

## ORIGINAL ARTICLE

## Pharmacokinetics and pharmacodynamics of interferon beta 1a in *Cebus apella*

Mónica Lahoz<sup>1</sup>, Marcelo Andrés Kauffman<sup>2</sup>, Julio Carfagnini<sup>2</sup>, Alejandro Vidal<sup>2</sup>, Mariana Papouchado<sup>2</sup>, Aída Sterin-Prync<sup>2</sup>, Roberto Alejandro Diez<sup>2</sup> & Carlos Nagle<sup>1</sup>

<sup>1</sup> Centro de Investigación en Reproducción Humana y Experimental (CEMIC), Buenos Aires, Argentina

<sup>2</sup> Bio Sidus S.A., Buenos Aires, Argentina

### Keywords

*Cebus apella* – Interferon beta –  
Pharmacodynamics – Pharmacokinetics

### Correspondence

Marcelo Andrés Kauffman, Bio Sidus S.A.,  
Constitución 4234, Buenos Aires 1254,  
Argentina.  
Tel.: (5411) 4909 8049;  
fax: (5411) 4924 3601;  
e-mail: m.kauffman@biosidus.com.ar

Accepted October 22, 2008.

### Abstract

**Background** Recombinant human interferon (hIFN $\beta$ ) is indicated for the treatment of multiple sclerosis. Its effect presents species restriction, thus lacking biological activity on most mammals. Although there have been previous studies of the pharmacology of IFN $\beta$  in Old World primates, no data exists on New World primates. Therefore, we explored its effect on *Cebus apella*, a New World monkey, describing the pharmacology of this molecule when injected by subcutaneous route in this species.

**Methods** Safety, pharmacokinetics and pharmacodynamics of IFN $\beta$  were evaluated in nine *Cebus apella* individuals.

**Results** A single subcutaneous injection of  $12 \times 10^6$  IU of hIFN $\beta$ 1a resulted in a median AUC<sub>(0-48)</sub> (area under the curve) of 14.82 ng/ml, a C<sub>max</sub> (maximum plasma concentrations) of 1.51 ng/ml and a T<sub>max</sub> (time to achieve maximum plasma concentrations) of 3 h. IFN $\beta$  was biologically active as demonstrated by an increase in neopterin levels. There were no safety concerns.

**Conclusions** New World non-human primates are a suitable animal model for the study of IFN $\beta$  pharmacology.

### Introduction

Interferon  $\beta$  (IFN $\beta$ ), a type I IFN produced by most cells, elicits antiviral, antiproliferative and immunomodulatory effects. Over the past 25 years and after initial studies with natural IFN $\beta$  (ATC code: L03AB02) [7], different recombinant forms of human IFN $\beta$  have been clinically evaluated in several disorders, proving efficacious for the treatment of multiple sclerosis [1]. IFN $\beta$ -1a (ATC code: L03AB07; CAS 220581-49-7) is a recombinant protein produced in Chinese hamster ovary cells that is glycosylated and has the same amino acid sequence as the natural human protein from which it is indistinguishable.

Alike IFNs and some other cytokines, the effect of human IFN $\beta$ 1a presents species restriction, thus lacking biological activity on most mammals [8]. As a consequence, the development of IFN $\beta$  products is

severely restricted by the need to perform pre-clinical studies in non-human primates such as macaques. Old World monkeys, mainly *Macaca fascicularis* and *Macaca rhesus*, have been used in such studies, as they are responsive to the regulatory effect of human IFN $\beta$  [9]. Moreover, although the sequence of *Cebus* IFN $\beta$  has not been described, *M. fascicularis* IFN $\beta$  share more than 90% amino acid identity with their *homo sapiens* homologue [13]. Furthermore, different studies that have investigated on other cytokines sequences have found more than 95% of homology between non-human primates and *homo sapiens* [2, 6, 15]. On the other hand, no data are available on the effect of human IFN $\beta$ 1 on New World primates. The aim of this research was to explore the effect of human IFN $\beta$ 1a on *Cebus apella*, a New World monkey, and to describe the pharmacokinetics of this molecule when injected by subcutaneous (s.c.) route in this species.

