

Comparative Pharmacokinetics and Pharmacodynamics of Two Recombinant Human Interferon Alpha-2b Formulations Administered Intramuscularly in Healthy Male Volunteers

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ABSTRACT

A randomized, double-blind, two-treatment study was designed to compare the pharmacokinetic, pharmacodynamic and safety profiles of INTRON A and HEBERON ALFA R—two recombinant interferons (IFN) alpha 2b—after a single 10×10^6 IU intramuscular injection in 24 healthy male volunteers. Subjects of 20–35 years old and body mass index of 19–29 kg/m² who gave informed consent, were eligible. Pharmacokinetic profiles were calculated from the serum levels of IFN antiviral activity. Temperature and serum beta-2-microglobulin concentration were taken as pharmacodynamic parameters. There were no differences between groups in the baseline variables. Observed peak serum activities (C_{max}) were 15 and 18 IU/mL after 5 and 7 h (t_{max}) for INTRON A and HEBERON ALFA R, respectively. There were no significant differences regarding clearances (CL = 18.6 vs. 18.7 L/h). However, INTRON A showed significantly smaller area under the curve from 0 to 24 h (AUC_{0-24} = 168 vs. 237 IU·h/mL; P = 0.049) and distribution volume (V_d = 190 vs. 209 L; P = 0.02), shorter mean residence time (MRT = 10 vs. 11 h; P = 0.04), mean input time (MIT = 10.2 vs. 13.8 h; P = 0.003), half value duration (HVD = 8.6 vs. 15.0 h; P = 0.003), and larger C_{max}/AUC ratio (CAV = 9.0 vs. 7.6 h⁻¹; P = 0.01). Antiviral activity was detected 24 h after injections. A depot effect at the administration site seemed to occur, which was more intense for HEBERON ALFA R. Both formulations induced similar significant increases in serum beta-2-microglobulin. There was a high correlation between the IFN titers and the temperature. Both products produced almost identical fever profiles. Side effects were also similar: fever and headache (100%), chills (71%), back pain (63%), arthralgias (33%), and vomiting (17%). Only one subject showed a moderate leukopenia. Despite the differences in some pharmacokinetic parameters, we conclude that both products have similar profiles, pharmacodynamic actions and safety patterns.

Keywords: IFN, interferon alpha-2b, formulation, pharmacodynamics, pharmacokinetics, recombinant

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RESUMEN

Farmacocinética y farmacodinamia comparadas de dos formulaciones de interferón alfa-2b humano recombinante administradas por vía intramuscular en hombres voluntarios sanos. Se diseñó un estudio aleatorizado y a doble ciegas para comparar la farmacocinética, la farmacodinamia y el perfil de seguridad de INTRON A y HEBERON ALFA R—dos formulaciones de interferón (IFN) alfa-2b humano recombinante— después de una inyección intramuscular única de 10×10^6 UI en 24 hombres voluntarios sanos. Se eligieron sujetos de 20-35 años de edad, con índice de masa corporal de 19-29 kg/m², que dieron su consentimiento informado de participación. Los perfiles farmacocinéticos se calcularon a partir de los niveles séricos de actividad antiviral del IFN. Se tomó la temperatura y la concentración de beta-2-microglobulina como parámetros farmacodinámicos. No hubo diferencias entre los grupos en las variables de base. Los valores máximos de actividad sérica (C_{max}) fueron 15 y 18 UI/mL a las 5 y 7 h (t_{max}) para INTRON A y HEBERON ALFA R, respectivamente. No hubo diferencias significativas en cuanto a los aclaramientos (CL = 18,6 vs. 18,7 L/h). Sin embargo, INTRON A mostró valores significativamente menores de área bajo la curva de 0 a 24 h (AUC_{0-24} = 168 vs. 237 IU·h/mL; P = 0.049), volumen de distribución (V_d = 190 vs. 209 L; P = 0,02), tiempo de residencia medio (MRT = 10 vs. 11 h; P = 0,04), tiempo medio de entrada (MIT = 10,2 vs. 13,8 h; P = 0,003), tiempo de duración del valor medio (HVD = 8,6 vs. 15,0 h; P = 0,003) y mayor relación C_{max}/AUC (CAV = 9,0 vs. 7,6 h⁻¹; P = 0,01). Se detectó actividad antiviral en suero a las 24 h después de las inyecciones. Parece que se produce un efecto de depósito en el sitio de administración que es más intenso para HEBERON ALFA R. Ambas formulaciones indujeron incrementos significativos similares en los niveles séricos de beta-2-microglobulina. Hubo una alta correlación entre los títulos de IFN y la temperatura. Ambos productos produjeron perfiles de fiebre casi idénticos. Los efectos adversos también fueron similares: fiebre y cefalea (100%), escalofríos (71%), dolor lumbar (63%), artralgiás (33%) y vómitos (17%). Sólo un sujeto tuvo una leucopenia moderada. A pesar de las diferencias en algunos parámetros farmacocinéticos, se puede concluir que ambos productos exhiben perfiles, acciones farmacodinámicas y patrones de seguridad similares.

