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Bioequivalence study of two formulations containing isotretinoin in fed condition in Colombian healthy volunteers

Abstract

This is a pharmacokinetic trial that evaluates under fed conditions two formulations containing 20 mg of Isotretinoin, with the aim to compare the Bioavailability, between the Test Product (Isoface® Isotretinoin from Procaps S.A. Laboratory, Colombia) and the Reference Product (Roaccutane® Isotretinoin from Catalent Germany Laboratory, Eberbach GMBH, Germany.), to be able to declare Bioequivalence between both formulations. The same formulations were previously studied by these authors under fasting conditions. For this trial, an open, cross-over, randomized study of two periods and two sequences was carried out, with a single dose of 40 mg of Isotretinoin in fed conditions, in 28 healthy male volunteers; washing period was 14 days between each period. For results presentation, Plasma concentration ratio Vs Time curves were runned until hour 72. In order to identify the concentration provided by the studied formulation, the baseline status of each volunteer which was constructed with 3 concentrations prior to the administration of the study drug was eliminated from the analysis. The analytical method employed was high performance liquid chromatography with tandem mass spectrometry detector, HPLC MS/MS, for identification and quantification of Isotretinoin in plasma. The main pharmacokinetic parameters for the Reference Product Vs the Test Product were: T_{max} 5.3 Vs 5.6hours, C_{max} 374.6 Vs 385.1ng/mL, AUC_{0-t} 6421.3 Vs 6590.0hr*ng/mL and AUC_{0-inf} 7484.9 Vs 7233.2hr*ng/mL.

Confidence interval calculation of data with logarithmic transformation showed confidence intervals for the variables C_{max}, AUC_{o-t}, and AUC_{0-Inf} within the values 80–125%, accepted by the F.D.A. and by the European Medicines Agency EMA in their bioavailability and bioequivalence guidelines to accept the Bioequivalence hypothesis between the two formulations under study, and thus, declare Bioequivalence and interchangeability of the Procaps S.A. Laboratory product (Test Product), with the Catalent Germany Laboratory, Eberbach GMBH product (Reference Product).

Keywords: bioequivalence, isotretinoin, acne, bioavailability, pharmacokinetics, health

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Introduction

Retinoids are intracrine and paracrine mediators that intervene in apoptosis, proliferation and reproduction of cell differentiation, by binding to nuclear retinoic receptors. Isotretinoin in oral presentation has as its main effect sebaceous secretion reduction, which is reached in 80% by the fourth week of treatment.[1] Isotretinoin´s gastrointestinal absorption exhibits a high variability, which is greatly influenced by the presence or absence of food.[2]

Pharmacokinetic studies of 40 mg of Isotretinoin in healthy volunteers who were given the medication in fasting conditions, reported concentrations of 167 to 459ng/mL at 3.2 hours.[3] But recommendation for Isotretinoin´s use is to be administered after eating food, as its bioavailability increases between 1½ to 2 times, even though elimination half-life after a dose of 40mg is 10 to 20 hours in volunteers, being up to 90 hours in patients[2,3]

The results obtained in our first study carried out in Colombian population under fasting conditions with these same formulations,[4] evidenced for Isoface® from Procaps Product the following values: Tmax 2.6 hours, Cmax 190.5ng/mL, AUC_{0-t} 3003.8hr*ng/mL and AUC_{0-inf} 3726.3hr*ng/mL.

According to the FDA and EMA, two products are bioequivalent if they contain the same active ingredient and if the kinetic studies do not show significant differences in the AUC and Cmax parameters, showing confidence interval in the range of 0.80 to 1.25.[5,6] Here are the results of a single dose study of 40mg of strength, comparing the bioavailability of two formulations of Isotretinoin, one from Procaps S.A. Colombia named Isoface® against the formulation of the Catalent Germany laboratory named Roaccutane®

Study design

An open, crossed, with a single dose regimen of 40 mg and randomized design was established, with two periods, two sequences, in fed condition, and a washout of 14 days between each period. A total of 28 healthy volunteers participated in the present Bioequivalence study. To calculate the number of volunteers for this Bioequivalence trial, the formula proposed by Marzo and Balant (1995)[7] was used $n \geq (15{,}68 \times CV^2)/\Delta$. Where intraindividual variation coefficient reported by the technical datasheet of the reference product is: 25%.[8] Therefore: $n \geq (15.68 \times 25^2)/20^2 = 24.5$. It was defined to conduct the study with 28 volunteers based on the high power and significance level that the formula gives, and 4 additional volunteers in order to prevent volunteer's dropouts or withdrawal.