## Material and methods

### Animals

Nine adult *Cebus apella* (seven males and two females), aged between 10 and 21 years, from the colony of CEMIC were used in this study. All specimens were born in captivity. The average weight of the animals at the time of treatments was 3.07 kg (range: 2.70–3.57). The monkeys were caged individually in air-conditioned and light-controlled rooms (14 hours light and 10 hours darkness) and fed with monkey chow supplemented with fresh fruit, eggs and biscuits. Water was available *ad libitum*. The experimental protocol was approved by Institutional Animal Ethics Committee and the studies were carried out in accordance with the principles and procedures described in the guidelines for the care and use of laboratory animals (Institute of Animal Laboratory Resources, 1996). No animal was euthanized.

### Experimental procedures

The *in vivo* part of the study was conducted sequentially in two phases. In the first phase, five male monkeys received a single s.c. injection of  $12 \times 10^6$  IU of IFN $\beta$ 1a (BLASTOFERON<sup>®</sup>; Bio Sidus SA, Buenos Aires, Argentina). This concentration is about 22 times higher than the usual human dose. We used this concentration because it is the one present in commercial formulations of the pharmaceutical and should provide IFN levels appropriate for pharmacokinetic calculation. Blood samples from the femoral vein, approximately 1 ml each, were drawn prior to each dosing and at 0.5, 1, 3, 6, 9, 12, 24, 48, 72 and 96 h following the IFN $\beta$ 1a injections to assay plasmatic IFN and neopterin (6-D-erythro-thrihydroxypropyl-pteridin) concentrations. According to the design of the study, if biological activity (as assessed by neopterin level) was found, the second phase should be performed to test product safety and immunogenicity. Thus, in the second phase, other three monkeys (two males and one female) were used in a repeated IFN $\beta$ 1a administration study to evaluate product safety and immunogenicity. These monkeys received s.c. injections of  $12 \times 10^6$  IU of IFN $\beta$ 1a, three times a week, for 2 weeks. Clinical signs including body weight, body temperature and qualitative food consumption were monitored pre-dose and at routine intervals throughout the dosing and recovery period up to 6 weeks. Blood was sampled by femoral venipuncture at pre-dose and on weeks 1, 2, 3, 4 and 6 after starting the treatment for the assay of anti-IFN $\beta$ 1a antibodies, complete blood count and clinical chemistry (including bilirubin, albumin, liver

transaminases, cholesterol and creatinine) evaluations. The other female monkey was used as a source of untreated plasma control for the analysis of antibodies. As used on a relatively small number of animals, safety results must be considered preliminary. The choice of monkey's sex depended on the availability in the colony at the time of our research.

### Assay methods

Plasma IFN $\beta$  concentrations were quantified using an ELISA (Toray-Fuji Bionics, Tokyo, Japan) according to the manufacturer's instructions. This ELISA kit has been previously used in other IFN $\beta$ 1a pharmacokinetics study performed in monkeys [9]. Plasma neopterin concentrations were measured by a commercially available ELISA (Catalog No. RE59355; IBL, Hamburg, Germany) used according to the instructions of the manufacturer. Anti-IFN $\beta$  antibodies were assessed by a sandwich ELISA performed in microtitre plates coated with 2  $\mu$ g/ml of the anti-IFN $\beta$  monoclonal antibody BO2 (Yamasa, Tokyo, Japan) to which IFN $\beta$  is bound in phosphate-buffered saline (PBS). Bound monkey Ig was detected by adding 100  $\mu$ l of horseradish peroxidase-conjugated goat anti monkey IgG (USBiological, Swampscott, MA, USA), diluted 1/5000 in a solution containing *skimmed milk and PBS with tween* and incubated for 1 hour at room temperature. Optical density readings were taken in an ELISA plate reader (Spectra Max 340, Molecular Devices, Sunnyvale, CA, USA) at 540 nm.

### Pharmacokinetic analysis

Interferon $\beta$  pharmacokinetic parameters were calculated from individual plasma concentrations by using a compartmental model and analysed with PKCALC [12]. The areas under the plasma concentration-time curve were calculated from time 0 to the last quantifiable time-point [area under the curve ( $AUC_{0-t}$ )] using the trapezoidal rule. From the terminal log-decay phase, a first-order clearance rate constant ( $K_e$ ) was estimated by linear regression and the terminal half-life ( $T_{1/2}$ ) was estimated using the equation:  $T_{1/2} = \ln 2 \times K_e^{-1}$ . The AUC from zero time to infinity ( $AUC_{0-\infty}$ ) included an extrapolated area from the last plasma drug concentration to infinity by using the  $K_e$  estimated from the slope ( $\beta$ ) of the terminal log-linear phase of the semilog plot of concentration versus time. The maximum plasma concentrations ( $C_{max}$ ) and time to maximum plasma concentrations ( $T_{max}$ ) were observed from the measured plasma concentrations following IFN administration.

