

Bioequivalence Study of Two Formulations Containing Irbesartan 300 Mg Tablets in Healthy Colombian Volunteers

Vargas M^{1*}, Bustamante C² and Villarraga Ea³

¹Pharmacology Unit Universidad De La Sabana, Bogotá-Colombia

²Universidad La Sabana, Bogotá - Colombia

³Pharmacology Unit Universidad De La Sabana, Bogotá-Colombia

Abstract

This is a pharmacokinetic study of two formulations containing Irbesartan 300 mg. Its objective was to compare the Bioavailability between the Test product (Irbesartan produced by Tecnoquímicas S.A., Colombia laboratory) and the Reference product (Aprovel[®] produced by Sanofi Aventis laboratory) in order to be able to state the Bioequivalence between them. For this, an open label, two periods, two randomized sequences, crossover, single fasting 300 mg dose study was performed with an 8-day washout period between each period in 24 healthy volunteers and collection of 12 plasma samples between 0 and 48 hours. The analytical method used was HPLC. The 90% confidence interval for the C_{max} parameter was between 83.0 – 113.9 with a 97.2 ratio; for the AUC_{0-t} parameter the 90% CI it is between 92.1 -116.7 with a 103.7 ratio, and for the $AUC_{0-∞}$ the 90% CI was found to be between 95.5 – 114.8 with a 104.7 ratio.

According to the European and FDA guidelines, the confidence interval falls within the permissible ranges for Bioequivalence and Interchangeability declaration of the Tecnoquímicas S.A. product with the Sanofi Aventis Reference product, Aprovel[®].

Keywords: Bioequivalence; Irbesartan; Antihypertensive; Pharmacokinetics

Introduction

Arterial Hypertension may be defined as the condition in which blood pressure in the arterial tree is higher than normal and may be considered as a disorder per se or as an expression of other diseases. In most cases, it is not possible to find a specific cause, thus, it is named as Essential or Primary Hypertension. In a lesser proportion (10 to 15% of cases) finding a specific causal factor is feasible, generally being a nephropathy, a vascular injury or an endocrine disorder. Studies performed worldwide noted that 15 to 20% of the population suffers from hypertension (when hypertension is considered as a blood pressure above 160/90 mm Hg). In Colombia, the available data through First National Morbidity Survey pointed out that 9.6% of the subjects older than 15 years had systolic blood pressure above 160 mm Hg and 9.2% of the subjects had diastolic pressure above 95 mm Hg. [1]

The objective of this study was to establish the Bioequivalence of two formulations containing Irbesartan 300 mg tablets by comparing its bioavailability after a single dose between the Test product produced by Tecnoquímicas S.A. (Colombia) and the Reference product, Aprovel[®], produced by Sanofi Aventis.

Materials and Methods

Study formulations

Test drug : Irbesartan 300 mg tablets, manufactured in Colombia by Tecnoquímicas S.A. Laboratories. Lot 1C1122.

Reference drug : Aprovel[®] Irbesartan 300 mg tablets, manufactured and distributed by Sanofi Aventis. Lot 1A715

Physical and chemical properties like assay of active ingredient and dose uniformity were evaluated for both the Test and Reference products and results are summarized in (Table 1) in order to state the Pharmaceutical Equivalence of these medicinal products before the conduction of the *in vivo* study.

Subjects: 24 healthy non-smoking subjects from both genders, 12 female and 12 male, aged between 19 and 40 years old with a Body Mass Index (BMI) of 18-25 kg/m², completed the study. All volunteers were assessed with a medical examination and laboratory tests before the clinical phase to confirm their health status. Alcoholism history, preexistent diseases compromising liver or kidney function, blood dyscrasia or proteinuria were considered as exclusion factors (Table 2).

Medical examinations and clinical laboratory tests: Performed clinical laboratory tests included complete blood count, total and direct bilirubin, creatinine, glycaemia, total protein, complete urinalysis, HIV ELISA test, antibodies against hepatitis B and C, electrocardiogram and blood pregnancy test for women.

Informed consent process: The protocol and the informed consent form were authorized by the La Sabana University Clinical Research Ethics Committee (CREC) which is ruled by the legal and ethical guidelines of the resolutions 008430 of 1993 and 002378 of 2008 of the Ministry of Social Protection (Colombia), World Conference on Harmonization for Good Clinical Practice of Institutions Conducting Investigation in Human Subjects and by the World Medical Assembly principles published in the Declaration of Helsinki, last review in 2008 [2].

