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Comparative pharmacokinetics and pharmacodynamics of two formulations of agalsidase beta (agalsidase Biosidus) and Fabrazyme® by intravenous infusion in healthy male volunteers

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A R T I C L E I N F O

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A B S T R A C T

Fabry disease is a rare X-linked lysosomal condition that leads to the accumulation of glycosphingolipids in various tissues, causing cellular dysfunction, tissue remodeling, progressive fibrosis, and organ failure. The disease results from a deficiency in the human α-galactosidase A enzyme, responsible for breaking down glycosphingolipids like globotriaosylceramide (GL-3 or Gb3) into galactose and dihexose ceramides. In individuals diagnosed with Fabry disease, treatment from 2 years of age onwards typically involves agalsidase beta, the normal recombinant form of the defective enzyme. Agalsidase beta from Biosidus has been developed as a biosimilar to Sanofi-Genzyme's Fabrazyme®. In the molecule's clinical journey, a phase I trial was designed to establish its similarity in terms of pharmacokinetics, pharmacodynamics, and immunogenicity compared to the reference medication. The study was conducted on 24 healthy male volunteers, aged between 18 and 40 years. All volunteers received a single 1 mg/kg bw dose of Fabrazyme® or Biosidus Agalsidase beta by continuous intravenous (IV) infusion over 5 h. The 90 % confidence interval (CI) of the maximum concentration (Cmax), area under the plasma concentration-time curve from time 0 to 12 h (AUC0-12 h) and area under the plasma concentration-time curve extrapolated from time 0 to infinity (AUC0-∞) ratios fell within the accepted range of 80–125 %. No differences were detected in adverse effects or antibody induction. This indicates that Biosidus agalsidase beta meets the criteria for being considered similar to the reference formulation Sanofi Genzyme's Fabrazyme®.

1. Introduction

Fabry disease (MIM 301500), a condition linked to the X-chromosome affecting both men and women, is caused by the deficiency of α-galactosidase A [1,2], which catalyzes hydrolysis of globotriaosylceramide (GL-3 or Gb3) and other neutral glycosphingolipids with terminal α-galactyl, to galactose and dihexose ceramides. This deficiency leads to the lysosomal accumulation of these substrates in the vascular endothelium and other cells, primarily affecting the kidneys, heart, and central nervous system and ultimately leading to the death of patients in the fourth or fifth decade of life [3].

The current treatment options for this disease include enzyme replacement therapy, pharmacological chaperone therapy and supportive therapy. Among the enzyme replacement therapy options, there are two formulations of α-galactosidase A: agalsidase alfa and agalsidase beta. Both treatments effectively decrease the accumulation of GL-3 in renal, cardiac, dendritic, and dermal cells, improve pain and maintain renal functions. The goal of enzyme replacement therapy is to restore enzyme activity to a sufficient level to eliminate the accumulated substrate in target tissues, preventing, stabilizing, or reversing the progressive decline in organ function before irreversible damage occurs [5,8,9].

Biosidus developed agalsidase beta as a biosimilar to Fabrazyme®. A biosimilar has to demonstrate similarity, meaning no clinically meaningful differences, between itself and its reference biologic product in terms of identity, safety, purity, and efficacy in accordance with

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provision 7729/2011 of ANMAT (Administración Nacional de Medicamentos, Alimentos y Tecnología Médica, the national drug regulatory agency of Argentina).

Biosidus' agalsidase beta, the recombinant form of human α-galactosidase A, is manufactured using recombinant DNA technology in Chinese Hamster Ovary (CHO) cells (Van [4]).

Agalsidase beta is a homodimeric glycoprotein with an approximate molecular weight of 100 kD. The mature protein comprises two subunits, each containing 398 amino acids (50 kD for the monomer and 100 kD for the dimer). Each subunit features three N-linked N-glycosylation sites (N139, N192, and N215) and is stabilized by five disulfide bridges [3].

Comprehensive studies have demonstrated that Biosidus' agalsidase beta exhibits a chemical structure, non-clinical pharmacokinetic and pharmacodynamic parameters, and safety profile equivalent to those of the reference product (Van [4]).

To continue with the molecule clinical development, Biosidus designed this phase I, randomized, parallel-arm study in healthy volunteers, to evaluate similarity in relation to biological activity in blood (as a pharmacodynamic marker), pharmacokinetics (according to bioequivalence criteria) and safety compared to the reference product.

The primary objective of this study was to compare the pharmacokinetic profile after the IV infusion over a period of 5 h of a single administration of Biosidus agalsidase beta dosed at 1 mg/kg of body weight (test formulation, "T") and an IV infusion of Sanofi Genzyme Fabrazyme® at the same dose (reference formulation, "R").

As secondary objectives, we have evaluated and compared plasma agalsidase beta enzymatic activity. Also, we evaluated the potential induction of anti-agalsidase beta antibodies. Through this study we have evaluated tolerance to infusion and the occurrence and frequency of adverse effects.