## Pharmacodynamic analysis

Plasma concentrations of neopterin were determined as a measure of IFN $\beta$  biological activity. The areas under the plasma or serum concentration-time curve ( $AUC_{0-48}$ ) were calculated from time 0 to the last quantifiable time point using the trapezoidal rule. The maximum neopterin plasma concentrations ( $C_{max}$ ) and time to maximum neopterin plasma concentrations ( $T_{max}$ ) were observed from the measured plasma concentrations following IFN administration.

## Statistical analysis

For the safety analysis we evaluated the effect of IFN on the different blood count and blood chemistry determinations by one-way ANOVA for repeated measures. The analysis considered three levels of time of treatment (basal, week 2 and week 6) with Tukey's multiple comparisons method for *post hoc* analysis. A *P*-value <0.05 was considered significant.

## Results

### Pharmacokinetics

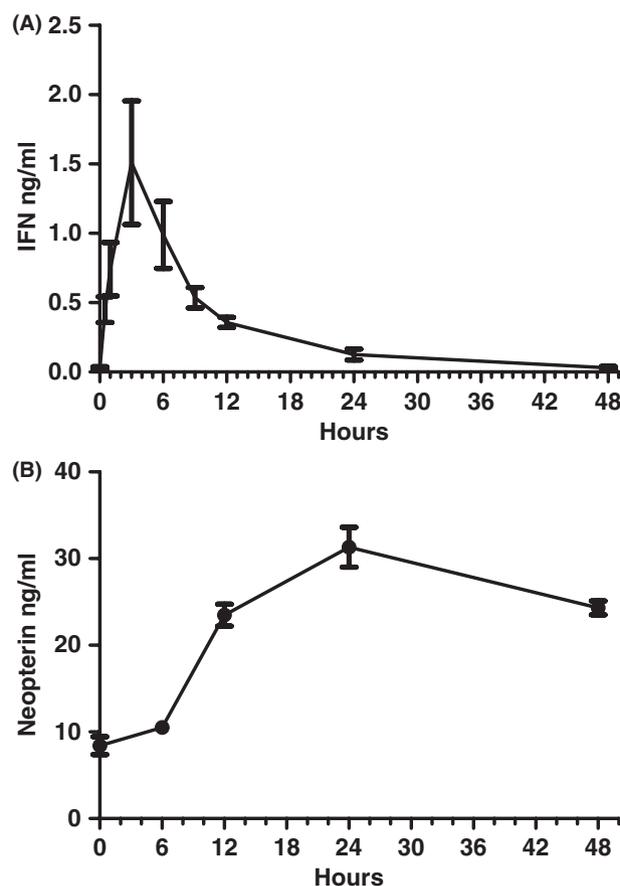
A single s.c. injection of  $12 \times 10^6$  IU of IFN $\beta$ 1a resulted in a typical concentration-time profile median  $AUC_{(0-48)}$  of 14.82 ng/ml, a  $C_{max}$  of 1.51 ng/ml and a  $T_{max}$  of 3 h. The mean plasma concentration-time profile of IFN $\beta$ 1a after s.c. administration is shown in Fig. 1A, whereas pharmacokinetic parameters are summarized in Table 1. Substantial variability was measured for the different parameters between the animals studied, except for  $T_{max}$ . By 48 h after injection, values were below the sensitivity of the assay.

### Pharmacodynamics

Pharmacodynamic effect was assessed determining the rate and extent of neopterin concentration increase in blood. The mean plasma concentration-time profiles of neopterin after s.c. administration of IFN $\beta$ 1a are shown in Fig. 1B. The overall profiles demonstrate a delayed onset and slow return toward baseline. The median time to peak plasma concentration was 3 hours, whereas the  $C_{max}$  was 30.6 ng/ml (90% CI: 25.7–35.6). The  $AUC_{0-48}$  was estimated at 1855.4 ng/ml (90% CI: 1114.3–2596.4).