Volunteers were explained in detail about the study, emphasizing on the type of medication to be used, dose, potential drug adverse reactions, blood volume to be collected at each study phase, the material

***Corresponding author:** Vargas M, Dentist, Pharmacologist, Scientific Director, Pharmacology Unit Universidad De La Sabana, Bogotá-Colombia, Tel: 861 5555; E-mail: mauricio.vargas@unisabana.edu.co

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Asysa	USP 36 NF 31 Specification	TEST PROD.	REF. PROD.
Description	Circular, biconvex, white, smooth on both sides tablet. Free of foreign particles	Circular, biconvex, white, smooth on one side with trade mark Free of foreign particles	Circular, biconvex, white, with mark on both sides, free of foreign particles
ID-Irbesartan	The retention time for main peak of Standard schedule should be the same as the sample.	Conforms	Conforms
Assessment - Potassium Irbesartan	90.0 – 110.0% of the labeled amount (300 mg /Tablet)	102.7% of the labeled amount (308.1 mg / Tablet)	100.7% of the labeled amount (302.1 mg / Tablet)
Dissolution - Potassium Irbesartan	Should be dissolved the 80% (Q) minimum of the labeled amount in 20 minutes	101% 102% 101% 101% 101% 101% Conforms S1	92% 93% 91% 87% 93% 93% Conforms S1
Uniformity of Dosage Units - Irbesartan	L1: ≤ 15	L1: 4 Average: 102.7%	L1: 4 Average: 100.7%
Related Compounds	There is no more than 0.2% of the related compound A of Irbesartan, there is no more than 0.2% of any individual impurity, and there is no more than 0.5% of total impurities.	Conforms	Conforms
		Not detected	Not detected

Table 1: Test and Reference Product Physical and Chemical Tests results.

Demographic Variable	Obtained mean (n=22)
Age (years)	28 ± 6.6
Height (cm)	165 ± 10.0
Weight (kg)	61 ± 9.7
BMI (kg/m ²)	22 ± 1.8

Table 2: Demographics of Volunteers Included in the Pharmacokinetic and Statistical Analysis.

to be used to collect such samples, the staff in charge of sampling and monitoring, diet restrictions to comply with, and all the information they requested to freely decide on their participation in the study. Subsequently, each one of them signed an informed consent form.

Study design: A randomized, open-label, two periods, two sequences, crossover design was used with an 8 days washout period between each period. Three days before each period initiation, volunteers must refrain from medications, alcohol and any food or beverage containing methylxanthines. These restrictions were maintained during the entire sampling period. All volunteers were randomized to be allocated to the treatment sequence.

Drug administration: Volunteers had 10 hours fasting prior administration of the drug, which was given with 200 mL of water at doses of Irbesartan 300 mg, [3] (i.e. 1 tablet of 300 mg) to each volunteer, and two hours later, each volunteer was given a standardized food. During hospital stay, they received three full meals (breakfast, lunch and dinner) and two snacks (one in the morning and one in the afternoon). These are shown in (Table 3).

The sampling team was comprised by a physician and one registered nurse. Using Vacutainer[®], a blood sample was obtained by venipuncture in the superior limb immediately prior to administering the medication. Such sample was called 'zero time point sample'. All volunteers received both the Test and Reference product based on randomization and 12 blood venous samples were collected according to the following time points: 0; 20 and 40 minutes; 1, 1.5, 2, 3, 6, 9, 12, 24 and 48 hours. Samples were labeled for identification and centrifuged at 3000 rpm for 30 minutes. Plasma was transferred to a previously labeled tube and frozen at -20°C for later analysis. After an 8-day washout period, administration was repeated completing the second study period.

Validation of analytical method: The bioanalytical method employed for Irbesartan quantification in plasma was high-performance liquid chromatography with UV detection (HPLC-UV). Irbesartan was quantified in plasma using a chromatographic bio-analytical method validated Acetonitrile was employed as proteins precipitating agent. Analyte separation was achieved with an L1 Phenomenex 4.6 x 15 mm,

5 μ m Column, at a temperature of 25°C employing an Agilent Infinity 1256 chromatographer. Isocratic elution was performed with a mobile phase comprised by buffer solution: Acetonitrile 60:40, at a constant flow rate of 1.0 mL/min. Total run time was 9.4 min. [4,5]

