Bioequivalence of Two Subcutaneous Pharmaceutical Products of Interferon Beta 1a

Guillermo Di Girolamo[1], Marcelo A. Kauffman[2], Eliseo González[3], Mariana Papouchado[2], Alejandra Ramírez[2], Guillermo Keller[1], César Carbonetto[2], Susana Dabsys[2], Alejandro Vidal[2], Aída Sterin-Prync[2], Roberto A. Diez[2]

[1] Facultad de Ciencias Médicas, Universidad Favaloro, Buenos Aires (Argentina)
[2] Bio Sidus S.A. , Buenos Aires (Argentina)
[3] Centro de Diagnóstico Molecular S.A. , Buenos Aires (Argentina)

Corresponding author: Marcelo Andrés Kauffman, MD, Bio Sidus S.A., Constitución 4234 (C1254ABX) Buenos Aires (Argentina); e-mail: m.kauffman@biosidus.com.ar

Abstract

Blastoferon®, in the following referred to as the test product, is a pharmaceutical product of interferon beta 1a (CAS 220581-49-7) currently marketed as a biosimilar to the innovator interferon beta 1a product (referred to as the reference product). Pharmacokinetics and pharmacodynamics assays are critically relevant to demonstrate similarity between biopharmaceuticals. The aims of the present study were to investigate the bioavailability (BA) of the test product (either absolute or relative to the innovator product) and to compare the extent of increase of neopterin concentration following administration of either product. Two studies were performed: initially, an absolute BA assay with i.v. and s.c. injection of test product to 12 healthy subjects. Second, a formal relative BA study with s.c. injections of 88 µg of both products to 24 healthy volunteers. Blood samples for pharmacokinetic and pharmacodynamic profiling were drawn at different intervals after injection. Interferon beta (IFNB) concentrations were determined by ELISA. In the absolute BA study, a single s.c. dose of 44 µg of the test product resulted in a median bioavailable fraction of 29 %, a median T_{max} of 4 h (4–6) and a C_{max} of 3.69 (3.27–4.41) IU × ml^{-1}.

In the relative BA study, values for the test product were: median T_{max} of 3 h (2–18), C_{max} of 5.39 (4.99–6.31) IU x ml^{-1}, $AUC_{(0-72)}$ of 142.86 (134.16–190.15) IU × h × ml^{-1} and $AUC_{(0-infinity)}$ of 190.95 (174.23–303.13) IU × h × ml^{-1}. The corresponding values for the innovator product were: T_{max} of 3 h (1–24), C_{max} of 4.44 (4.12–5.40) IU x ml^{-1}, $AUC_{(0-72)}$ of 128.77 (121.18–170.92) IU × h × ml^{-1} and $AUC_{(0-infinity)}$ of 192.61 (183.04–286.46) IU × h × ml^{-1}. The $AUC_{(0-72)}$ ratio was 111 % (CI 90 %: 106–116), the $AUC_{(0-infinity)}$ was 99 % (CI 90 %: 92–107) and the C_{max} ratio was 121 % (CI 90 %: 112–131). IFNB1a increased neopterin levels in both studies. Both products induced side-effects commonly reported for IFN with no serious adverse events. This study presents pharmacokinetics parameters of the test product and demonstrates similar bioavailability of IFNB1a for both pharmaceutical products.

Key words

- **Blastoferon®, bioequivalence, pharmacokinetics**
- **CAS 220581-49-7**
- **Immunomodulators**
- **Interferon beta 1a**

