.

Table 7. Comparison of safety profiles of Heberon® Alfa R liquid and lyophilized formulations*

Adverse event	Occurrence	Lyophilized		Liquid	
		Patients	%	Patients	%
Fever	Yes	1974	54.76	903	41.02
	No	1631	45.24	1302	58.98
Myalgia	Yes	1336	37.05	516	23.44
	No	2269	62.95	1685	76.56
Arthralgia	Yes	1080	29.96	387	17.58
	No	2525	70.04	1814	82.42
Chills	Yes	1076	29.85	360	16.36
	No	2529	70.15	1841	83.64
Headache	Yes	1018	28.24	1076	48.89
	No	2587	71.76	1125	51.11
Asthenia	Yes	629	17.45	526	23.90
	No	2976	82.55	1675	76.10
All	Yes	3247	90.07	2170	98.59
	No	358	9.93	31	1.41
Total		3605		2201	

*Heberon® Alfa R lyophilized and liquid formulations' data showed statistically significant differences (χ^2 test; $p < 0.05$) in the number of patients showing each adverse event and in general, with less patients having them with the liquid formulation.

*D*iscussion

We considered that a review of a large number of patients and trials was pertinent because data from a broad group of physicians were compiled; and the comparisons between different formulations and routes of administration will be useful to facilitate improving and maintaining the use of Heberon® Alfa R in the treatment of viral and malignant diseases in the future.

The methodology used for the nomenclature of events in safety profile of Heberon® Alfa R was correct because WHO-ART is the most commonly alternative recommended by regulatory agencies and pharmaceutical companies. Moreover, the CIOMS had functioned, since 1977, as a forum for discussion between international drug regulatory authorities and pharmaceutical companies; and in 1994 an unambiguous international medical terminology for regulatory purposes [51] was established.

Entirely expected in any safety analysis of IFN alpha, including some previous studies performed with Heberon® Alfa R [52-54], the most frequent

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adverse events were those related to pseudoinfluenzal syndrome. Administration of exogenous IFN alpha releases a cascade of cytokines, including tumor necrosis factor alpha (TNF alpha), interleukin (IL)-1, IL-2, IL-6, IFN gamma and IFN-inducible protein-10 [55]. Many of the cytokines are mediators of the cellular and inflammatory immune response to a viral infection; consequently, they can cause similar constitutional symptoms and activate another complex cascade of processes responsible for several IFN alpha toxicities. For example, the neurologic effect comprising fatigue, mood disorders, cognitive changes, depression and anorexia appear as the result of the interaction of IFN with the opioids receptors located at hypothalamic level [56].

Meanwhile, fever is associated with a stimulated basic mechanism of temperature increase because the cytokines released in response to exogenous IFN are important endogenous pyrogens capable of activating receptors on thermosensitive neurons in the preoptic area of the hypothalamus (*i.e*., the fever center of the brain). Moreover, IL-1 and TNF alpha trigger production of prostaglandin E2 by glial cells, which can reset the central thermostat [57].

Alterations in the hematological parameters were caused by myelosuppression after the interaction between IFN and specific receptors at the cellular level [58]. Anemia, one the most frequent events, was influenced by the hemolytic effect of ribavirin received by 404 patients from those treated for chronic hepatitis C [59].

It was confirmed that IFN alpha toxicities affect numerous organ systems and are associated with the individual dose regimen [60]. In the subject population studied, most of them were analyzed after long-term treatment regimens and with high cyclic doses weekly of Heberon® Alfa R administered, only the flu-like symptom classified as very frequent events. This finding corroborates that the events are mainly acute and often demonstrate progressive blunting within days on continued therapy [60]. This phenomenon known as tachyphylaxis is triggered because the first parenteral administrations of Heberon® Alfa R inhibit the IFN-alpha-specific IgG antibody response and induced peripheral tolerance to subsequent parenteral administration [61]. Clinically means that acute toxicity occurs at the beginning of the treatment and later diminishes in number and intensity until disappears. Another positive finding for Heberon® Alfa R was the low incidence of chronic toxicities such as weight loss, bone marrow depression, drowsiness and psychiatric depression, considered in association with cumulative dose or therapy duration [62].

All authors agree that the majority of IFN alpha side effects can be managed with appropriate supportive care [60-62]. Our experience confirmed the utility of prophylactic administration of non-steroidal anti-inflammatory drugs for reduce flu-like symptoms and improve adherence of patients to treatment.