Palabras claves: farmacocinética, farmacodinamia, formulación, IFN, interferón alfa-2b, recombinante

Introduction

Studies comparing different preparations of the same drug substance have gained considerable importance over the last few years. Two formulations can differ in their extent and rate of drug absorption, biological effects, and tolerability due to their components. Furthermore, the integration of pharmacokinetic and phar-

macodynamic characterizations into drug development provides a scientific framework for its rational and efficient application [1].

The interferons (IFNs) have been accepted as antiviral and antitumor agents for several years. While many of their characteristics and properties have been identi-

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fied, others are still under study at the same time that clinical trials are being carried out to assess their efficacy. As biological response modifiers, IFNs have several properties that ought to be taken into account. Firstly, the IFN system is a naturally occurring defense mechanism; secondly, IFNs have multiple pathways of action; and thirdly, IFNs are naturally produced at critical body sites and have a unique pharmacodynamics. These features indicate that IFNs are part of a complex, interactive, physiological system with special molecular, biological and pharmacological characteristics that have to be understood in order to optimally exploit their therapeutic potential [2].

Several recombinant IFN α -2 preparations have been used in clinics essentially with the same results in terms of efficacy and safety. Some examples are viral diseases such as chronic hepatitis B [3–5] and C [6, 7], and condylomata acuminata [8, 9], as well as benign [10–12] and malignant [13, 14] neoplasias. However, the components of the formulations can be responsible for some differences that have arisen, for example, in immunogenicity [15, 16].

Some recent works have focused on the effect of differences in the composition of IFN formulations on its pharmacokinetic and pharmacodynamic properties [17, 18]. The aim of this work was to compare these characteristics, as well as the tolerability of two formulations of recombinant IFN α -2b administered intramuscularly to healthy male volunteers.

Materials and Methods

Study design

A randomized, double-blind, two-treatment study was designed. Twenty-four men aged 20 to 35 years, weighing 55 to 93 kg, with a body mass index between 19 and 29 kg/m², and who gave their written, informed consent, were eligible. Each subject was healthy by medical history, physical examination and laboratory tests. All of them received a 10 x 10⁶ IU dose of one of the formulations. Blood samples for serum IFN determinations were collected by separated venipunctures from an antecubital vein, just before injection and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16, and 24 h post-injection. Pharmacodynamics was assessed by recording body temperature profiles and by evaluating the serum β_2 -microglobulin (β_2 -m) concentration. Tumor necrosis factor alpha (TNF- α) was also measured in serum. Clinical monitoring lasted 24 h and included temperature, heart rate, blood pressure, and symptomatic side effects. Adverse events were recorded throughout the study. Hematological and blood chemistry tests were performed prior and 24 h after injection. They included leukocyte, neutrophil, lymphocyte, monocyte, eosinophil and platelet counts, and hemoglobin determination. Hepatic (serum aspartate and alanine aminotransferases), and renal (creatinine) functions were assessed too.