Arzneimittel-Forschung (Drug Research) 2008;58(4):193–198

1. Introduction

Interferon beta (IFNB), a type I interferon produced by most cells, elicits antiviral, antiproliferative and immunomodulatory effects. Over the past 25 years, and after initial studies with natural IFNB (ATC code L03AB02) [1], different recombinant forms of human IFNB have been clinically evaluated in several disorders, proving efficacious for the treatment of multiple sclerosis [2]. IFNB1b (ATC code L03AB08) is a recombinant protein produced in *E. coli*; thus, it is non-glycosylated and contains a genetically engineered serine substitution for cysteine at position 17. IFNB1a (ATC code L03AB07; CAS 220581-49-7), a recombinant protein produced in Chinese hamster ovary (CHO) cells, is glycosylated and has the same amino acid sequence as the natural human protein, from which it is indistinguishable. Two innovator IFNB1a products have been developed, one to be administered by i.m. injection (Avonex®, Biogen Idec, Cambridge, USA, on a weekly basis of 30 µg in a single dose) and the other by s.c. injection (three doses of 44 µg per week, for a total weekly dose of 132 µg). In addition, there are currently two commercial IFNB1a products licensed in Latin America for s.c. route administration: the aforesaid innovator product and Blastoferon® (Bio Sidus S.A., Buenos Aires, Argentina), which was designed as a biosimilar to the former one.

The optimal dosage, route of administration and the resulting pharmacodynamic properties of IFNB are matters of ongoing discussions [3]. There have been a few previous studies investigating the pharmacokinetics of IFNB, some of them being controversial due to methodological criticism [4–6]. The disposition of IFNB seems to follow a multi-exponential decline with an absolute bioavailability around 27 %–30 % and a terminal half-life of about 66 h [7]. Even though there is no single biological marker of IFNB action in multiple sclerosis, several classic surrogate markers of IFNB response do exist, such as proteins codified by IFNB inducible genes or their stable end-products [8]. Commonly used markers have included the enzyme 2',5'-oligoadenylate synthetase [9], the metabolic cofactor neopterin [10], and the class I major histocompatibility complex light chain $ß_2$-microglobulin [11].

The concept of biosimilars is currently under debate [12]. Biosimilarity needs to rely on comparability criteria, including at least molecular characterization, biological activity relevant for the therapeutic effect and relative bioavailability ("bioequivalence"). The purposes of this study were to evaluate the pharmacokinetic and pharmacodynamic properties of the IFNB1a test product, and its bioavailability relative to the innovator product after a single subcutaneous administration to healthy volunteers.

2. Patients and methods

2.1 Subjects

Thirty-six healthy volunteers (18 males and 18 females) were enroled in this study. Twelve (4 males and 8 females) were included in the absolute bioavailability study, whereas 24 (8 males and 16 females) volunteered to participate in the relative bioavailability study. Body weights ranged from 53 to 78 kg (mean ± SD: 68.2 ± 6.23 kg; Body Mass Index ranged from 20 to 27 kg × m^{-2}) and ages ranged from 21 to 46 years. All subjects were in good physical condition as determined by complete physical and clinical examinations including medical history, blood pressure and ECG, chest X-ray, urinalysis, and blood biochemical, virological and hematological examinations before the study. Subjects were instructed to abstain from any drug for at least 2 weeks prior to and during the study, except for paracetamol, which was used to alleviate predictable adverse effects of IFNB1a administration, such as flu-like syndrome. Subjects with a history of drug or alcohol abuse or drug hypersensitivity were excluded. Women were using an effective contraceptive method (intrauterine device or oral contraceptives) during the whole study and for one month after the end of the study. The study was explained to, and informed consent was obtained from, each subject before enrolment in the study. The study protocol was approved by an independent Ethics Committee and the national drug regulatory authority (ANMAT dispositions #4067/05 and #6170/06).

2.2 Drugs

The absolute bioavailability study involved the use of Blastoferon (44 µg/0.5 ml pre-filled syringes; lot No.: F12-JE05-C; manufacturer: Bio Sidus S.A., Buenos Aires, Argentina). For the relative bioavailability study, Blastoferon. (44 µg/0.5 ml pre-filled syringes; lot No.: F12-J012-H) was used as test product and the innovator product (44 µg/0.5 ml pre-filled syringes; lot No.: Y00B786301) was used as reference product. The formulations of both productsaresimilar and contain 4 mg of albumin, 27.3 mg of mannitol, NaOH and acetic acid, in addition to 44 µg of IFNB1a. The reference preparation was purchased from a local commercial supplier.