2. Materials and methods

2.1. Study design

This clinical trial was designed to compare the putative biosimilar with the innovator product as a phase I, randomized, open-label for the clinical staff but blinded for tests performed in the laboratory, balanced two-arm, adaptive study conducted at a single site. The initial sample size ($n = 20$) included the possibility of additional volunteers.

This calculation was based on a phase I study conducted with JR-051[4], the first agalsidase beta biosimilar approved in Japan, with 20 volunteers (10 per treatment group). This, in turn, was based on the fact that previous studies with this molecule assumed the geometric mean C_{max} and AUC_{0-24} to be 1.0 and the coefficient of variation to be 0.15.

Therefore, with a power of 0.80, at a significance level of 0.05 the 90 % confidence interval of the AUC ratio (for $AUC_{0\text{-}12hs}$ and $AUC_{0\text{-}\infty}$) should be within the range 0.80–1.25, to accept bioequivalence. 9 volunteers per group were required for the study. Estimating the possibility of 1 volunteer lost to follow-up per group, the calculation resulted in a total of 20 volunteers.

This clinical study was conducted in accordance with the current Good Clinical Practice (GCP) Guidelines, and the relevant ICH (International Council on Harmonization, former International Conference on Harmonization) Guidelines and ANMAT Provision 6677/2010 and 9929/19 (Disposition DI-2019-5344-APN-ANMAT#MSYDS). The trial was registered in clinicaltrials.gov under code NCT05343715.

2.2. Subjects

The study initially involved 35 enrolled volunteers, of which 18 failed the selection process, but 7 re-enrolled after meeting reversible criteria. Selection failures included body mass index (BMI) issues, high blood pressure, COVID vaccine timing, medication use, positive COVID-19 PCR, and exceeding the 21-day limit from the selection date. After randomization at visit 2, 24 volunteers participated, all successfully completing the study visits. These 24 healthy volunteers met the following specified criteria.

Male individuals aged 18 to 40, with a BMI between 19 and 25 kg/m^2, qualified for study inclusion. Volunteers should have undergone pre-inclusion assessments (ECG, chest X-ray, blood and urine tests for clinical chemistry, PCR for COVID-19), with results within normal limits or deemed clinically insignificant at the investigator's discretion. Criteria included systolic blood pressure between 110 and 139 mmHg, diastolic pressure between 70 and 89 mmHg, and a heart rate between 50 and 90 beats per minute after 5 min in a sitting and standing position (including extreme values). Eligible volunteers might willingly comply with the study and have signed the approved informed consent before recruitment.

All volunteers in this study were of Latin Hispanic heritage; detailed demographic information is provided in Table 1.

Exclusion criteria for this study prioritize safety and research integrity. Ineligible participants included those with significant allergies, substantial blood pressure drops upon position change, medication use (prescription or over the counter) within two weeks prior to the study, autoimmune diseases, CNS disorders, infections, or recent vaccinations. Exclusions also encompassed allergies to formulation components, active smoking, severe digestive disorders, organ surgeries, various health conditions, recent drug/alcohol abuse, and recent participation in clinical studies. Ineligibility extended to the use of interfering drugs, recent blood donation, heavy consumption of certain beverages, abnormal electrocardiogram, positive PCR/serology for specific infections, abnormal clinical results, and uncooperative behavior.

All volunteers were randomly assigned to the Test (Biosidus Agalsidase beta) or Reference (Fabrazyme® by Sanofi Genzyme) product using a randomization table (Table 2). Randomization was made in balanced blocks to ensure a balance between treatment groups (1:1).

The table was generated using the RANDOM program from Randomness and Integrity Services Ltd. Premier Business Centres, 8 Dawson Street, Dublin 2, D02 N767, Ireland.

2.3. Procedures and timelines

The clinical study spanned up to 56 days per randomized volunteer, comprising a 21-day screening period and a 35-day follow-up until the end-of-study visit. The first visit involved obtaining informed consent, screening, and conducting admission examinations.

Table 1
Summary of demographic information.

Characteristic	Measures of central tendency	Biosidus Agalsidase beta	Fabrazyme®	Total (n = 24)
		($n = 12$)	(n = 12)	
Age (years)	**Mean**	**27,9**	**27,1**	**27,5**
	SD	7,6	7,3	7,3
	Min value	18	20	18
	Median	31,5	27,5	28,5
	Max value	38	40	40
Weight (kg)	**Mean**	**71**	**64,5**	**67,6**
	SD	5,7	7,9	7,4
	Min value	60,5	50,5	50,5
	Median	72,7	67,25	70
	Max value	81	78	81
Height (cm)	**Mean**	**174,5**	**169,5**	**172**
	SD	6,6	9,3	8,2
	Min value	162	150	150
	Median	174,5	169,5	173
	Max value	187	187	187
BMI (Kg/m^2)	**Mean**	**23,2**	**22,4**	**22,8**
	SD	23,1	1,7	1,6
	Min value	23	20,6	20,6
	Median	22,8	21,7	23,1
	Max value	22,7	24,9	25,1

Table 2
Randomization table for subject treatment.