Study drugs

INTRON A (Shering-Plough, Innishannon, Ireland) was available as a sterile lyophilized powder containing IFN α -2b, NaH₂PO₄·H₂O (0.55 mg/mL), Na₂HPO₄ (2.27 mg/mL), human serum albumin (1 mg/mL), and glycine (20 mg/mL).

HEBERON ALFA R (Heber Biotec S.A., Havana, Cuba) was supplied as sterile lyophilized powder containing IFN α -2b, NaCl (7 mg/mL), NaH₂PO₄·H₂O (6 mg/mL), Na₂HPO₄ (11.4 mg/mL), human serum albumin (1.5 mg/mL), manitol (5 mg/mL), and low molecular weight dextran (10 mg/mL).

Each product was stored at 2–8 °C in vials containing 10 x 10⁶ IU. Their activity was accepted as stated on the label. Nevertheless, both batches were checked for antiviral activity. They were reconstituted with 1 mL of sterile water immediately before each administration.

Assay methods

Levels of IFN α in serum were quantified using a cytopathic effect inhibition (CPEI) bioassay [19, 20]. The CPEI assay detected the ability of IFN α to protect HEP-2 cells (human laryngeal carcinoma; ATCC No. CCL 23) from lysis due to Mengo virus infection. Cell monolayers were incubated in 96-well polystyrene plates (Costar, Cambridge, MA) at 37 °C, 5% CO₂, 95% humidity for 24 h with serial twofold dilutions of serum samples or standards to allow the induction of the antiviral state. Then, Mengo virus (approximately 10^{7.2} TCID per well) was added and incubation proceeded in the same conditions for 18 h more. Cell lysis was determined using a crystal violet staining and reading the absorbance at 570 nm in a plate reader (Tecnosuma, Havana, Cuba). Appropriate wells without IFN (virus controls), or without IFN or virus (cell controls), and a laboratory IFN α standard, were included in each plate. This standard was calibrated against the WHO international standard 69/19 of natural human leukocyte interferon [21]. An IFN unit is defined as the concentration that protects 50% of the cell monolayer from lysis. Serum samples and standards were tested twice in two replicate plates. Titers were calculated from Probit regression analysis of the absorbance *versus* sample dilution curves and standardized with the corresponding reference preparation. The weighed geometric means of the titers were taken.

Serum β_2 -m concentrations were measured with a quantitative competitive radioimmunoassay, as previously described [22].

TNF- α was measured by means of a bioassay with the murine fibroblast cell line L929 as target [23]. A specific sandwich-type ELISA [24], kindly provided by M Ojeda (Pharmaceutical Division, Center for Biological Research, Cuba), was also used. Ninety-six well, flat-bottom plates (Nunc, Denmark) were coated with a murine monoclonal anti-human TNF- α antibody. A rabbit polyclonal anti-human TNF- α serum was used as a second antibody, and a peroxidase-labeled goat anti-rabbit serum conjugate was used to develop the reaction.

Pharmacokinetic analysis and statistical methods

A standard descriptive analysis of serum IFN activity data for both IFN α -2b products, was conducted. The pharmacokinetic profiles were characterized by using the area under the curve from 0 to 24 h (AUC_{0–24}) post-dosing, using the trapezoidal algorithm, the observed peak serum activity (C_{max}), the observed time

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to peak serum activity (t_{max}), the terminal rate constant (β) by logarithmic linear regression, the half life ($t_{1/2}$) by $\ln 2/\beta$, the mean residence time (MRT), the C_{max}/AUC ratio (CAV), the clearance (CL), the distribution volume (Vd), and the half value duration (HVD) time during which the serum concentration deviates from the maximum concentration by less than 50%. They were calculated according to previously described methods [25–27].