We confirmed that thyroid dysfunction (TD) is the most common endocrinopathy associated with hepatitis C and may be a condition exacerbated or induced by the treatment with IFN alpha [63]. Studies have shown that in patients who developed TD, immune markers levels such as IL-6 and TNF alpha are relatively higher, the condition consistent with a phenomenon secondary to the administration of exogenous IFN alpha [64]. Our results were in correspondence with many similar studies with high frequency of hypothyroidism [65].

Heberon® Alfa R showed the lowest immunogenicity. The comparison between different products of the same biological molecule is usually controversial because many factors may be involved. We consider that the manufacturing and purification procedure of the Cuban IFN alpha-2b has contributed to a significant reduction in contaminating immunogenic molecular species [66].

The three formulations compared were lyophilized and contained human serum albumin (HSA) as a stabilizer. Since the HSA can covalently interact with other proteins, a formation of heterotypic IFN-HSA aggregates has been described. Furthermore, during lyophilization a homotypic IFN-IFN aggregates can be formed. Both types of protein aggregation are considered impurities contributing to the formation of antibodies [67]. Marketed IFN alpha products contain 10 to 5000 times more HSA than IFN, depending on the formulation; therefore it is feasible to consider further formation of IFN-HSA complexes; consequently ROFERON® with 5-fold more HSA is 10-fold more immunogenic [68].

Heberon® Alfa R with 1.5 mg/mL HSA content was slightly less immunogenic than Intron A® with

Table 8. Comparison of safety profiles of Heberon® Alfa R and PEG-Heberon®*

Adverse event	Occurrence	Lyophilized		Liquid	
		Patients	%	Patients	%
Fever	Yes	2877	50.40	148	27.00
	No	2929	49.60	400	73.00
Myalgia	Yes	1852	31.90	104	18.98
	No	3954	68.10	444	81.02
Arthralgia	Yes	1467	25.27	103	18.80
	No	4339	74.73	445	81.20
Chills	Yes	1436	24.73	44	8.03
	No	4370	75.27	504	91.97
Headache	Yes	2094	36.07	124	22.63
	No	3712	63.93	424	77.37
Asthenia	Yes	1155	19.89	160	29.20
	No	4651	80.11	388	70.80
All	Yes	5417	93.30	524	95.62
	No	389	6.70	24	4.38
Total		5806		548	

*Heberon® Alfa R lyophilized and liquid data showed statistically significant differences (χ^2 test; $p < 0.05$; $p = 0.04$ for the overall data comparison) in the number of patients showing each adverse event and in general, with less spatients having among those treated with PEG-Heberon®. PEG-Heberon® is a liquid formulation, while data of Heberon® Alfa R comprise patients treated with both formulations (3605 with lyophilized and 2201 with liquid formulations).

Table 9. Efficacy profile of Heberon® Alfa R

Biological effect	Trials	Patients treated	Respondent patients	%
Antiviral	56	2902	1511	52
Anti-proliferative	31	657	509	81
Anti-angiogenic	4	131	94	77
Anti-fibrotic	4	139	95	68
Immunomodulatory	3	47	38	81
Central Nervous System	17	696	418	60
General	115	4572	2665	58

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1.0 mg/mL HSA. Although this finding could be related to minimal post-transcriptional changes and small differences in their three-dimensional structures, it is much more probable to consider the possible formation of aggregates favored by inadequate storage conditions, the pH used for the formulation, as well as for the process of INTRON A® lyophilization [69]. The development of HSA-free Heberon® Alfa R liquid formulation was a very important contribution to the clinical safety profile and to the reduction of immunogenicity by eliminating the two sources of IFN–HSA and IFN–IFN aggregates formation [70].

The lower incidence of adverse events reported by individual studies [71] with PEG-Heberon® is one of the traditional advantages reported for pegylated versus conventional formulation [72]. The new IFN alpha & gamma formulation is expected to demonstrate clinical results that coincide with the reduction in number and intensity of adverse events with respect to both individual products [73].

In summary, from the large number of trials reviewed and the low percentage of patients with clinically relevant adverse events, we consider the Cuban IFN alpha produced by CIGB a well-tolerated, effective and safe drug. In particular, the HAS-free liquid formulation of Heberon® Alfa R offers a positive benefit/risk ratio safety profile.

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