Volunteer Number	Treatment
1	Fabrazyme® by Sanofi Genzyme
2	Biosidus Agalsidase beta
3	Biosidus Agalsidase beta
4	Fabrazyme® by Sanofi Genzyme
5	Biosidus Agalsidase beta
6	Fabrazyme® by Sanofi Genzyme
7	Fabrazyme® by Sanofi Genzyme
8	Biosidus Agalsidase beta
9	Biosidus Agalsidase beta
10	Fabrazyme® by Sanofi Genzyme
11	Fabrazyme® by Sanofi Genzyme
12	Fabrazyme® by Sanofi Genzyme
13	Fabrazyme® by Sanofi Genzyme
14	Biosidus Agalsidase beta
15	Biosidus Agalsidase beta
16	Fabrazyme® by Sanofi Genzyme
17	Biosidus Agalsidase beta
18	Fabrazyme® by Sanofi Genzyme
19	Biosidus Agalsidase beta
20	Biosidus Agalsidase beta
21	Biosidus Agalsidase beta
22	Biosidus Agalsidase beta
23	Fabrazyme® by Sanofi Genzyme
24	Fabrazyme® by Sanofi Genzyme

Subsequent visits included the administration of a single IV infusion of either Sanofi-Genzyme Fabrazyme® or Biosidus Agalsidase beta at a dose of 1 mg/kg of body weight, which was administered intravenously over a period of 5 h. For both products, two dosages were used to adjust the dose to the weight of each participant: 35 mg and 5 mg. The 35 mg dosage of both Fabrazyme® and Biosidus Agalsidase beta was reconstituted with 7.2 ml of water, while the 5 mg dosage of both products was reconstituted with 1.1 ml of water, following the manufacturer's instructions and the investigator's brochure (IB), respectively. After reconstitution, each vial was further diluted with 0.9 % Sodium Chloride Injection, to a total volume based on the participant's weight. The infusion rate was carefully controlled and did not exceed 0.25 mg/min (15 mg/h) in any instance to minimize the risk of infusion-associated reactions.

Venous blood was drawn at various intervals for pharmacokinetic quantification, enzyme activity, and immunogenicity assays. Participants were followed up via telephone on days 3, 7, 14, and 21 post-visit 2 to assess medication tolerance and adverse events. On day 35 after injection, a serum sample was collected for immunogenicity analysis, along with laboratory tests and a physical examination for safety analysis.

2.3.1. *Agalsidase beta enzyme activity and immunogenicity*

The enzymatic activity of agalsidase was analyzed using the fluorimetric method with the synthetic substrate 4-methylumbelliferyl-α-d-galactopyranoside (4-MU-Gal) at a final concentration of 3.0 mmol/l. In this procedure, 25 μL of the plasma sample, which had been diluted 1/40 in a reaction buffer consisting of 35.3 mM citric acid, 62 mM sodium phosphate, pH 4.6, and 0.1 % Bovine Serum Albumin (BSA), were combined with 75 μL of the fluorimetric substrate. This substrate was dissolved in a 0.05 mol/l citrate–phosphate buffer at pH 4.6, also containing 0.1 % (*w/v*) BSA. After a 15-min incubation period, enzymatic reactions were stopped by introducing 100 μL of stop buffer (0.2 M NaOH/ 0.2 M glycine buffer at pH 10.6), following the detailed protocol outlined by Mayes et al. [7]. Subsequently, the resulting fluorescent product, 4-methylumbelliferone, was quantified using a Synergy H1 Multi-Mode Reader (BioTek, Winooski, VT, USA). with measurements taken at 455 nm. The specific activity of each sample was determined by assessing the calculated concentration. The assessment of enzyme activity included a time-course analysis at different intervals: 0 h (pre-dose), 1 h, 3 h, 5 h, 5.5 h, 6 h, 7 h, 8 h, 10 h, and 12 h from the initiation of the infusion.

2.3.2. *Neutralizing anti-agalsidase antibodies*

To assess the presence of anti-AGA antibodies in the serum, 5 μl of samples were incubated with 1 ng of agalsidase beta for 15 min at room temperature. Subsequently, the activity of the enzyme was evaluated using the 4-MU-Gal substrate, which was allowed to incubate for 20 min at 37 °C. The reaction was halted with 150 μl of stop buffer, and fluorescence measurements were taken by exciting at 365 nm and recording the readings at 455 nm using a plate spectrophotometer. The percentage of inhibition in the samples was calculated by comparing the activity value of 1 ng of agalsidase beta alone with that of agalsidase treated with the serum samples. Samples with a percentage of inhibition greater than 50 % were categorized as positive [10]. Sampling time points for immunogenicity measurement included 0 h (pre-dose), 12 h, and 35 days post-infusion.