Pharmacokinetic parameters were calculated and normalized with respect to the dose/m² received by each individual. The parametric statistical Student's *t* test was used when normal distribution and variance homogeneity permitted it. Alternatively, the non-parametric Mann-Whitney's U test was used. Pearson's correlation analysis was performed between serum IFN concentrations and body temperature at each time point. β_2 -m values were compared within groups (differences with $t = 0$) by analysis of variance and Duncan's test for multiple comparisons. Treatment effects on hematological and blood chemistry variables were assessed using the paired Student's *t* test comparing pre-treatment and post-treatment values. The significance level considered was $P = 0.05$, except for the comparison of IFN concentrations at the different times after injection. In that case, in order to correct the increase of the error due to multiple serial tests, the *P* value was corrected by the number of comparisons ($0.05/14 = 0.00357$).

Results

There were no statistically significant differences between the groups in the demographic characteristics, baseline variables, or in the dose received/m² (Table 1).

Figure 1A shows the mean serum IFN concentration profiles throughout the follow-up period. After 12 h post-injection, the mean serum IFN activity tended to be higher with HEBERON ALFA R than following INTRON A administration, but it was statistically significant only at 24 h ($P = 0.0032$). C_{max} were 15 and 18 IU/mL after 5 and 7 h (t_{max}) for INTRON A and HEBERON ALFA R administration, respectively ($P > 0.05$). Clearances did not differ significantly as well. None of the volunteers had IFN in serum before treatment (< 1 IU/mL) and antiviral activity (range: 1–12; median: 4 IU/mL) was found in all but one individual 24 h after injection of either formulation. Consequently, the AUC_{0-24} value was $< 80\%$ of the total extrapolated area ($AUC_{0\infty}$). Table 2 summarizes the pharmacokinetic parameters and characteristics of each product. INTRON A yielded significantly smaller AUC_{0-24} value, larger CAV, and shorter MRT, mean input time (MIT), HVD and smaller Vd. The $t_{1/2}$ value was longer for HEBERON ALFA R, although not statistically significant. The terminal rate constants (β) were not different.

Both products induced almost identical fever profiles (Figure 1B). There was a highly significant correlation between serum IFN titers and body temperature ($r = 0.435$; $P < 0.000001$). Approximately 1 IU/mL corresponded to a 0.1 °C increase in temperature. Interestingly, the temperature was slightly higher for the same IFN level during the descending phase than during the ascending phase of the IFN curves (result not shown). Increases in the serum β_2 -m levels (Figure 1C)

Table 1. Summary of demographic and baseline variables. The data are the mean \pm standard deviation (SD).

	INTRON A	HEBERON ALFA R	P
Age (years)	26.8 \pm 4.06	26.5 \pm 5.23	0.86
Weight (kg)	73.4 \pm 12.3	69.6 \pm 7.9	0.37
Height (m)	1.75 \pm 0.07	1.74 \pm 0.05	0.47
Body surface (m ²)	1.88 \pm 0.18	1.82 \pm 0.11	0.35
Body mass index (kg/m ²)	23.82 \pm 3.12	23.08 \pm 2.13	0.51
Dose received (IU/m ²)	5.37 \pm 0.52	5.51 \pm 0.33	0.44
Body temperature (°C)	36.7 \pm 0.43	36.5 \pm 0.22	0.20
Heart rate (beats/min)	78 \pm 7.34	72 \pm 7.52	0.08
Systolic blood pressure (mm Hg)	123 \pm 10.73	125 \pm 10.87	0.69
Diastolic blood pressure (mm Hg)	80 \pm 4.50	80 \pm 4.26	0.77
Hemoglobin (g/L)	150 \pm 7.19	150 \pm 10.95	0.88
Hematocrit (%)	48 \pm 0.20	48 \pm 0.30	1.00
Total leucocytes ($\times 10^9$ cells/L)	7.0 \pm 2.00	6.8 \pm 1.98	0.77
Neutrophils ($\times 10^9$ cells/L)	4.1 \pm 1.49	3.7 \pm 1.47	0.59
Lymphocytes ($\times 10^9$ cells/L)	2.3 \pm 0.64	2.5 \pm 0.51	0.48
Monocytes ($\times 10^9$ cells/L)	0.4 \pm 0.16	0.3 \pm 0.10	0.08
Eosinophils ($\times 10^9$ cells/L)	0.3 \pm 0.33	0.3 \pm 0.18	0.95
Platelets ($\times 10^9$ cells/L)	188 \pm 32.8	183 \pm 21.32	0.44
Alanine aminotransferase (U/L)	17 \pm 12.6	16 \pm 14.17	0.55
Gamma-glutamyltransferase (U/L)	24 \pm 12.1	21 \pm 8.49	0.50
Creatinin (μ mol/L)	75 \pm 9.44	78 \pm 8.33	0.92