2.3 Study design

2.3.1 Absolute bioavailability study

The study was conducted according to an open-label, blinded (for laboratory determinations), balanced, randomized, single-dose (44 µg of IFNB1a by subcutaneous and intravenous route), two-period, cross-over design in 12 healthy volunteers under fasting conditions. After an overnight fast, a single dose of the test product (44 µg) was administered by subcutaneous and intravenous route in each phase of the study according to a randomized crossover design with a washout period between the phases of at least two weeks. No food was allowed until a standardized meal was given 5 h after test product administration. Vital signs were checked before, during, and after the end of the study. Volunteers remained hospitalized for 24 h. Blood samples (10 ml) from a suitable antecubital vein were collected into EDTA-preloaded polypropylene tubes before and 0.25, 0.5, 0.75, 1 , 2, 4, 6, 8, 12, 24, 48, 72 and 96 h after dosing. Plasma samples were collected and stored at –20 °C until tested.

2.3.2 Relative bioavailability study

The study was conducted according to an open-label, blinded (for laboratory determinations), balanced, randomized, single-

Arzneimittel-Forschung (Drug Research) 2008;58(4):193–198

dose (subcutaneous 88 µg dose of IFNB1a), two-period, crossover design in 24 healthy volunteers under fasting conditions. After an overnight fast, IFNB1a in two 44 µg pre-filled syringes of (i.e., 88 µg) was injected by the subcutaneous route into the arm according to a randomized crossover design with at least a two-week washout period between phases. No food was allowed until a standardized meal was given 5 h after drug administration. Vital signs were checked before, during, and after the end of the study. Volunteers remained hospitalized for 24 h. Blood samples (10 ml) from a suitable antecubital vein were collected into polypropylene tubes before and 1, 2, 3, 4, 5, 6, 7, 8, 12, 18, 24, 48 and 72 h after dosing. Serum samples were collected and stored at –20 °C until tested.

2.4 Assay method

Serum or plasma IFNB1a concentrations were quantified using an ELISA (Immuno-Biological Laboratories, Minneapolis, MN, USA) according to the manufacturer's instructions with minor modifications. In brief, an internal Bio Sidus IFNB1a standard curve (comparable with the kit standard) was used in each assay plate and a heterophilic blocking agent (HBR-1, Scantibodies Laboratory, Santee, CA, USA) was added at a final concentration of 0.8 mg/ml to the antibody-enzyme conjugate to reduce matrix interferences, which could lead to falsely elevated concentrations [13]. This ELISA kit has been previously used in another IFNB1a pharmacokinetics study [7].

Serum or plasma neopterin concentrations were measured by a commercially available ELISA (IBL, catalog #RE59355, Hamburg, Germany) used according to the instructions of the manufacturer.

Optical density readings were done in an ELISA plate reader (Spectra Max 340, Molecular Devices, Sunnyvale, CA, USA).

2.5 Pharmacokinetic analysis

IFNB1a pharmacokinetic parameters were calculated from individual plasma (absolute BA study) or serum (relative BA study) concentrations by using a non-compartmental model and analyzed with WinNonlin® (Pharsight Corporation, Mountain View, CA, USA). The areas under the plasma or serum concentration-time curve were calculated from time 0 to the last quantifiable time point ($AUC_{0\text{-}t}$) using the trapezoidal rule. From the terminal log-decay phase, a first-order clearance rate constant (Ke) was estimated by linear regression and the terminal half-life ($T_{1/2}$) was estimated using the equation $T_{1/2} = \ln 2 \times Ke^{-1}$. The AUC from zero time to infinity ($AUC_{0\text{-}\infty}$) included an extrapolated area from the last plasma/serum drug concentration to infinity by using the Ke estimated from the slope (β) of the terminal log-linear phase of the semilog plot of concentration versus time. The maximum plasma/serum concentrations (C_{max}), and time to maximum plasma/serum concentrations (T_{max}) were observed from the measured plasma/serum concentrations following interferon administration. The bioavailable fraction (BF) of the test product administered subcutaneously was calculated according to the following equation:

$$BF = AUC_{(0\text{-}96)s.c.}/AUC_{(0\text{-}96)i.v.}$$

Where s.c. means subcutaneous and i.v., intravenous.

2.6 Pharmacodynamic analysis

Serum or plasma concentrations of neopterin at 0, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 h and at 0, 6, 12, 24, 48, 72 h in the absolute and relative bioavailability studies, respectively, were determined as the measure of the biological activity of IFNB1a. Arbitrarily, an increase of 50 % over the basal neopterin concentrations had been defined as evidence of IFNB1a activity. The areas under the plasma or serum concentration-time curve ($AUC_{0\text{-}t}$) were calculated from time 0 to the last quantifiable time point using the trapezoidal rule.