From the results of plasma agalsidase beta concentration at different sampling time points, the following variables were estimated: AUC0-t (area under the curve between time 0 and time t, in this case, 12 h), AUC0-∞ (area under the curve, resulting from adding the extrapolation between time t and time ∞ to AUC0-t), Cmax (maximum concentration of agalsidase beta), Tmax (time of maximum concentration of agalsidase beta), and Cmax / AUC0-∞ (ratio between maximum concentration and the area under the curve between time 0 and ∞, serving as an index of less variability than Tmax). Samples were coded, and the laboratory performing the determinations executed them in a blind manner, reporting results by volunteer to the principal investigator.

2.4. *Safety*

2.4.1. *Adverse events*

The safety and tolerability of Biosidus agalsidase beta and Fabrazyme® were evaluated during the entirety of the study, until day 35 after the infusion. The 24 volunteers that were included were analyzed.

2.4.2. *Immunogenicity*

Immunogenicity was tested as described above.

2.5. *Statistical methods*

2.5.1. *Approach for the analysis*

Participants 01 and 02 were exclusively included in the intent to treat (ITT) evaluation due to the manual infusion using a burette rather than an infusion pump. Recognizing that gravity infusion devices introduced greater variability in infusion rates and fluctuations in administration and since the primary objective of the study was a pharmacokinetic comparison, a decision was made to employ a continuous infusion pump (Biocare iP 12B, Shenzhen Biocare Bio-Medical Equipment Co., Shenzhen, China) for subsequent volunteers. This choice aimed to reduce variability, ensuring a uniform infusion rate and guaranteeing accurate drug administration under consistent conditions for all volunteers when conducting the preliminary analysis of the results.

2.5.2. *Pharmacokinetics*

The analysis of the pharmacokinetic behavior was made based on the specific enzyme activity.

From the results of plasmatic agalsidase beta concentration at different sampling time points, the following variables were estimated:

$$\mathrm{AUC0-t, AUC0-\infty, Cmax, Tmax, Cmax/AUC0-\infty}$$

The drug elimination rate constant and elimination half-life in the body were estimated. Summary measures (arithmetic and geometric means, standard deviation, CV%, and range) were presented for each infusion, as well as individual results for each volunteer.

The following pharmacokinetic parameters were calculated:

AUC_T/AUC_R and $C_{max\ T}/C_{max\ R}$

Specific pharmacokinetic software (EquivTest version 2.0, 2012, Statistical Solutions Ltd., Cornwall, UK) was used to calculate the pharmacokinetic parameters in accordance with the regulations of ANMAT under Provision 5040/2006 and its amendments.

2.5.3. *Pharmacodynamics*

For the determination of the biological activity of agalsidase beta in the plasma samples, the fluorometric method of synthetic substrate 4-MU-Gal hydrolysis was used.

As a pharmacodynamic estimator of the biological activity of agalsidase beta, variation in the enzymatic activity of agalsidase beta from blood samples drawn at the end-of-infusion (5 h) and pre-infusion time (baseline activity) was calculated.

The similarity criterion was that the quotient of the variation of the activity in both groups is close to 1 and that the CI 90 % of the quotient is between 0.8 and 1.25. An ANOVA test calculated with IBM SPSS, version 25.0, 2017, was performed to detect significant differences between the two formulations.

3. Results

3.1. *Subjects*

24 healthy male volunteers met the inclusion criteria, with an average age of 27.5 ± 7.3 years, body weight of 67.6 ± 7.4 kg, height of 172 ± 8.2 cm, and BMI of 22.8 ± 1.6 kg/m2. No volunteers were discontinued during the trial.

The statistical analysis of pharmacodynamics and pharmacokinetic behavior was conducted in two distinct approaches: per-protocol (PP) and intention-to-treat (ITT).

3.2. *Pharmacokinetic analysis*

Samples for plasma enzyme activity measurements were taken immediately before infusion (baseline value) and at 1 h, 3 h, 5 h, 5.5 h, 6 h, 7 h, 8 h, 10 h, and 12 h thereafter, for both formulations. Subsequently, two distinct statistical analyses were conducted, taking into account the PP population (n = 22) and the ITT population (n = 24), based on these data.

3.2.1. *Per-protocol analysis*

Table 3 presents the average enzymatic activity values of agalsidase beta at each sampling time point for both formulations. Additionally, Fig. 1 illustrates the mean curve of these values for each formulation.

The plasma concentration-time profiles following the intravenous administration of the two products under investigation are depicted in Fig. 1. As expected from the pharmacokinetics of intravenously administered drugs, the concentration of agalsidase beta typically increases after the start of the infusion. The three points measured during the infusion (hours 1, 3 and 5) are significantly higher than the basal value, without statistically significant differences among them (one way ANOVA, 95 % CI, p-value adjusted according to the Bonferroni method) in both products. Additionally, no significant differences were observed between the groups in the PP analysis.