Pharmacokinetic analysis: The pharmacokinetic analysis was performed using WinNonlin 5.3 (Pharsight Corporation, Cary USA) software, by means of a non-compartmental analysis. Peak concentration (C_{max}) and time to peak concentration (t_{max}) were directly obtained from results of plasma concentrations, as currently recommended by the FDA [6] and the European Medicines Agency (EMA) for drug assessment [7]. AUC_{total} was calculated by the sum of partial AUC: a) AUC_{0-t} , between zero time point and the last time point with detectable concentrations, calculated through the trapezoidal rule and guaranteeing the calculation of at least 80% of the AUC with the last sample, b) $AUC_{t-\infty}$, calculated as the C/K ratio, where C is the last detectable concentration and K the slope obtained by linear regression from the points corresponding to the drug elimination phase through a linear regression of the natural logarithm of concentrations [8]. Bioavailability-adjusted elimination constant (Ke), half-life ($t_{1/2}$), clearance (Cl) and mean residence time (MRT) were calculated after performing the non-compartmental analysis. The results of pharmacokinetic variables are summarized in (Table 4) with C_{max} , AUC_{0-t} , $AUC_{0-\infty}$, T_{max} values and the elimination rate (Ke) of each one of the studied formulations.

Statistical analysis

An analysis of variance (ANOVA) was used to determine possible effects for each variation factor by sequence, period or subject. For this, F-test with a statistical significance level of 5% ($\alpha=0.05$) was used. Statistical comparison of transformed pharmacokinetic parameters of both formulations was performed using the statistical software WinNonlin version 5.3. The following Bioequivalence criterion was established in the protocol: The 90% confidence interval of Test C_{max} / Reference C_{max} and last Test AUC/last Reference, ratios that should be within the range 80-125% acceptability. In addition, the last AUC parameter should not be less than 80% of total AUC parameter.

Adverse events report: Adverse events were recorded according

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Content Of Mean Calories And Nutrients Evaluation Pattern List										
NORMAL, SEMI-BLAND, LOW-SALT DIET And/Or PATTERN										
Nutrients	Portion	Quantity	Kcal	Prot.	Fat	Cholesterol	Cho	Fiber	Calcium	Phosphorus
Type of food	SERVED	g or c.c.		g.	g.	mg.	g.	g.	mg.	mg.
Breakfast										
Fruit juice	200 cc	60.0	22.2	0.5	0.1	0.0	5.6	0.5	9.7	11.6
Hot milk beverage	180 cc	180.0								
Milk (100%)	180 cc	180.0	90.0	6.1	4.5	25.2	7.0	0.0	216.0	171.0
Bev. (1) powder prep.	10 g	10.0	40.3	0.9	0.9	0.0	7.3	0.1	22.8	27.2
Protein (1) egg, cheese	80 g	80.0	141.4	14.2	7.9	139.8	1.9	0.0	118.4	153.4
Carbohydrates (2) bread /subst.	60 g	60.0	195.2	5.0	4.9	0.0	33.8	0.5	40.0	69.4
Accompaniments										
Butter	10 g	10.0	73.2	0.1	8.2	21.9	0.1	0.0	2.2	2.0
Jam	8 g	8.0	22.1	0.0	0.0	0.0	5.6	0.0	1.0	1.0
Total breakfast			584.3	26.9	26.5	186.9	61.4	1.2	410.1	435.6
Lunch										
Fruit juice	200 cc	60.0	22.2	0.5	0.1	0.0	5.6	0.5	9.7	11.6
Soup or cream	200 cc	200.0								
Carbohydrates (5) tub, plant, root	50 g	50.0	58.3	0.7	0.1	0.0	14.5	0.5	5.9	20.5
Cooked vegetables	40 g	40.0	14.4	1.0	0.1	0.0	2.7	0.5	30.7	24.1
Cereal soup	25 g	25.0	85.6	2.8	0.5	0.0	18.1	0.3	10.4	78.1
Protein (3) meats, offal	100 g	110.0	146.6	21.8	5.7	96.8	0.4	0.0	14.9	226.6
Raw/cooked vegetables	75 g	75.0	23.3	1.5	0.1	0.0	4.5	0.9	56.3	36.2
Carbohydrate: rice	100 g	50.0	179.5	3.9	0.2	0.0	39.4	0.2	4.5	70.0
Carbohydrates (5) tub, plant, root		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Candy or dessert	70 g	70.0	197.7	2.2	1.8	0.0	44.9	0.1	56.9	48.6
Total lunch			705.2	33.8	8.5	96.8	130.0	2.5	179.6	504.1
Dinner										
Fruit juice	200 cc	60.0	22.2	0.5	0.1	0.0	5.6	0.5	9.7	11.6
Soup or cream	200 cc	200.0								
Carbohydrates (5) tub, plant, root	50 g	50.0	58.3	0.7	0.1	0.0	14.5	0.5	5.9	20.5
Cooked vegetables	40 g	40.0	14.4	1.0	0.1	0.0	2.7	0.5	30.7	24.1
Cereal soup	25 g	25.0	25.0	2.8	0.5	0.0	18.1	0.3	10.4	78.1
Protein (3) meats, offal	100 g	110.0	146.6	21.8	5.7	96.8	0.4	0.0	14.9	226.6
Raw/cooked vegetables	75 g	75.0	23.3	1.5	0.1	0.0	4.5	0.9	56.3	36.2
Carbohydrate: rice		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carbohydrates (5) tub, plant, root	100 g	120.0	139.8	1.7	0.2	0.0	34.7	1.1	14.2	49.2
Candy or dessert	70 g	70.0	197.7	2.2	1.8	0.0	44.9	0.1	56.9	48.6
Total meal			627.2	32.1	8.6	96.8	125.3	4.0	199.1	494.9
Sugar (day)		40.0	153.6	0.0	0.0	0.0	39.7	0.0	0.0	0.0
Fat (day) vegetable oil		30.0	265.2	0.0	30.0	0.0	0.0	0.0	0.0	0.0
Total contribution day normal diet			2335.5	92.8	73.6	380.5	356.4	7.6	788.8	1434.5
Nutrients			Kcal	Prot.	Fat	Cho				
Units			G.	G.	G.					
Average recommended			2400.0	84.5	78.0	390.0				
Adjustment percentage (%)			97.3	109.8	94.4	91.4				
Normal diet tcv distribution (%)			2335.5	15.9	28.4	61.0				
Ratio animal/vegetal protein (%)			68.9	ANIMAL	31.5	VEGETAL				