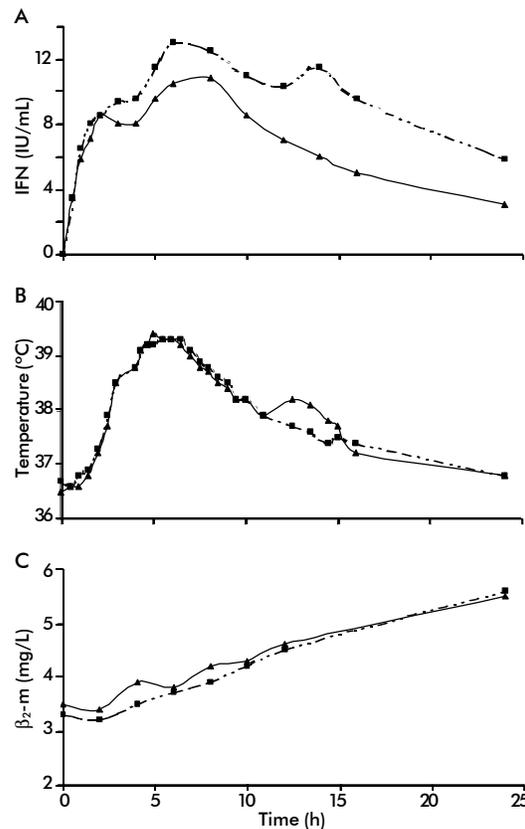


Figure 1. Variables measured at different times after a single intramuscular injection of 10×10^6 IU of two IFN α -2b formulations to healthy volunteers. Solid line, INTRON A; dashed line, HEBERON ALFA R. A) Mean serum IFN antiviral activities. B) Mean body temperature. C) Mean serum β_2 -microglobulin (β_2 -m) concentration. Standard deviations are not shown. Only the 24-h values were statistically significant ($P = 0.0032$) in A; there were no significant differences between groups in B or C. For both treatments, the increase in β_2 -m concentration was statistically significant, as compared to the $t = 0$ values.

were also similar for both formulations. They became statistically significant, as compared with pre-injection values, after 24 h ($P < 0.001$). TNF- α in serum was not induced by either IFN injection in any subject (results not shown).

There were no clinically significant differences in the frequency and intensity of adverse events produced by both treatments (Table 3). Injection-site re-

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Table 2. Summary of pharmacokinetic parameters and characteristics of both formulations of IFN α -2b. The data are the mean \pm standard deviation (SD).

	INTRON A n = 12	HEBERON ALFA Rn = 12	P
C_{max} (U/mL)	14.4 \pm 6.7	17.6 \pm 6.3	0.25
t_{max} (h)	5.2 \pm 2.7	7.0 \pm 2.8	0.20
Vd (L)	190.0 \pm 17.9	209.3 \pm 21.2	0.025
CL (L/h)	18.6 \pm 0.2	18.7 \pm 0.2	0.50
AUC ₀₋₂₄ (U·h/mL)	158.3 \pm 70.0	237.3 \pm 91.6	0.027
CAV (h ⁻¹)	9.12 \pm 1.44	7.58 \pm 2.00	0.006
MRT (h)	10.2 \pm 0.97	11.2 \pm 1.09	0.028
MIT (h)	10.2 \pm 3.44	13.8 \pm 1.82	0.003
HVD (h)	8.62 \pm 3.86	15.0 \pm 5.41	0.003
β (h ⁻¹)	0.13 \pm 0.13	0.08 \pm 0.04	0.11
$t_{1/2}$ (h)	7.8 \pm 3.5	11.3 \pm 5.1	0.064