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Materials and methods

Study formulations

Test drug

Isoface® 20mg Isotretinoin, for oral administration, manufactured and distributed by Procaps Laboratories S.A., Colombia, Lot 1104962

Reference drug

Roaccutane® 20mg Isotretinoin, for oral administration, manufactured and distributed by Catalent Germany, Germany. Lot B2025B01

Subjects

Healthy volunteers diagnosed by physical examination and laboratory tests to determine their correct hepatic, cardiac, renal function, etc. and that they met the inclusion criteria reflected in the study´s protocol.

Obtaining informed consent

Volunteers signed the informed consent form authorized by the Ethics Committee of Universidad de La Sabana, which complies with the national regulations of Resolutions 008430 of 1993 and 002378 of 2008 of the Ministry of Social Protection (Colombia),[9] and the Helsinki Declaration.[10]

All volunteers signed informed consent

The 28 selected healthy volunteers met the inclusion criteria of the study: male volunteers, average age 29.7 years, average weight of 69.3kg, average height of 171.5cm and average BMI of 23.6kg/m^2.

Drug administration

For the medicine administration, volunteers received a standardized diet. Breakfast was programmed with high lipids content, approximately 50 percent, and high caloric content, approximately 800 to 1000kcal.[11] This meal was administered 30 minutes before the investigational drug was administered, that is 2 capsules of 20mg each, to each volunteer. The dose was ingested with 200mL of water. After this, volunteers remained seated for the next 4hours[12]

During the hospitalization period in the clinic, two meals (breakfast and lunch) and two snacks (one in the morning and one in the afternoon) were provided to the volunteers.[13]

All volunteers received Isoface® or Roaccutane® according to randomization. Blood samples were taken in the following times: -10, -2 and 0 hours before drug administration, and 1, 2, 2.5, 3, 3.5, 4, 6, 12, 24, 48 and 72 hours after. Plasma was separated from the samples and stored at -20 Celsius degrees for further analysis. After 7 half-lives of the active study medication, that is to say 14 days of washout, the second study period took place.[14]

Validation of the analytical method

The validation procedure of bioanalytical methodology established by QUASFARM&F S.A. was performed. In order to quantify the active metabolite Isotretinoin, the metabolites product of the elimination do not have important active behaviors, reason why those metabolites were not quantified.[15] Conditions were the same as the ones in the previous study published by us.[4]

Pharmacokinetic analysis

The WinNonlin 5.3 program (Pharsight Corporation, Cary USA) through a non-compartmental analysis was used. The pharmacokinetic parameters evaluated were Maximum concentration (C_{max}) and the time to reach it (t_{max}), as currently recommended by the FDA[5] and the European Agency for evaluation of medicines (EMA),[6] AUC_{total} and AUC_{0-t}, between time zero and the last time with detectable concentrations.[16]

Statistic analysis

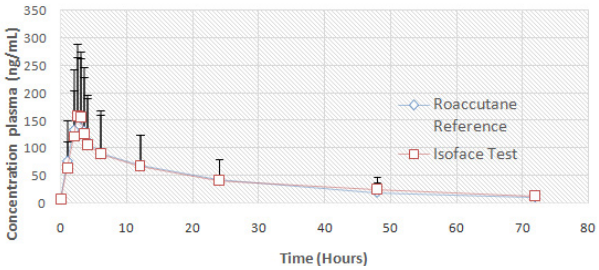
Possible effects for the variables sequence, period, volunteer or treatment, were evaluated by variance analysis, applying an F-test with 0.05% alpha.

Bioequivalence criteria were established with confidence interval of 90% of the relations of C_{max} and AUC that must be within the range of 80-125% of acceptability.[16,17] These analysis were performed using the WinNonlin version 5.3 software.

Results

28 healthy male volunteers completed the two periods and were included in the pharmacokinetic and statistical analysis.

Graph 1 shows the bioavailability of both Isotretinoin´s formulations (Isoface® from Procaps S.A., Colombia) and (Roaccutane® from Catalent Germany) in 28 healthy male volunteers Table 1.



Graph 1 Bioavailability curve (concentration vs. time) obtained after an Isotretinoin dose in fed condition of the Test product (Isoface® from Procaps S.A., Colombia) and the Reference product (Roaccutane® from Catalent Germany, Eberbach GMBH, Germany).

Table 1 Analytical conditions

Column	Fortis 1.7µm C1850x2.1mm
Column Code	U-L1-c-1-ex
Precolumn	N.A.
Column Temperature	30°C
Mobile Phase Flow	0.2mL/min
Column Pressure	80Bar
Mobile Phase	Acetonitrile:Water 90:10
Autosampler Temperature	N.A.
Injection Volume	10µm
Run Time	3minutes
Holding time	1.1min Acitretine, 1.6min Isotretinoine
Integration Parameters	Agilent
Extraction Type	Liquid-liquid
Extraction Agent	Hexane:Isopropanelol 95:5

Quantification limit was 1ng/mL.[13–15]

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In Table 2**,** the mean values of the pharmacokinetic parameters obtained from all the volunteers (mean±SD) with Isoface® from Procaps S.A., Colombia and Roaccutane® from Catalent Germany, can be seen. For the pharmacokinetic analysis a mean value was obtained. Basal points were taken at-10-2 and 0hours. This value was eliminated from the individual values obtained for each volunteer of the plasma concentrations contributed by the studied formulations. The main pharmacokinetic parameters for the Reference Product Vs the Test Product were: T_{max} 5.3 Vs 5.6 hours, C_{max} 374.6 Vs 385.1ng/mL, AUC_{0-t} 6421.3 Vs 6590.0hr*ng/mL and AUC_{0-inf} 7484.9 Vs 7233.2hr*ng/mL.