### Safety analysis

The dose administered to animals was about 22 times higher than usual human dose. In spite of this high



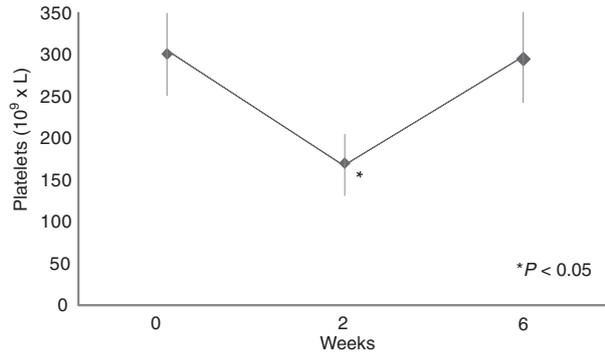
**Fig. 1** Pharmacokinetic and pharmacodynamic profiles of interferon $\beta$  (IFN).

dose, the acute and chronic tolerance of the product was good. All animals exhibited mild malaise and a transient rise in body temperature during the first 24 hours that followed IFN administration. A transient reduction of about 5% of the animal's weight was registered during the first 96 hours. The serial blood counts performed during the chronic exposition phase of the study showed only a decrease in platelets count of about a 50% at week 2, returning values to normal at week 6 (view Fig. 2). The values of the other

**Table 1** Summary of pharmacokinetic parameters estimated after s.c. administration of recombinant human IFN $\beta$  1a to five *Cebus apella* monkeys

Parameter	Estimation (90% CI)
$AUC_{(0-48)}$ (ng/ml)	14.82 (8.93–20.71)
$C_{max}$ (ng/ml)	1.51 (0.28–2.74)
$T_{max}$ (hours)	3 (3–6)

s.c., subcutaneous; AUC, area under the curve; IFN, interferon.



**Fig. 2** Blood platelets after repetitive administration of interferon  $\beta$ .

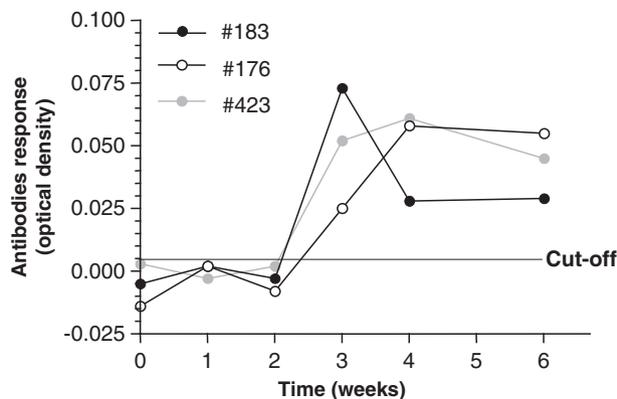
blood counts and chemistry determinations did not change during the study.

### Immunogenicity

As expected, considering the heterologous nature of the human IFN administered to the monkeys, all animals developed IgG antibodies against IFN  $\beta$  at week 4, with persistent elevation until the last point measured at week 6 (view Fig. 3).

### Discussion

Pharmacokinetic and pharmacodynamic parameters of IFN $\beta$ 1a in a primate animal model were investigated. Our results demonstrated that *Cebus apella* is relevant species to investigate IFN $\beta$ 1a. A few previous studies have investigated the pharmacology of IFN $\beta$ 1a in non-human primates [9, 10, 14], but none of them were performed in New World primates. Nevertheless, our results are in good agreement with previous reports [10].



**Fig. 3** Antibodies response after repetitive administration of interferon  $\beta$ .

Pharmacology studies performed on New World primates are also relevant because the recent availability of biosimilar or follow-on pharmaceuticals in developing countries [5] has called, by regulatory authorities, for the requirement of safety studies performed with appropriated animal models in these regions of the world.

Pharmacokinetics-pharmacodynamic modelling of IFN $\beta$ 1a is a complex task that currently deserves considerable interest [9]. In our study, pharmacodynamics was assessed using the increase in neopterin level, a classical surrogate marker of IFN $\beta$  response [4]. Neopterin 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 IFN $\beta$  [11]. In agreement with previous reports, neopterin blood level increased after several hours following IFN $\beta$ 1a injection and elevated level persisted even when IFN $\beta$ 1a concentration was already undetectable [10]. Considering that the neopterin response observed is lower than expected for a dose 22 times higher than usual human dose, the IFN $\beta$  response pathway might be saturated at this level of stimulation. Furthermore, a similar situation has been observed in studies performed in humans by us and other groups [3, 5].