2.7 Safety analysis

Adverse events were registered during the studies. Two weeks after the end of both studies, safety laboratory tests were performed, including complete blood count, blood chemistry (including fasting glucose, BUN, creatinine, ASP, ALT, and bilirrubin) and urinalysis.

2.8 Statistical analysis

A linear analysis of variance (ANOVA) was used to analyze the logarithmically transformed C_{max}, $AUC_{0\text{-}96}$, $AUC_{0\text{-}72}$ and $AUC_{0\text{-}\infty}$ of IFNB1a data, taking into account sources of variation due to formulation, subject and study period. Values for T_{max} were compared using the Wilcoxon signed rank sum test. Residual variances from ANOVAs were used to calculate confidence intervals for the difference in formulation means on the log scale, 90 % confidence intervals (CI 90 %) were constructed for C_{max}, $AUC_{0\text{-}96}$, $AUC_{0\text{-}72}$ and $AUC_{0\text{-}\infty}$. Untransformed values for partial areas under the concentration-time curve (AUCs) and $T_{1/2}$ were analyzed by a similar ANOVA model. Bioequivalence was accepted if the CI 90 % of mean ratio (T/R) for C_{max}, $AUC_{0\text{-}96}$, $AUC_{0\text{-}72}$ and $AUC_{0\text{-}\infty}$ lay within 0.8–1.25. Taking into account the possibility of high C_{max} variability (CV > 30 %), this interval could be increased to 0.75–1.33, as accepted by several regulatory agencies [14–16]. To set up bioequivalence limits, Schuirmann and Hauck & Anderson tests were used. The statistical analysis was performed using Statistica® 6.0 (Statsoft, Tulsa, OK, USA).

3. Results

3.1 Pharmacokinetics

The pharmacokinetic parameters for each route of administration are summarized in Table 1. A mean C_{max} of 3.69 IU × ml^{-1} was measured for the subcutaneous route, whereas a mean C_{max} of 808.19 IU × ml^{-1} was observed after intravenous administration. The median bioavailable fraction of the test product was 29 % for subcutaneous administration of a single dose of 44 µg of IFNB1a.

On the other hand, the relative bioavailability study showed that no significant differences in IFNB1a serum concentration were observed between the two commercial products. The mean serum IFNB1a concentration-time profile for each preparation is shown in Fig. 1. The pharmacokinetic parameters for each preparation are summarized in Table 2.

Bioequivalence between test and reference products was assessed by using $AUC_{0\text{-}72}$ and $AUC_{0\text{-}\infty}$ for the extent of absorption and C_{max} for the rate of absorption. Results are shown in Table 3. The logarithmically transformed parametric 90 % confidence intervals for $AUC_{0\text{-}72}$, $AUC_{0\text{-}\infty}$ and C_{max} values lie within the bioequivalence interval.

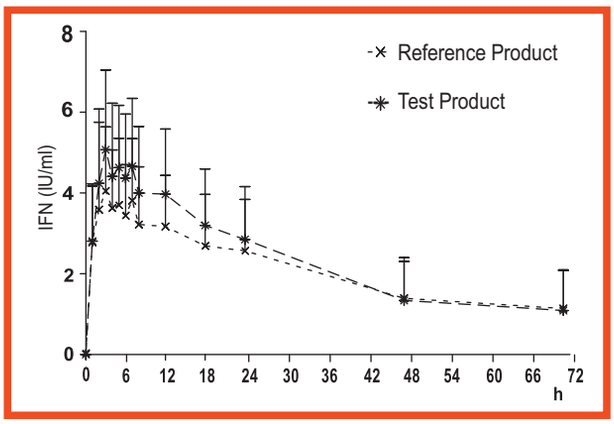

Fig. 1: Mean concentrations of IFNB1a vs time after s.c. administration of test and reference products.