Results obtained in the previous study carried out by these same researchers, in Colombian volunteers in fasting conditions, and with the same drugs, showed the following results for the Test Product Vs the Reference Product: T_{max} 2.6 Vs 2.8 hours, C_{max} 190.5 Vs 186.5ng/mL, AUC_{0-t} 3003.8 Vs 2933.5hr*ng/mL and AUC_{0-inf} 3726.3 Vs 3521.2hr*ng/mL[4]

Table 3 shows the confidence intervals for the variables C_{max}, AUC_{0-t}, and AUC_{0-Inf} within the values 80–125%

The coefficient of intrasubject variation for each variable obtained in this study was: C_{max} CV% intrasubject 35.4%, AUC_{0-t} CV% intrasubject 25.8% and AUC_{Inf} CV% intrasubject 26.3%.

Table 2 Isotretinoin pharmacokinetic parameters of the Test product (Isoface® from Procaps S.A., Colombia) and the Reference Product (Roaccutane® from Catalent Germany, Eberbach GMBH, Germany) followed by a single oral dose in fed condition. Pharmacokinetic parameters summary data adjusted to baseline

Treatment	Elimination Speed (1/hr)	Half Life(hr)	$T_{max(hr)}$	C_{max} (ng/mL)	AUC_{0-t} (hr*ng/mL)	AUC_{0-Inf} (hr*ng/mL)
Reference	0.041	13.1	5.3	374.6	6421.3	7484.9
Test	0.046	13.2	5.6	385.1	6590	7233.2

Table 3 90% confidence intervals for Isotretinoin formulations (Test and Reference products) after their administration to healthy volunteers in fed conditions

		Standard CI 90% (Test/Reference)	
Parameter	Units	Ratio%	80 125
Ln(C_{max})	ng/mL	102.62	87.72 120.06
Ln(AUC_{all})	hr*ng/mL	95.25	84.81 106.97
Ln($AUC_{inf-obs}$)	hr*ng/mL	92.96	82.59 104.63

Discussion

Bioequivalence studies are the subrogated evidence that generic drugs will have similar efficacy and safety profile.[17] The use of generic medications favors health resources control.

Performance of the in vitro study allowed us to compare the two formulations studied in order to declare the Pharmaceutical Equivalence and qualify the quality attributes of the same. But in fact, the in vivo studies are the ones that allow us to declare the interchangeability of the active principles that do not comply with bio-exemption.[18] With this, we would achieve financial resources control as developed countries do with high regulatory requirements for generic drugs; countries who increase year after year the use of generics which demonstrate bioequivalence.

The study was completed with the 28 volunteers initially included, and the adverse events related in Table 4 ocurred. The washout period was enough and guaranteed the absence of carryover effect.[19]

We can show how food administration before the drug ingestion impacts on a greater quantity of active ingredient available to generate pharmacodynamic effects, when compared to fasted conditions.[4]

In the case of Isotretinoin, the incorporation of a bioequivalent formulation to the pharmacological treatment for nodule-cystic acne or with a severe scar component, contributes to the clinical objective and costs control of this pathology.

Table 4 Distribution of adverse reactions reported by medication and the causal relationship related to each subject

Subject´s code	Date: 08/13/17	Product	Outcome	Causal relationship	Instituted treatment
NFFG	Post-intake heartburn	Test	Recovery without sequel	Probable	None Observation
JFR	Mild headache	Test	Recovery without sequel	Probable	None Observation
Subject´s Code	Date: 07/30/17	Product	Outcome	Causal Relationship	Instituted treatment
NFFG	Dry skin	Reference	Recovery without sequel	Probable	None Observation
CCGD	Mild frontal headache	Reference	Recovery without sequel	Probable	None Observation
GQP	Drowsiness	Reference	Recovery without sequel	Probable	None Observation

Conclusion

The Isoface® (Test Product),manufactured by Procaps S.A., Colombia, and the Roaccutane®, (Reference Product), manufactured by Catalent Germany, show pharmacokinetic parameters that allow to declare Bioequivalence between both formulations.

Acknowledgements

None.

Conflict of interest

The author declares there is no conflicts of interest in this work.

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