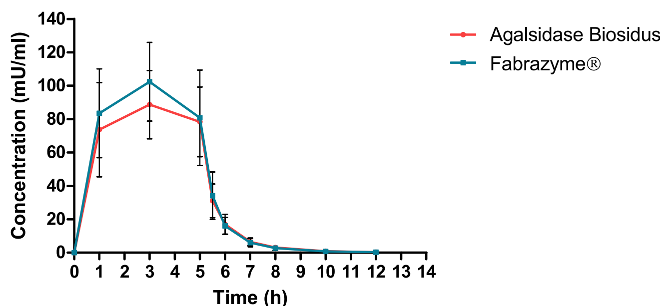

Fig. 1. Mean curve and standard deviation of agalsidase enzyme activity at each time point, PP analysis (n = 22).

A summary of the pharmacokinetic parameters of agalsidase beta in 22 volunteers, comparing Biosidus' with Fabrazyme®, is presented in Table 4. In terms of maximum concentration (Cmax), Biosidus' averaged 95.51 mU/ml with a standard deviation (SD) of 24.9, while Fabrazyme® had a mean of 109.65 mU/ml with an SD of 24.32. The time to reach Cmax (Tmax) for Biosidus was 3.83 h (mean) and 1.53 (SD), compared to 3.36 h (mean) and 1.21 (SD) for Fabrazyme®.

Regarding the area under the curve over 12 h (AUC0-12 h), Biosidus' showed a mean value of 418.21 mU*h /ml with an SD of 84.81, whereas Fabrazyme® recorded 472.15 mU*h /ml (mean) and 100.47 (SD). The terminal half-life (T1/2) of Biosidus' was 1.95 h (mean) with an SD of 1.33, as opposed to 1.7 h (mean) and 0.91 (SD) for Fabrazyme®.

In terms of the elimination constant (Ke), Biosidus' had a mean of −0.48 1/h with an SD of 0.24, whereas Fabrazyme® presented −0.49 1/h (mean) and 0.19 (SD). The area under the curve to infinity (AUC0-∞)

Table 4
Summary of the pharmacokinetic parameters in the 22 volunteers studied (PP Population) for the two formulations evaluated.

Pharmacokinetic parameter	Units	TEST Biosidus Agalsidase		REFERENCE Fabrazyme®	
		Mean	SD	Mean	SD
C_{max}	mU/ml	**95.51**	24.9	**109.65**	24.32
T_{max}	h	**3.83**	1.53	**3.36**	1.21
$AUC_{0\text{-}12h}$	mU/ml*h	**418.21**	84.81	**472.15**	100.47
$T_{1/2}$	h	**1.95**	1.33	**1.7**	0.91
Ke	1/h	**−0.48**	0.24	**−0.49**	0.19
AUC0-∞	mU/ml*h	**419.81**	85.1	**473.51**	100.87

Table 3
Mean values of agalsidase beta enzyme activity (in mU/ml) at each time point, PP population (n = 22).

Treatment	Parameter	Sampling time point (h)									
		0	1	3	5	5.5	6	7	8	10	12
Biosidus Agalsidase (Test)	Arithmetic mean	0.00	73.68	89.58	78.38	31.04	16.96	6.52	3.08	0.90	0.35
	Geometric mean	0.00	69.24	87.78	75.42	29.63	16.03	6.20	2.91	0.84	0.00
	SD	0.00	26.92	18.72	19.92	9.60	5.79	2.15	1.11	0.35	0.27
	%CV	–	36.50	20.90	25.40	30.90	34.20	33.00	36.10	39.40	79.40
	Median	0.00	70.60	88.84	84.90	25.90	16.11	6.37	2.95	0.80	0.39
Fabrazyme® (Reference)	Arithmetic mean	0.00	83.53	102.41	80.81	34.76	16.08	6.00	2.70	0.80	0.26
	Geometric mean	0.00	80.13	99.91	75.97	32.24	15.51	5.61	2.45	0.73	0.00
	SD	0.00	25.37	22.45	27.27	14.11	4.74	2.39	1.30	0.38	0.29
	%CV	–	30.40	21.90	33.70	40.60	29.50	41.75	48.50	47.40	115.30
	Median	0.00	75.44	96.99	75.40	31.35	14.70	5.00	2.40	0.70	0.00

for Biosidus' was 419.81 mU*h /ml (mean) with an SD of 85.1, compared to Fabrazyme® with 473.51 mU*h /ml (mean) and 100.87 (SD).

3.2.1.1. Bioequivalence analysis. Table 5 provides a summary of the statistical analysis of relative bioavailability, including individual ratios (T/R) of the of the log-transformed data (In) of Cmax, AUC0-12 h, and AUC0-∞ for Biosidus agalsidase and Fabrazyme®.

The three pharmacokinetic parameters evaluated and their $CI_{90\%}$ fall within the accepted bioequivalence range of 0.80–1.25.

An ANOVA test was utilized to evaluate the impact of the formulation on AUC and Cmax parameters, utilizing log-transformed raw data. For Cmax, no significant formulation effect was detected (F = 1.210; p = 0.284), with a negligible difference of 0.110 (90 % CI: −0.062 to 0.282; $p < 0.001$). The Schuirmann test conclusively affirmed bioequivalence ($p < 0.001$). Similarly, for the AUC0-12 h parameter (F = 1.270; p = 0.273), the observed difference was 0.097 (90 % CI: −0.051 to 0.245; p < 0.001), and the Schuirmann test underscored bioequivalence (p < 0.001). Regarding the ABC0-∞ parameter (F = 1.240; p = 0.279), the noted difference was 0.096 (90 % CI: −0.053 to 0.244; p < 0.001), and the Schuirmann test confirmed bioequivalence (p < 0.001).