C_{max} , observed peak serum activity; t_{max} , time to peak serum activity; Vd, distribution volume; CL, clearance; AUC₀₋₂₄, area under the curve from 0 to 24 h; CAV, C_{max} /AUC ratio; MRT, mean residence time; MIT, mean input time; HVD, half value duration; β , terminal rate constant; $t_{1/2}$, half time.

actions were not found. Both products produced a slight, but statistically significant decrease in lymphocyte and eosinophil, and an increase in monocyte counts (Table 4). These changes attained the pathological ranges only in some cases, as shown in Table 3. Main side effects were fever, headache, chills, back pain, arthralgias, and vomiting. All of these symptoms were mild, since they disappeared spontaneously or with common antipyretic medication. One subject from the INTRON A group had moderate leukopenia and granulocytopenia. The other hematological alterations shown in Table 3 correspond to different individuals. The blood cell counts recovered spontaneously in all cases.

Discussion

Randomization assured that both groups of volunteers were comparable regarding the main demographic and baseline variables. The clinical assessments as well as all the analytical determinations were performed following a double-blind design. Therefore, the internal validity of the data is high.

In published data, there is a considerable variability concerning the pharmacokinetic parameters and characteristics of IFN α -2 [reviewed in 28]. The maximum serum IFN α concentrations following intramuscular and subcutaneous injection of 5×10^6 IU/m² of INTRON A have been 18 to 116 IU/mL and occurred at 3 to 12 h after administration [29]. Zhi *et al.* obtained C_{max} values of 58–67 IU/mL at 7–8 h after a subcutaneous injection of 18×10^6 IU of IFN α -2a [18]. In addition, $t_{1/2}$ of 5–8.2 h [30, 31] have been found after an intramuscular administration of recombinant IFN α -2. Our results reside within these reported ranges for recombinant IFN α .

In order to better characterize the pharmacokinetics of both IFN α formulations, other parameters which are not often included in the published reports on IFN were calculated. The large Vd values are indicative of IFN binding to receptors in extravascular tissues throughout the body. The little skewed IFN concentration profiles suggested a depot effect at the administration site. This is characterized by the MIT, MRT and, better, by the HVD. The latter characteris-

Table 3. Frequency of adverse reactions after interferon α -2b treatment. The data are presented as the number of occurrences (%).

Adverse event	INTRON A	HEBERON ALFA R
Clinical		
Fever (temperature > 38 °C)	12 (100)	12 (100)
Headache	12 (100)	12 (100)
Chills	9 (75)	8 (67)
Tachycardia (heart frequency > 100)	9 (75)	8 (67)
Back pain	7 (58)	8 (67)
Hypotension (systolic blood pressure < 100 mm Hg)	8 (67)	8 (67)
Arthralgias	3 (25)	4 (33)
Vomiting	3 (25)	1 (8)
Hypertension (diastolic blood pressure > 90 mm Hg)	1 (8)	0
Myalgias	2 (17)	0
Photophobia	1 (8)	1 (8)
Hematological		
Increase in monocyte count (> 0.6×10^9 cells/L)	5 (42)	1 (8)
Anemia (hemoglobin < 120 g/L)	0	1 (8)
Leukopenia (< 4×10^9 cells/L)	1 (8)	0
Granulocytopenia (< 2×10^9 cells/L)	1 (8)	1 (8)
Lymphopenia (< 1×10^9 cells/L)	1 (8)	0 (8)

Table 4. Changes in white blood cell counts after IFN administration. Data express the mean difference between the values before and 24 h after injection for the corresponding group of volunteers. The sign indicates decrease (minus) or increase (plus) after treatment. The statistical analysis was done using the paired Student's *t* test within each group.

