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RESEARCH ARTICLE

INTRA SUBJECT VARIABILITY OF PROGESTERONE 200 MG SOFT CAPSULES IN INDIAN HEALTHY POSTMENOPAUSAL FEMALE SUBJECTS UNDER FED CONDITIONS

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ABSTRACT

The aim of this study was to evaluate the intra subject variability of progesterone 200 mg soft capsules of Test product with Prometrium® (Progesterone USP) capsules 200 mg (Reference) marketed by Solvay Pharmaceuticals Inc., Marietta, GA in healthy adult, human, post menopausal female volunteers. This study was an open label, randomized, balanced, single-dose, two sequence four period, full replicate, crossover oral bioequivalence study was conducted in 30 healthy adult, human, post menopausal female volunteers under fed conditions in two equal groups. Subjects received progesterone 200 mg of either test or reference formulation with a washout period of 11 days. After study drug administration, serial blood samples were collected over a period of 36 hours post dose. The plasma concentrations of progesterone were determined using validated LC/MS/MS method. Pharmacokinetic parameters C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, Kel and $T_{1/2}$ were determined for both the formulations. The 95% upper confidence bound of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of progesterone was found to be -0.37246%, -0.02535%, and 0.04630% respectively were less than zero (0.0000) and the T/R ratios for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of progesterone were found to be 77.25%, 76.20%, and 61.64% respectively were lie outside the acceptance range of 80.00-125.00%. Within subject standard deviation of reference product (S_{WR}) for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ was found to be 1.13304, 0.72073 and 0.78637 respectively were found to be >0.294. The test formulation in this study fails to show the bioequivalence in comparison with that of reference formulation in terms of scaled average bioequivalence criteria under fed conditions.

INTRODUCTION

Bioequivalence (BE) studies are an integral component of the ANDA (Abbreviated New Drug Application) approval and marketing of generic drug products. BE studies are generally designed to determine if there is a significant difference in the rate and extent to which the active drug ingredient, or active moiety, becomes available at the site of drug action. According to the criteria developed by the U.S. (United States) Food and Drug Administration (FDA) and generally applied by other regulatory agencies, two pharmaceutically equivalent products are judged bioequivalent if the 95% upper confidence bound for $\mu T - \mu R)^2 - \theta * \sigma^2_{WR}$ must be <0.000 and the geometric mean ratio (GMR) of AUC and C_{max} fall within 80.00-125%.00[1]. Since the reference listed drug is Prometrium® (Progesterone USP) capsules 200 mg, it has been selected to conduct bioequivalence studies under fasting and fed conditions. As a consequence fed study was conducted intially[2].

Progesterone is a steroid hormone indicated in the treatment of causing a menstrual period in premenopausal women with absent menstrual periods (secondary amenorrhea) and preventing abnormal overgrowth of the lining of the uterus (endometrial hyperplasia) in postmenopausal women taking estrogen hormone therapy. It plays an important role in the preparation and maintenance of pregnancy[3].

The pharmacokinetic properties of progesterone

Absorption

After oral administration of progesterone as a micronized soft-gelatin capsule formulation, maximum serum concentrations were attained within 3 hours. The absolute bioavailability of micronized progesterone is not known. Table 1 summarizes the mean pharmacokinetic parameters in postmenopausal women after five oral daily doses of Prometrium (Progesterone) Capsules 100 mg as a micronized soft-gelatin capsule formulation[3].

Table 1 Pharmacokinetic Parameters of Prometrium (progesterone) Capsules

Parameter	Mean ± SD		
	100 mg	**200 mg**	**300 mg**
C_{max} (ng/mL)	17.3 ± 21.9	38.1 ± 37.8	60.6 ± 72.5
T_{max} (hr)	1.5 ± 0.8	2.3 ± 1.4	1.7 ± 0.6
AUC $_{(0-10)}$ (ng*hr/mL)	43.3 ± 30.8	101.2 ± 66.0	175.7 ± 170.3

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Serum progesterone concentrations appeared linear and dose proportional following multiple dose administration of Prometrium (progesterone) Capsules 100 mg over the dose range 100 mg/day to 300 mg/day in postmenopausal women. Although doses greater than 300 mg/day were not studied in females, serum concentrations from a study in male volunteers appeared linear and dose proportional between 100 mg/day and 400 mg/day. The pharmacokinetic parameters in male volunteers were generally consistent with those seen in postmenopausal women[3].