3.2 Pharmacodynamics

Pharmacodynamic effect was assessed determining the rate and extent of neopterin concentration increase in blood. The mean plasma neopterin concentration-time profile for each route of administration is shown in Fig. 2A. No statistically significant differences in AUC and C_{max} between i.v. and s.c. routes were found. Neopterin levels achieved after injection of either the reference or the test product are summarized in Table 4. The mean serum neopterin concentration-time profile for each preparation is shown in Fig. 2B. The extent and rate of neopterin increase was similar for both commercial products.

3.3 Safety and tolerability

Local tolerability of IFNB1a injections was good, even in the relative bioavailability study, despite the high dose used. Safety and tolerability were comparable for both commercial formulations. In spite of the use of paracetamol, the most commonly observed adverse event was a flu-like syndrome, which followed 94 % of injections. The most often reported complaints were headache, myalgia and arthralgia. There were no clinically significant abnormalities or changes in vital signs or laboratory tests. In the relative BA study, complaints were more intense in the first phase of the study, no matter the commercial product administered.

Table 1: Pharmacokinetic parameters of 44 µg of the test product administered by s.c. route to 12 healthy volunteers.

Parameter	Subcutaneous route	Intravenous route
T_{max} (median, h)	4 (4–6)	0.25 (0.25–0.25)
T_{last} (median, h)	24 (24–48)	24 (0.75–24)
C_{max} (geometric mean, IU × ml^{-1})	3.69 (3.27–4.41)	808.19 (684.46–1030.33)
C_{last} (geometric mean, IU × ml^{-1})	1.72 (1.49–2.14)	0.88 (0.64–3.13)
Ke (geometric mean, h^{-1})	0.0216 (0.014–0.046)	0.282 (-0.035–3.32)
$T_{1/2}$ (geometric mean, h)	32.05 (21.50–68.35)	2.45 (2.60–12)
AUC_{0-96} (geometric mean, IU × h × ml^{-1})	66.51 (59.25–79.41)	228.45 (193–203.54)

Table 2: Pharmacokinetic parameters of 88 µg of test and reference products administered by s.c. route to 24 healthy volunteers.

Parameter	Test product	Reference product
T_{max} (median, h)	3 (range 2–18)	3 (range 1–24)
T_{last} (median, h)	72 (range 24–72)	72 (range 18–72)
C_{max} (geometric mean, IU × ml^{-1})	5.39 (CI 90 % 4.99–6.31; CV 31.8 %)	4.44 (CI 90 % 4.12–5.40; CV 39.5 %)
C_{last} (geometric mean, IU × ml^{-1})	0.93 (CI 90 % 0.89–1.55; CV 98 %)	1.10 (CI 90 % 1.02–1.60; CV 67.3 %)
Ke (geometric mean, h^{-1})	0.0263 (CI 90 % 0.024–0.0395)	0.0203 (CI 90 % 0.016–0.0367)
$T_{1/2}$ (geometric mean, h)	26.35 (CI 90 % 24.11–39.54)	34.12 (CI 90 % 32.74–46.49)
AUC_{0-72} (geometric mean, IU × h × ml^{-1})	142.86 (CI 90 % 134.16–190.15; CV 59 %)	128.77 (CI 90 %121.18–170.92; CV 58.9 %)
$AUC_{0-\infty}$ (geometric mean, IU × h × ml^{-1})	190.95 (CI 90 % 174.23–303.13; CV 77.5 %)	192.61 (CI 90 % 183.04–286.46; CV 76.2 %)

Table 3: Summary of bioequivalence analysis of the test product respect to the reference product.

Test/Reference	Geometric mean (%)	90 % Confidence interval
C_{max} ratio	121.16	112.07–130.99
AUC_{0-72} ratio	110.94	106.01–116.10
$AUC_{0-\infty}$ ratio	99.14	91.77–107.10

4. Discussion

Pharmacokinetic and pharmacodynamic parameters of the test product alone or in comparison to the innovator product were investigated. Our results expand the data reported about the pharmacokinetics and pharmacodynamics of IFNB1a and show that test product and reference product are bioequivalent with respect to the ex-