	INTRON A		HEBERON ALFA R	
	Mean change	P	Mean change	P
Total leukocytes ($\times 10^9$ cells/L)	- 0.44	0.44	- 0.63	0.27
Neutrophils ($\times 10^9$ cells/L)	+ 0.01	0.99	- 0.10	0.84
Lymphocytes ($\times 10^9$ cells/L)	- 0.47	0.027	- 0.59	0.001
Monocytes ($\times 10^9$ cells/L)	+ 0.16	0.038	+ 0.14	0.011
Eosinophils ($\times 10^9$ cells/L)	- 0.14	0.095	- 0.12	0.034

tic measures the plateau time during which the serum concentration deviates from C_{max} by less than 50%. These characteristics are more suitable than t_{max} [27]. It seems that this depot effect is more intense for HEBERON ALFA R, since its Vd and HVD values were significantly higher.

The statistically significant differences obtained for AUC₀₋₂₄, CAV, MRT, and MIT suggest that HEBERON ALFA R has a greater bioavailability (149%) than INTRON A, given by a slower absorption pattern and a longer persistence in blood.

Contrary to other reports [18, 28, 29], small but significant amounts of IFN were found in serum at 24 h after injection, probably because the antiviral bioassay was used for the IFN measurements. This method is more sensitive than the immunoassays used by others, which have detection limits around 10 IU/mL and with which we would have not detected any IFN at 24 h in any individual. In addition, the behavior of one of the main pharmacodynamic actions of IFN is measured at the same time with the bioassay. However, the bioassay is less precise, which reduces the power of the statistics, and less specific, since other cytokines such as TNF- α can exert some antiviral activity as well. In this work, TNF- α determinations did not reveal any induction during the experiment. In any case, the amount of IFN found at 16 and 24 h post-injection limited the pharmacokinetic analysis, mainly of the elimination phase, since AUC₀₋₂₄ was smaller than 80% of the extrapolated AUC_{0- ∞} .

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The results of this study indicate that two preparations with identical IFN α -2b proteins that are similar in their biochemical properties [32] differ in their pharmacokinetic profiles after intramuscular administration. The most probable explanation for this difference is that they have different excipients. The fact that there were no differences in clearance also suggest that the differences in AUC₀₋₂₄, CAV, MIT, MRT, Vd and HVD values are not due to the active principles, but to the other components of the formulations. This is also supported by immunogenicity screenings in treated patients, where both molecules have performed similarly [33, 34]. INTRON A contains glycine, whereas HEBERON ALFA R has NaCl, manitol, low molecular weight dextran, a higher phosphate concentration, and a slightly higher amount of albumin. These differences in the formulations could contribute to modify the absorption of IFN α -2b by affecting binding to the extracellular matrix and/or influencing inactivation by pH-dependent proteases.

An important point to consider is whether the differences in the formulations and in the pharmacokinetics have any clinical repercussion. Pharmacodynamic studies are an approach to this problem. The temperature profiles were indistinguishable for the two treatments. The highly significant correlation between serum IFN concentration and temperature shows that the latter is a good pharmacodynamic parameter for IFN action. Our results reproduced the counterclockwise hysteresis described by Zhi *et al.* [18] for IFN α -2a, which is indicative of a delay mechanism between the IFN concentration and its flu-like effect. Both preparations induced a similar time-dependent increase in serum β_2 -m concentration, a marker for IFN-inducible genes.

The clinical side effects were also similar for both products. Their frequency and intensity were those

commonly reported for IFN α at this dose level [35]. The effects observed on the white blood cell counts agree with the results reported previously for IFN α -2b [36], probably due to the increase in cortisol levels after a single injection of IFN α -2b, which was observed by the same group in another study [37].

Finally, it is worth noting that the pharmacokinetic pattern of IFNs, as for other biological response modifiers, should be taken only as an indication that they remain shortly in the circulation, while their biological effects last longer depending on the lifespan of the cells stimulated or the "memory" of the biochemical mechanisms. Probably, the differences found in this study have no clinical significance. For example, the levels of the major histocompatibility complex class I-related protein β_2 -m, which was chosen as a marker of IFN-inducible genes, play an important role in the control of tumor growth and metastasis [38]. The same probably happens for other members participating in the IFN-inducible mechanisms, as found for neopterin induction by IFN β preparations with different pharmacokinetic properties [17]. In fact, IFN has been applied for a wide variety of human disorders using very different schedules and dose regimens, most of them empirically.

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