3.2.2. Intention-to-treat analysis (n = 24)

In accordance with the ITT analysis, Fig. 2 illustrates the mean curve of enzyme activity values for each formulation of agalsidase beta. Though the graph seems to depict a difference between the values of the third and fifth hour for each product, the difference is not significant.

3.2.2.1. Bioequivalence analysis. Table 6 summarizes the ITT bioequivalence analysis. All three pharmacokinetic parameters evaluated again fall within the accepted bioequivalence range of 0.80–1.25.

In order to evaluate the treatment effect (formulation), an ANOVA test based on log transformation of the raw data before analysis was used for the parameters AUC and Cmax.

No significant formulation effects were observed for the Cmax parameter (F = 1.845, p = 0.188), indicating a marginal Cmax difference of 0.133 (90 % CI: −0.035 to 0.302; $p < 0.001$). The Schuirmann test reliably confirmed bioequivalence ($p < 0.001$). Similarly, the AUC0-12 h parameter showed no formulation effect (F = 1.979; p = 0.173), with an AUC0-12 h difference of 0.115 (90 % CI: −0.025 to 0.256; p < 0.001), supported by the Schuirmann test for bioequivalence (p < 0.001). For the AUC0-∞ parameter, there was no formulation effect (F = 1.942; p = 0.177), and the AUC0-∞ difference of 0.114 (90 % CI: −0.027 to 0.255; p < 0.001) confirmed bioequivalence per the Schuirmann test. Since the 90 % confidence intervals for the Cmax ratio, AUC0-12 h, and AUC0-∞ fall within the established range of 80–125 %, as per ANMAT provision 1746/07 and documented in international guidelines like ICH or EMA, the conclusion is that Biosidus agalsidase beta qualifies as a product bioequivalent to the Reference formulation Sanofi Genzyme's Fabrazyme®.

3.3. Pharmacodynamic analysis

The enzyme activity levels obtained at the initiation (0 h.) and the conclusion of the agalsidase infusion (5 h.) were compared for both treatment groups.

In both populations, the enzyme activity value prior to the start of the infusion (0 h.) was undetectable. For the PP population, the average enzyme activity difference for the Biosidus Agalsidase product was 78.38 ± 19.92 mU/ml and for Fabrazyme® 80.81 ± 27.27 mU/ml. These results show a ratio of the enzyme activity difference at 5 h for the Test/Reference of 0.97 (Table 7).

The ITT results reveal a ratio of the enzyme activity difference at 5 h. for the Test/Reference of 0.96 (Table 8).

Differences obtained for the PP (n = 22) and ITT (n = 24) populations are depicted in Fig. 3.

The values reached at 5 h after the initiation of the infusion were significantly higher than at baseline and the ratio was within the range of 0.8 to 1.25. However, in both analyses, no significant differences were observed between the two treatment groups at 5 h (p = 0.8221 and p = 0.7776 for the PP and ITT approaches, respectively).

3.4. Adverse events

In total, 7 adverse events were documented in the cohort treated with Biosidus agalsidase, encompassing heightened diastolic blood pressure, headache, elevated proteinuria, an increased erythrocyte sedimentation rate, and hypotension. Alike, within the Fabrazyme®-treated group, 9 adverse events were observed, headache, hypertension, and proteinuria. Additionally, an elevated level of glutamic-pyruvic transaminase (GPT) was specifically reported in the Fabrazyme® group. None of these events was serious.

3.5. Immunogenicity results

Additionally, the immunogenicity of both products was assessed by comparing the presence of antibodies in the baseline sample (pre-treatment), at 12 h and 35 days post-administration among the 24 randomly assigned participants. All samples returned negative results, indicating that under the conditions employed (single dose in normal

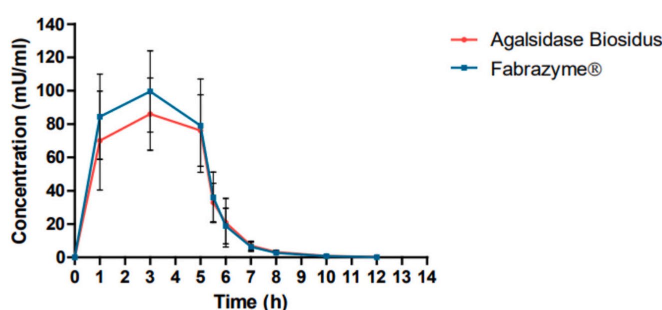

Fig. 2. Mean curve and standard deviation of agalsidase enzyme activity at each time point, ITT (n = 24).

Table 5
Bioequivalence Analysis. Estimation of the 90 % CI of the T/R ratio in the PP analysis (n = 22).