Distribution: Progesterone is approximately 96 percent to 99 percent bound to serum proteins, primarily to serum albumin (50 to 54 percent) and transcortin (43 to 48 percent)[3].

Excretion: The glucuronide and sulfate conjugates of pregnanediol and pregnanolone are excreted in the bile and urine. Progesterone metabolites are eliminated mainly by the kidneys. Progesterone metabolites which are excreted in the bile may undergo enterohepatic recycling or may be excreted in the feces[3].

Food effect

Concomitant administration of progesterone capsules with food increased the bioavailability of progesterone capsules relative to a fasting state when administered to postmenopausal women at a dose of 200 mg[3].

Study Design and Objectives

This study was an open label, randomized, balanced, single-dose, two sequence four period, full replicate, crossover oral bioequivalence study was conducted in 30 healthy adult, human, post menopausal female volunteers under fed conditions.

The primary objective of this study was to identify the intra subject variability of progesterone soft capsules 200 mg and to compare the rate and extent of absorption of Progesterone USP capsules 200 mg (Test) manufactured by Aurobindo Pharma Limited and Prometrium® (Progesterone USP) capsules 200 mg (Reference) marketed by Solvay Pharmaceuticals Inc., Marietta, GA when given in equal doses of single oral dose in 30 healthy, human, post menopausal female subjects under fed conditions in two equal groups. The secondary objective was to monitor the adverse events and to ensure the safety of the subjects.

MATERIAL AND METHODS

The investigational products were supplied by the Aurobindo Pharma Ltd., India for the conduct of this bioequivalence study. Reference Product (R) of Prometrium® (Progesterone USP) Capsules 200 mg (batch no: 510299) of Solvay Pharmaceuticals Inc., Marietta, GA.or the Test Product (T) of Progesterone USP Capsules 200 mg (batch no: 7204798) of Aurobindo Pharma Limited, India were used in the present bioequivalence study.

Screening

Volunteers aged from 40-55 years with a body mass index (BMI) in the range of 18-29.9 Kg/m^2 were selected according to the inclusion and exclusion criteria. They were assessed to be healthy according to medical, systemic and physical examination including vital signs, and normal laboratory test results [haematology, biochemistry, urine analysis], Follicle stimulating hormone (FSH), prothrombin time (PT), activated partial thromboplastin time (APTT), estradiol, papanicolaou smear, mammogram, ultra sound pelvis, 12-lead ECG (Electrocardiogram), chest X-ray (PA view) and screening for infectious diseases including negative HIV 1 & 2, Hepatitis B, Hepatitis C, RPR (Rapid Plasma Reagin) tests. Drugs of abuse (Benzodiazepines, Opioids, Amphetamines, Cannabinoids, Cocaines and Barbiturates) in urine, urine pregnancy test and alcohol breath analysis test were performed during the study check-in of each period and who tested negative were checked-in.

Informed consent was obtained from all study participants prior to enrolment in the study and the subjects were free to withdraw at any time during the study. The study was conducted in compliance with the ICH-GCP (International Conference on Harmonization-Good Clinical Practice), ICMR (Indian Council of Medical Research) guidelines, and declaration of Helsinki at the research facility.

Drug Administration

After an overnight fasting of at least 10 hours, 30 minutes after serving of a high fat high-calorie meal a single oral dose of Progesterone USP capsules 200 mg, Test (T) or Reference (R) product were administered in sitting posture as per randomization schedule with 240 mL of drinking water at room temperature under fed conditions. The break-up of high fat high-calorie meal is as mentioned below.