The production of antibodies against human IFN $\beta$  was an expected finding because of the heterologous nature of the protein. Our assay only detected binding ability of such antibodies. As pharmacodynamic responses were measured before the development of antibodies against IFN $\beta$ , we were not able to evaluate the potential neutralizing activity of the elicited immune response. Moreover, the limited volume of samples such as neutralization hindered the evaluation of bioactivities of the antibodies.

In summary, this study expands the data reported about the pharmacokinetic and pharmacodynamic of IFN $\beta$ 1a and demonstrates that New World non-human primates are a suitable animal model for the study of IFN $\beta$ 1a pharmacology. Further independent studies are necessary to confirm our findings.

### References

- 1 Bermel RA, Rudick RA: Interferon-beta treatment for multiple sclerosis. *Neurotherapeutics* 2007; **4**:633–46.
- 2 Bostik P, Watkins M, Villinger F, Ansari A: Genetic analysis of cytokine promoters in nonhuman primates: implications for Th1/Th2 profile characteristics and SIV disease pathogenesis. *Clin Dev Immunol* 2004; **11**:35–44.

- 3 Buchwalder P, Buclin I, Trincharid I, Munafo A, Biollaz J: Pharmacokinetics and pharmacodynamics of IFN-beta 1a in healthy volunteers. *J Interferon Cytokine Res* 2000; **20**:857–66.
- 4 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; **109**:61–5.
- 5 Di Girolamo G, Kauffman MA, Gonzalez E, Papouchado M, Ramirez A, Keller G, Carbonetto C, Dabsys S, Vidal A, Sterin-Prync A, Diez RA: Bioequivalence of two subcutaneous pharmaceutical products of interferon beta 1a. *Arzneimittelforschung* 2008; **58**:193–8.
- 6 Heraud J, Lavergne A, Kazanji M: Molecular cloning, characterization, and quantification of squirrel monkey (*Saimiri sciureus*) Th1 and Th2 cytokines. *Immunogenetics* 2002; **54**:20–9.
- 7 Jacobs L, Salazar AM, Herndon R, Reese PA, Freeman A, Jozefowicz R, Cuetter A, Husain F, Smith WA, Ekes R et al.: Intrathecally administered natural human fibroblast interferon reduces exacerbations of multiple sclerosis. Results of a multicenter, double-blind study. *Arch Neurol* 1987; **44**:589–95.
- 8 Lee S, Parthasarathy R, Botwin K, Kunneman D, Rowold E, Lange G, Klover J, Abeqq A, Zobel J, Beck T, Miller T, Hood W, Monahan J, McKearn J, Jansson R, Voliva C: Biochemical and immunological properties of cytokines conjugated to dendritic polymers. *Biomed Microdevices* 2004; **6**:191–202.
- 9 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; **306**:262–70.
- 10 Martin P, Vaidyanathan S, Lane J, Rogge M, Gillette N, Niggemann B, Green J: Safety and systemic absorption of pulmonary delivered human IFN-beta 1a in the nonhuman primate: comparison with subcutaneous dosing. *J Interferon Cytokine Res* 2002; **22**:709–17.
- 11 Murr C, Widner B, Wirleitner B, Fuchs D: Neopterin as a marker for immune system activation. *Curr Drug Metab* 2002; **3**:175–87.
- 12 Shumaker R: PKCALC: a BASIC interactive computer program for statistical and pharmacokinetic analysis of data. *Drug Metab Rev* 1986; **17**:331–48.
- 13 Sommereyns C, Michiels T: N-Glycosylation of murine IFN- $\beta$  in a putative receptor-binding region. *J Interferon Cytokine Res* 2006; **26**:406–13.
- 14 Sung C, Nardelli B, LaFleur D, Blatter E, Corcoran M, Olsen H, Birse C, Pickeral O, Zhang J, Shah D, Moody G, Gentz S, Beebe L, Moore P: An IFN-beta-albumin fusion protein that displays improved pharmacokinetic and pharmacodynamic properties in nonhuman primates. *J Interferon Cytokine Res* 2003; **23**:25–36.
- 15 Villinger F, Brar S, Mayne A, Chikkala N, Ansari A: Comparative sequence analysis of cytokine genes from human and nonhuman primates. *J Immunol* 1995; **155**:3946–54.