		C_{max}	$AUC_{0\text{-}12h}$	$AUC_{0\text{-}\infty}$
Estimated point:		0.976	0.984	0.984
90 % CI	Lower value obtained	0.956	0.968	0.968
	Upper value obtained	0.998	1.001	1.000

Table 6
Bioequivalence analysis in the ITT approach (n = 24).

		C_{Max}	$AUC_{0\text{-}12h}$	$AUC_{0\text{-}\infty}$
Estimated point:		0.971	0.981	0.981
90 % CI	Minimum value obtained	0.951	0.966	0.966
	Maximum value obtained	0.993	0.997	0.997

Table 7
Mean difference in enzyme activity ± SD between 0 h and 5 h. after infusion in the per protocol population.

	Biosidus Agalsidase	Fabrazyme®	Test/ Reference
Difference of enzyme activity between 0 and 5 h.	78.38 ± 19.92 mU/ml	80.81 ± 27.27 mU/ml	**0.97**

Table 8
Mean difference in enzyme activity ± SD between 0 h and 5 h. after infusion in the ITT ($n = 24$).

	Biosidus Agalsidase	Fabrazyme®	Test/ Reference
Difference of enzyme activity between 0 and 5 h.	76.06 ± 21.47 mU/ml	78.98 ± 28.00 mU/ml	**0.96**

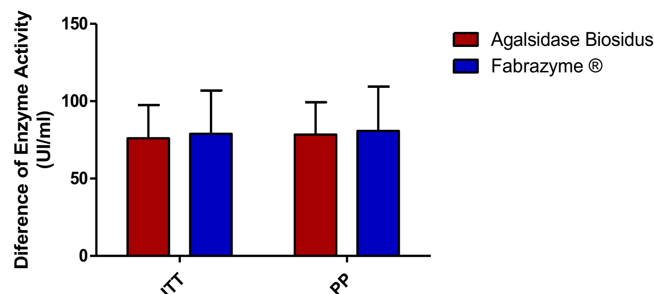

Fig. 3. Difference in enzyme activity between the beginning and the end of infusion (5 h), for both arms of the study (PP and ITT).

subjects), both products demonstrated low immunogenicity.

4. Discussion

The data presented herein reveal a comparable profile in pharmacokinetics, pharmacodynamics, and safety, including immunogenicity, between Biosidus agalsidase beta and the innovator product (Sanofi Genzyme's Fabrazyme®). The study's design opted for a parallel and non-crossover approach, primarily due to the inherent discomfort, catheter-related complications and potential risks associated with a prolonged (5 h) intravenous administration that were deemed inappropriate to repeat for healthy volunteers if a cross-over design had been selected. Fabrazyme® label warns about infusion-associated reactions, some of which were severe, which were the most common adverse reactions reported with Fabrazyme® in clinical trials and affecting more than 50 % of the patients (Fabrazyme® 59 % vs placebo 27 %). As a consequence, Fabrazyme® label also recommends careful administration, due to possible infusion-associated reactions, beginning with a rate of infusion below 0.25 mg/min (15 mg/h) until tolerance of the patient is established and allowing faster infusions in the subsequent doses. Since the infusion in the trial was the first (and only one) to be administered to the volunteers, the calculation of 5-h duration was considered safe for young subjects weighting close to 70-kg. On the other hand, volunteers were not prophylactically treated with antipyretics (as also recommended in the label) to avoid interference to the pharmacokinetic evaluation, though subjects were thoroughly monitored. Interestingly, both formulations were well tolerated by the volunteers and the rate of adverse events was low, without serious events, probably reflecting the different clinical condition of healthy subjects vs patients with Fabry's disease, and the good tolerance of prolonged infusion.

This study was aimed to determine similarity between a biosimilar candidate (Biosidus agalsidase beta) and the innovator product Fabrazyme®, but kinetics of both products suggest intriguing issues to be analyzed in future studies. Agalsidase beta's pharmacokinetics is not easy: Fabrazyme®'s label demonstrates that the pharmacokinetics of this protein is not linear, thus hindering a direct comparison with the kinetic parameters described in the label, though in general terms our results with both formulations are consistent with those data, as well as with values described in the PKPD evaluation of the first biosimilar of Fabrazyme®, JR-051 [9]. One of the striking findings in all these reports, and also herein, is the short duration on the enzyme in blood (terminal half-life <2 h), that however produces results allowing a dosing interval of 2-weeks in patients. Non-clinical studies of agalsidase beta have highlighted the swift clearance of the protein from the systemic circulation, primarily ascribed to tissue sequestration through the mannose 6-phosphate receptor [14]. The current interpretation is that the final destination of endocyted agalsidase is directioning to lysosomes, which are its site of action. Additionally, the protein's conformational characteristics may contribute to the low AUC of agalsidase. This phenomenon became evident in the concurrent administration of migalastat, a pharmacological chaperone, approved for treating Fabry's disease, in conjunction with agalsidase alfa or beta. The inclusion of migalastat resulted in a twofold increase in the systemic exposure of agalsidase in Fabry disease [12].