High Fat High Calorie Meal And Its Composition

Food Items [4,5,6]	Quant-ity	Protein (g)	Fat (g)	Carbo-hydrates (g)	Energy (Kcal)
Whole milk	240 mL	7.9	8.9	11.3	156.9
French fries	70g	2.7	11.3	26.9	220.1
Chicken fry	35 g	10.2	4.1	0.9	81.3
Bread Toast Butter	45g	3.9	0.4	26.0	123.2
(two slices of toast with butter)	20g	0.0	16.2	0.0	145.8
Egg fry (two eggs fried in butter)	90g	13.3	17.4	0.0	209.8
Total		38	58.3	65.1	937.1
Calories		152	524.7	260.4	
Percentage		16.2	56.0	27.8	

Blood Sampling Schedule

A total of 21 blood samples (4 mL each) in each period were collected in a pre-labeled vacutainer tubes containing K_2 EDTA. The blood samples were withdrawn pre-dose at -24.00, 0.00 hours and at 0.33, 0.67, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 8.00, 10.00, 12.00, 18.00 24.00 and 36.0 hours post dose in each period. The collected samples were centrifuged and separated plasma samples were transferred into pre labeled polypropylene tubes as single

aliquot and were stored in a deep freezer maintained at -80°C or colder until bio-analysis.

Washout Period

A period of eleven (11) days was given between the any of the four periods.

Sample Size

A total of 30 subjects were enrolled in the study. The total subjects were divided into two groups consisting group 1: subject no. 1-15 and group 2: subject no. 16-30). All the 30 subjects were dosed and except subject nos. 2, 3, 6 and 8 from group1, remaining 26 subjects completed all four periods of the study in both groups.

Analytical Method

Progesterone was analyzed using validated LC-MS/MS method. The method conditions are as mentioned below.
Run time: 3.4 minutes.
Polarity: +ve mode.
Column: Zodaic Sil 120-3-C18, AQ 3.0µ 4.6 × 100.
Mobile Phase: 2mM Ammonium Formate (pH 6.2): Methanol: Acetonitrile (ACN) in the ratio of 10:20:70.
Flow rate: 1.0 mL.
Injection volume: 10µL,
Retention time for progesterone: 2.55.
Internal standard (Progesterone d9) retention time: 2.50.

	Q1	Q3
Progesterone	315.5	97.3
Progesterone d9	324.5	100.3

The MRM (Multiple Reaction Mode) used in the detection of progesterone and its deuterated internal standard (Progesterone d9) was 315.5/97.3 and 324.5/100.3 respectively.

50µL of internal standard was taken (500 ng/mL) and mixed with 0.4mL of plasma and then 0.4 mL 2% OPA solution was added and vertexed for 10 minutes. Extracted the solution with Solid Phase Extraction using HCB Barry/ICC and conditioned with 1mL methanol and 1mL of milliQ water. The plasma sample was loaded and washed with 1mL of 0.2% ammonia solution. The obtained solution was washed with 1mL of 10% methanol and then the cartridges dried for 2 minutes. The solution was eluted with 1mL of Acetonitrile (ACN) and evaporated for 10 minutes and finally reconstituted with 0.4 mL mobile phase and injected the sample on LC/MS/MS System. The calibration curve range used during sample analysis was 1.00 ng/mL-80.00 ng/mL.

Pharmacokinetic and Statistical Analysis

Calculation of pharmacokinetic parameters of C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, K_{el} and $T_{1/2}$ was done for progesterone baseline-corrected data using drug concentration time data by non-compartmental method using WinNonlin professional software version 5.0.1 (Pharsight Corporation, USA). Statistical analysis of the pharmacokinetic parameters of the two formulations was carried out using PROC GLM (Generalized Linear Model) of SAS® release 9.1.3 (SAS Institute Inc., USA) to assess the bioequivalence of progesterone baseline-corrected data.

Bioequivalence Criteria

The test drug must pass both conditions before it is judged bioequivalent to the reference product using average bioequivalence criteria[8].

- A 95% upper confidence bound for $(\mu T - \mu R)^2 - \theta * \sigma^2_{WR}$ must be ≤ 0.
- The point estimate (test/reference geometric mean ratio) must fall within [0.80, 1.25].

Regulatory constants

$$\sigma_{W0}=0.25 \qquad \theta = \frac{(\ln \Delta)^2}{\sigma^2_{W0}} \qquad \Delta \text{ is } 1.25$$

θ =Scaled average bioequivalence limit,
μT = log-transformed average of test product,
μR = log-transformed average of reference product and
σ^2_{WR} = Within subject standard deviation of reference product.