Arzneimittel-Forschung (Drug Research) 2008;58(4):193–198

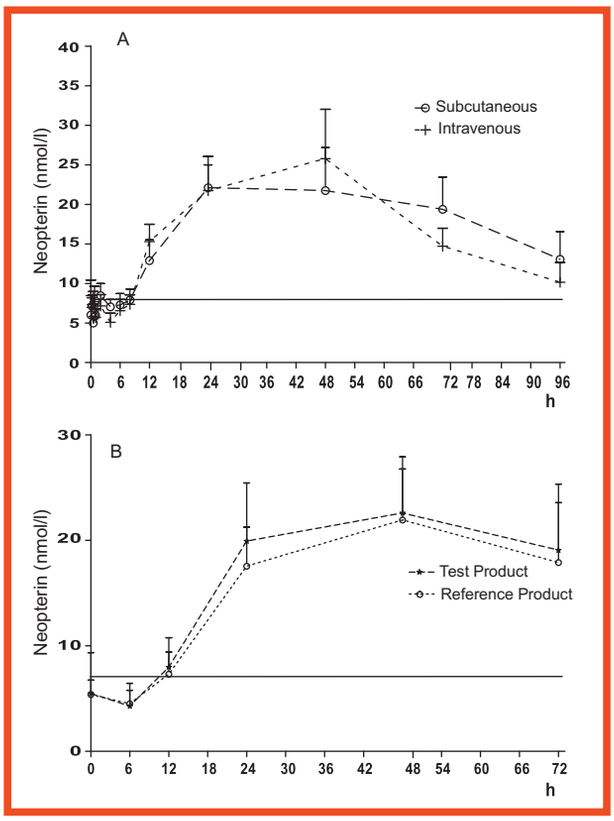

Fig. 2: Effect of IFNB1a administration on neopterin. (A) Mean concentrations of plasma neopterin vs time after s.c. and i.v. administration of 44 µg of the test product. (B) Mean concentrations of serum neopterin vs time after s.c. administration of 88 µg of test and reference products. A horizontal line is showing neopterin concentrations 50% greater than basal concentrations.

tent and rate of absorption after a single subcutaneous dose of 88 µg of IFNB1a.

Parameters such as absolute bioavailability, AUC, T_{max} and $T_{1/2}$ are in good agreement with a previous report from Buchwalder *et al.* [7], where no substantial differences were observed between their estimates of AUC, T_{max} and $T_{1/2}$ and ours. However, data from both studies differ from recently published data by Brearley *et al.* [17]. Furthermore, our results show a significant intra- and inter-subject variability in the pharmacokinetic data, in agreement with previous reports [7, 17]. Such variability is to be expected following extra-vascular administration of a cytokine and could probably explain the differences observed between the different studies, as suggested by Breatley *et al.* [17] On the other hand, we found systematically lower C_{max} values of IFNB1a than previously reported [7]. At present, the reason is unknown, but the use of serum samples instead of plasma samples in the relative BA study and the use of the heterophilic blocking agent could be contributing factors.

To investigate the relative bioavailability of the test product and the reference product we used a dose of 88 µg of IFNB1a. This dose was selected taking into consideration the sensitivity of the ELISA kit used for determining IFNB1a serum concentrations and after a preliminary assay in dogs. A similar approach has been used to study other drugs when analytical constrains can hinder an appropriate evaluation [18, 19]. Even though IFNB1a serum concentrations of about 1 UI x ml^{-1} could be accurately measured using adequate calibration curves, the choice of a higher dose of IFNB1a resulted in serum concentrations more amenable to be detected over that concentration by the mentioned ELISA kit. In spite of the higher dose, the safety and tolerability of IFNB1a was adequate.

PK-PD modeling of IFNB1a is a complex task that currently deserves considerable interest [5]. In our study, pharmacodynamics was assessed using the increase in neopterin level, a classical surrogate marker of IFNB1a response [10]. Neopterin (6D-erythro-thrihydroxypropyl-pteridin) is a low molecular mass metabolite, synthesized from guanosine-triphosphate by GTP-cyclohydrolase I, the key enzyme of pteridine biosynthesis. Unlike other species, humans and other primates only secrete neopterin in response to immune activation. The kinetics of neopterin makes this substance suitable for monitoring the therapeutic action of different cytokines, such as IFNB1a [20]. In agreement with previous reports [7], neopterin blood level increased after several h following IFN injection and persisted elevated even when IFN concentration was already undetectable. The neopterin concentrations achieved after the administrations of 12 and 24 MIU of IFNB1a were comparable, indicating the probable saturation of interferon regulated neopterin synthesis pathway. This is in agreement with a previous *in vitro* study which already demonstrated a similar pharmacodynamic response elicited by the test product and the reference product by whole genome microarray analysis [21]. Furthermore, both products induced a similar increase in neopterin levels.