Regarding the antibody response, notable distinctions in immune tolerance has been observed between healthy volunteers and affected patients. Healthy volunteers exhibited an absence of antibodies, whereas patients frequently develop antibodies against agalsidase, probably since they failed to develop tolerance due to the absence of the native protein [11]. In individuals with Fabry disease, the majority of men exhibit minimal to no endogenous α-galactosidase A enzyme activity, a factor that significantly contributes to the severity of their condition. In contrast, heterozygous women typically retain some degree of residual α-galactosidase A enzyme activity, which can modulate the clinical presentation of the disease [13] and the immunogenicity of the treatment. Anti-agalsidase antibodies are almost exclusively observed in men with the severe classic Fabry phenotype and are often associated with elevated plasma levels of Lyso-Gb3 [6].

In the current study, the quantification of antibodies was conducted at the 5-week mark, allowing for an adequate period for the development of antibodies. It is noteworthy that the immune response resulting in antibodies production typically begins after two/three weeks, so the absence of a response at five weeks suggests low immunogenicity of agalsidase beta in normal subjects, without difference between both formulations. The low immunogenicity observed in this study could also be attributed to the fact that the subjects were healthy individuals who naturally possess a functional version of the α-galactosidase A enzyme. Unlike Fabry disease patients, who may have no enzyme or either a deficient or defective version of it, making them more prone to develop an immune response, the healthy volunteers' immune systems likely recognized the administered enzyme as antigenically similar to the endogenous one, reducing the likelihood of antibody production.

This study presents some limitations. Since it was conducted with healthy volunteers, the results are highly reliable for pharmacokinetic assessments but may be less dependable for evaluating pharmacodynamics and immunogenicity. As a result, a Phase III study is currently underway to assess the efficacy and safety of the biosimilar candidate in patients with Fabry's disease. Although the participation of healthy subjects instead of patients with Fabry's disease demonstrates similarity according to current regulations, direct extrapolation to patients with Fabry's disease is limited due to potential physiological differences. Consequently, additional research involving patients is necessary to confirm that similar efficacy and safety outcomes are achieved. A second issue is immunogenicity, which is probable the most difficult property to evaluate during clinical development of a biologic. The assay used herein detects antibodies that neutralize the in vitro activity of agalsidase, probably targeting the active domain of the enzyme. However, this assay does not detect other antibodies, able to either bind the enzyme, changing its pharmacokinetic parameter, or binding other critical parts of the protein, such as the receptor binding domain, required to be endocyted, resulting thus in another form of neutralization. Probably none of such antibodies have been present during the clinical phase of the study, since all kinetics parameters were very similar, complying with regulatory requirements for biosimilarity, but their presence cannot be excluded at 5-week. This point also requires further evaluation in the clinical trial of efficacy and safety already mentioned.

5. Conclusion

This phase I study included in Biosidus agalsidase beta development plan implied the first-in-human administration of this product. It was intended to determine how similar Biosidus agalsidase beta was to Fabrazyme®, in pharmacokinetic and pharmacodynamic terms and in the safety profile.

The results indicate that the 90 % confidence intervals for the Cmax, AUC0-12 h, and AUC0-∞ ratios fell within the accepted range of 80–125 %. Following ANMAT provision 1746/07, as well as ICH and EMA guidelines, it can be concluded that, based on the data obtained, Biosidus agalsidase beta is bioequivalent to the reference formulation Sanofi Genzyme Fabrazyme®.

Regarding safety, Biosidus agalsidase beta showed a behavior similar to that of the comparator, both in terms of the number of adverse events and their categorization and severity. Notably, there were no serious adverse events, and all non-serious adverse events were classified as mild, with complete resolution. Adverse events classified as "possible" in terms of their causal relationship with the investigational drug were anticipated and documented in the investigator's brochure submitted to the regulatory authority and the independent ethics committee.

In summary, based on the comprehensive data obtained, Biosidus agalsidase beta is deemed similar to the reference formulation Fabrazyme®, marking a significant advancement in its development.

CRediT authorship contribution statement

Viridiana Berstein: Supervision, Investigation. **Eduardo M. Pirotzky:** Investigation. **Hernán D. Taconelli:** Investigation. **M. Gabriela Gobbi:** Supervision. **Lara Beider:** Writing – original draft. **Natali D. Salgueiro:** Writing – original draft. **Laila Dome:** Writing – original draft. **Roberto A. Diez:** Writing – review & editing. **Hugo Sotelo:** Supervision. **Sabrina Coppola:** Supervision.

Declaration of competing interest

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G.G., L.B., N.S., L.D., H.S., S.C. and V.B. on behalf of Biosidus. R.D, H.T. and E.P have received honoraria from Biosidus. All authors were involved in the interpretation of data, writing and critical review of the manuscript. All authors have reviewed and approved the contents of this manuscript in accordance with the International Committee of Medical Journal Editors guidelines.

Data availability

The data that has been used is confidential.

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