Table 3: Diet Given to the Study Volunteers.

to INVIMA guidelines Provision No. (1067/08), which defines them as serious or not serious and then, according to its definition, as likely, potential or non-related with the study medication. Since the sample size does not have enough statistical power, cases are informed as received from the investigation unit only and without any statistical estimation.

Results

Analytical results of active ingredient content, dose uniformity and

dissolution test met the required specifications for the Pharmaceutical Equivalence statement.

The study involved the participation of 24 healthy Colombian volunteers of both genders (50% women and 50% men) who completed both periods and were included in the pharmacokinetic and statistical analysis. Both treatments were well tolerated and no adverse events were observed (Table 4) shows the mean pharmacokinetic parameters obtained from all volunteers (mean \pm SD) and 90% confidence intervals

Parameter (unit)	Test Product	Reference Product
C_{max} (ng/mL)	4454.1 ± 3684	4415.9 ± 2705
AUC_{0-t} (ng*h/mL)	11935 ± 6315	11354 ± 5327
$AUC_{0-∞}$ (ng*h/mL)	12846 ± 6092	12112 ± 5224
T_{max} (h)	1.7 ± 0.8	1.4 ± 0.7
Ke (1/h)	0.463 ± 0.291	0.496 ± 0.227

Table 4: Pharmacokinetic Parameters for Test Product Irbesartan (Tecnoquímicas S.A.) and Reference Product (Aprovel®) followed by a 300 mg Single Oral Dose Under Fasting.

	Units	%Ratio ref	Standard CI 90% (Test/Reference)	
Ln (C_{max})	ng/mL	97.3	83.1	113.9
Ln(AUC_{0-t})	h.ng/mL	103.7	92.1	116.8
Ln($AUC_{0-∞}$)	h.ng/mL	104.7	95.5	114.9

Table 5: 90% Confidence Intervals of Logarithmically Transformed Pharmacokinetic Parameters of Two Formulations Containing Irbesartan (Test and Reference Products) after Administration to Healthy Volunteers.