Table 4: Pharmacodynamics of test and reference products assessed by neopterin increase in serum.

Parameter	Test product	Reference product
T_{max} (median, h)	48 (range 24–72)	48 (range 24–72)
T_{last} (median, h)	72 (range 72–72)	72 (range 72–72)
C_{max} (geometric mean, nmol/L)	21.86 (CI 90% 20.66–24.11)	22.91 (CI 90% 21.51–26.03)
C_{last} (geometric mean, nmol/L)	17.01 (CI 90% 15.91–19.89)	18.2 (CI 90% 16.88–21.26)
$AUC_{0\text{-}72}$ (geometric mean, nmol × h × ml^{-1})	1138 (CI 90% 1078–1246)	1207 (CI 90% 1140–1342)

Pharmacodynamic properties also underlie the profile of adverse reactions induced by IFNB1a administration. The safety and tolerability of IFNB1a were satisfactory both locally and systemically. Both formulations elicited the most common features of flu-like syndrome, such as fever, headache, muscle pain and arthralgias. The most frequent adverse event was headache. Taking into account that tumor necrosis factor alfa (TNFα) is involved in the pathophysiology of different headache disorders [22], the known induction of TNFα by IFNB1a [23] could be a mechanism responsible for such adverse reaction.

In summary, this study showed the pharmacokinetics of a new IFNB1a product and included a comparison with the innovator commercial formulation of IFNB1a for s.c. injection. The bioavailability of the test product is similar to that of the reference product, thus providing pharmacokinetic data for the comparability exercise.

Acknowledgements

Guillermo Di Girolamo, Guillermo Keller and Eliseo Gonzalez were contracted by Bio Sidus to perform these studies. Mariana Papouchado, Alejandra Ramírez, César Carbonetto, Susana Dabsys, Alejandro Vidal, Aída Sterin-Prync and Roberto Alejandro Diez are staff of Bio Sidus. Marcelo Kauffman is a part-time advisor to Bio Sidus.

Literature

[1] Jacobs L, Salazar AM, Herndon R, Reese PA, Freeman A, Jozefowicz R, *et al.* Intrathecally administered natural human fibroblast interferon reduces exacerbations of multiple sclerosis. Results of a multicenter, double-blind study. *Arch Neurol.* 1987 Jun;44(6):589–595.

[2] Bermel RA, Rudick RA. Interferon-beta Treatment for Multiple Sclerosis. *Neurotherapeutics*. 2007 Oct;4(4):633–646.

[3] Neuhaus O, Kiesseier B, Hartung H. Pharmacokinetics and pharmacodynamics of the interferon-betas, glatiramer acetate, and mitoxantrone in multiple sclerosis. *J Neurol Sci*. 2007 Aug 15;259(1–2):27–37.

[4] Gruber R. [Comparison of pharmacokinetic and pharmacodynamic data of interferon-beta 1a preparations after intramuscular and subcutaneous administration]. *Arzneimittel-Forschung (Drug Research)*. 1998 Oct;48(10):1043–1046.

[5] Mager D, Neuteboom B, Efthymiopoulos C, Munafo A, Jusko W. Receptor-mediated pharmacokinetics and pharmacodynamics of interferon-beta 1a in monkeys. *J Pharmacol Exp Ther*. 2003 Jul;306(1):262–270.

[6] Rogge MC, Simonian NA, Jones WE. Comparative pharmacokinetics and pharmacodynamics of recombinant human interferon beta-1a after intramuscular and subcutaneous administration. *Eur J Neurol.* 1999 May;6(3):375–377.

[7] Buchwalder P, Buclin I, Trinchard I, Munafo A, Biollaz J. Pharmacokinetics and pharmacodynamics of IFN-beta 1a in healthy vounteers. *J Interferon Cytokine Res*. 2000 Oct; 20(10):857–866.

[8] Kappos L, Achtnichts L, Dahlke F, Kuhle J, Naegelin Y, Sandbrink R, *et al.* Genomics and proteomics: role in the management of multiple sclerosis. *J Neurol.* 2005 Sep;252 Suppl 3:iii21-iii27.

[9] Hovanessian AG, Justesen J. The human 2'-5'oligoadenylate synthetase family: unique interferon-inducible enzymes catalyzing 2'-5' instead of 3'-5' phosphodiester bond formation. *Biochimie.* 2007 Jun-Jul;89(6–7):779–788.

[10] Casoni F, Merelli E, Bedin R, Sola P, Bertolotto A, Faglioni P. Is serum neopterin level a marker of responsiveness to interferon beta-1a therapy in multiple sclerosis? *Acta Neurol Scand.* 2004 Jan;109(1):61–65.

[11] Scagnolari C, Duda P, Bagnato F, De Vito G, Alberelli A, Lavolpe V, *et al.* Pharmacodynamics of interferon beta in multiple sclerosis patients with or without serum neutralizing antibodies. *J Neurol.* 2007 May;254(5):597–604.

[12] Mellstedt H, Niederwieser D, Ludwig H. The challenge of biosimilars. *Ann Oncol.* 2007 Sep 14.

[13] Morgan B, Tarter T. Serum heterophile antibodies interfere with prostate specific antigen test and result in over treatment in a patient with prostate cancer. *J Urol*. 2001 December;166(6):2311–2312.

[14] Tothfalusi L, Endrenyi L, Midha KK. Scaling or wider bioequivalence limits for highly variable drugs and for the special case of C(max). *Int J Clin Pharmacol Ther*. 2003 May;41(5):217–225.

[15] Tothfalusi L, Endrenyi L, Midha KK, Rawson MJ, Hubbard JW. Evaluation of the bioequivalence of highly-variable drugs and drug products. Pharm Res. 2001 Jun;18(6):728–733.

[16] Boddy AW, Snikeris FC, Kringle RO, Wei GC, Oppermann JA, Midha KK. An approach for widening the bioequivalence acceptance limits in the case of highly variable drugs. *Pharm Res.* 1995 Dec;12(12):1865–1868.

[17] Brearley C, Jaber A, Bertolino M, Priestley A, Seiberling M. Assessment of the safety, tolerability, and PK/PD properties of two new formulations of subcutaneously administered IFN-beta1a: a double-blind, placebo-controlled comparison with the currently available formulation. *Int J Clin Pharmacol Ther*. 2007 Jun;45(6):307–318.

[18] Hao K, Cao YG, Zhao YN, Mao GG, Liu XQ, Wang GJ. Bioequivalence of two tablet formulations of helicidum adminstered in single dose to healthy Chinese volunteers. *Arzneimittel-Forschung (Drug Research)*. 2007;57(8):522–525.

[19] Boonleang J, Panrat K, Tantana C, Krittathanmakul S, Jintapakorn W. Bioavailability and pharmacokinetic comparison between generic and branded azithromycin capsule: a randomized, double-blind, 2-way crossover in healthy male Thai volunteers. Clin Ther. 2007 Apr;29(4):703–710.

[20] Murr C, Widner B, Wirleitner B, Fuchs D. Neopterin as a marker for immune system activation. *Curr Drug Metab.* 2002 Apr;3(2):175–187.

[21] Sterin-Prync A, Yankilevich P, Barrero P, Bello R, Marangunich L, Vidal A, *et al.* Two recombinant human interferon-beta 1a pharmaceutical preparations produce a similar transcriptional response by whole genome microarray analysis. *Int J Clin Pharmacol Ther.* 2008;in press.

[22] Rozen T, Swidan SZ. Elevation of CSF tumor necrosis factor alpha levels in new daily persistent headache and treatment refractory chronic migraine. *Headache.* 2007 Jul-Aug;47(7):1050–1055.

[23] Jin S, Kawanokuchi J, Mizuno T, Wang J, Sonobe Y, Takeuchi H, *et al.* Interferon-beta is neuroprotective against the toxicity induced by activated microglia. Brain Res. 2007 Nov 7;1179:140–146.