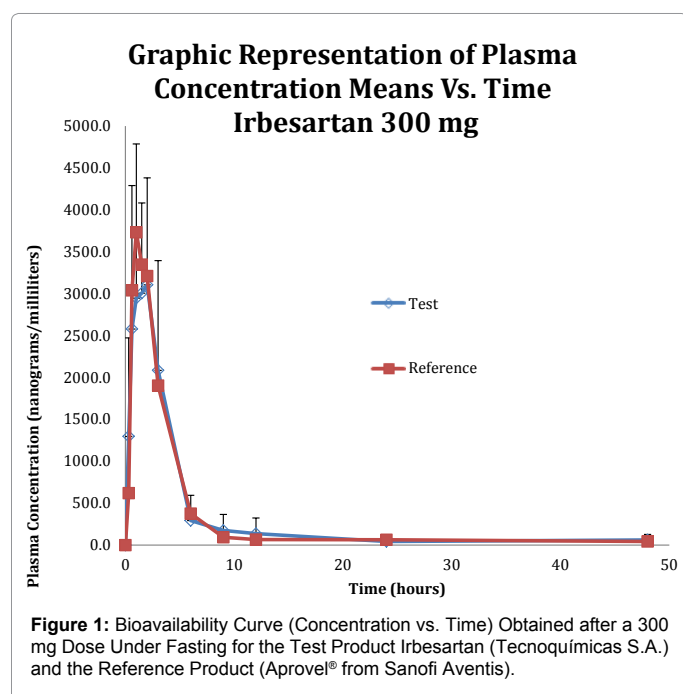


Figure 1: Bioavailability Curve (Concentration vs. Time) Obtained after a 300 mg Dose Under Fasting for the Test Product Irbesartan (Tecnoquímicas S.A.) and the Reference Product (Aprovel® from Sanofi Aventis).

of the log-transformed pharmacokinetic parameters, these analyses were performed to determine Bioequivalence between the Test product produced by Tecnoquímicas S.A. and Aprovel® produced by Sanofi Aventis, as shown in (Table 5).

The analytical method showed to be specific, since no interferences arising from the matrix components were found in the identification and quantification of Irbesartan. It showed linearity in a concentration range from 500 ng/mL to 7000 ng/mL with a correlation coefficient of 0.9977. The precision was proven with variation coefficients lower than 2.0% for low levels (500 ng/mL), and lower than 1.5% for the medium (3000 ng/mL) and high levels (7000 ng/mL). The accuracy was determined by comparing the ratio between the areas of the samples against 5 calibration curves of the system obtaining deviations of less than 2.0% for the lowest concentration of the curves, and less than 1.5% for the rest of the concentrations, showing compliance with the specification. The system has a quantification limit of 60 ng/mL for Irbesartan and a detection limit of 30 ng/mL for Irbesartan. The lower limit of quantification (LLOQ) was 60 ng/mL, the lower quality control

(LQC) 7000ng/mL, the minimum quantifiable concentration (MQC) was $\leq 2.0\%$ and the high quality control (HQC) was $\leq 2.0\%$.

Discussion

The reduction in costs of cardiovascular pathologies treatment using multisource products is a desired aim by governments [8] and, accordingly, Bioequivalence studies allow suggesting the interchangeability of generic products versus reference products without repeating clinical trials in patients [6,7].

WHO recommends in its guidelines for the Conduction of Comparative Bioavailability Studies to carry out *in vivo* testing in multisource products to assess one dose and a sudden increase of the medication in plasma concentration, which was evaluated in this study [9]. These findings are consistent with other studies, which assess the pharmacokinetics changes of Irbesartan when administered without food [4,5,10].

The Pharmaceutical Equivalence Statement allowed qualifying the *in vitro* quality attributes of both formulations. These two periods, two sequences, crossover, single-dose design with healthy volunteers minimizes the variability and allows to assess the formulation effects. The analytical method used was selective, precise, accurate and robust. All 24 volunteers completed the study and did not exhibit adverse events with any of the formulations. The washout period was higher than the recommended 7 elimination half-lives and guaranteed the absence of carryover effect between periods.

The objective of this study was to assess the Bioequivalence of two Irbesartan 300 mg formulations. (Figure 1) shows the curves of the graphic representation of mean plasma concentration vs. time where similarity can be observed. Furthermore, the mean AUC_{0-t} and C_{max} were not significantly different and the 90% confidence intervals of ratios (Test/Reference) to the mean criteria of AUC_{0-t} and C_{max} comply with the interval requested by the FDA and the EMA [7] (Table 4).

In response to this evidence, this study conducted between the Test product manufactured by Tecnoquímicas S.A. and Aprovel® manufactured by Sanofi Aventis in Colombian population, demonstrates that it will be possible to exchange these two formulations.

Conclusions

The Irbesartan formulation manufactured by Tecnoquímicas S.A. (Test Product) and Aprovel® manufactured by Sanofi Aventis (Reference Product) has pharmacokinetic parameters that allow stating Bioequivalence between both formulations.

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