RESULTS AND DISCUSSION

The descriptive statistics, ANOVA, 95% upper confidence bound, within subject standard deviation (σ_{WR}) of reference product were computed for the pharmacokinetic parameters of progesterone baseline-corrected data were as mentioned below.

Table 2

Parameter (Unit)	Mean ± SD** (Un-transformed data) Progesterone baseline-corrected	
	Test Product (T)	Reference Product (R)
C_{max} (ng/mL)	24.21 ± 26.44	42.63 ± 55.10
$AUC_{0\rightarrow t}$ (hr. ng/mL)	38.11 ± 35.82	55.72 ± 55.91
$AUC_{0\rightarrow\infty}$ (hr. ng/mL)	38.96 ± 36.19	62.25 ± 59.03
T_{max} (hr)	2.00 (1.00-5.00)	3.00 (1.00-18.00)
K_{el} (hr^{-1})	0.33204 ± 0.430626	0.25589 ± 0.268375
$t_{½}$ (hr)	4.51 ± 3.07	5.80 ± 5.27

Table 3 Scaled average bioequivalence criteria

Parameter	Ln-transformed data (T/R) Ratio %	95% Upper confidence bound	Within subject SD** of reference (σ_{WR})	ISCV* of reference (%)
C_{max}	77.25	-0.37246	1.13304	161.6
$AUC_{0\rightarrow t}$	76.20	-0.02535	0.72073	82.5
$AUC_{0\rightarrow\infty}$	61.64	0.04630	0.78637	92.5
ANOVA p-value		C_{max}	$AUC_{0\rightarrow t}$	$AUC_{0\rightarrow\infty}$
Lntransformed	Sequence	0.0809	0.0816	0.1954

*Intra subject coefficient of variation. ** Standard Deviation.

Since baseline concentrations were not detected with the calibration curve range of 1.00 ng/mL-80.00 ng/mL, the obtained concentration after post dose were considered as baseline corrected data. The obtained pharmacokinetic parameters (Table 2) of C_{max} and AUC for test product was

found to be lower than that of literature, whereas for reference product more or less similar in comparison.

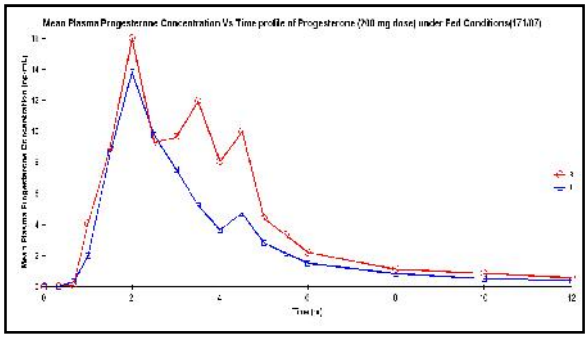

Figure 1 Mean Plasma Concentration versus Time Profile of Progesterone (ng/mL) Under Fed Conditions for all 26 subjects in linear scale.

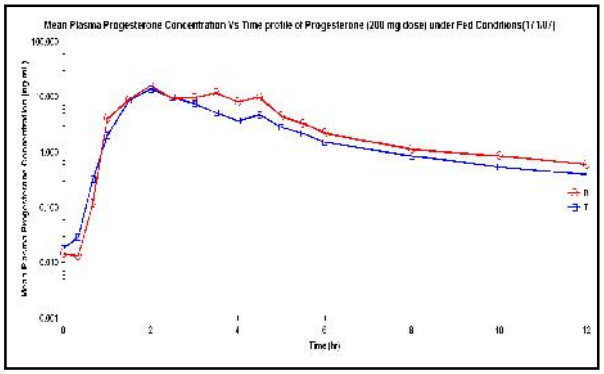

Figure 2 Semi-log Mean Plasma Concentration versus Time Profile of Progesterone (ng/mL) Under Fed Conditions for all 26 subjects in linear scale.

The T/R ratios obtained using scaled average bioequivalence criteria for Ln- transformed data of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were found to be 77.25%, 76.20% and 61.64% respectively. The obtained 95% upper confidence bound for $C_{max,}$ AUC_{0-t} & $AUC_{0-\infty}$ was found to be 1.13304, 0.72073 and 0.78637 respectively. None of the T/R ratios were found be within 80.00-125.00%. However the 95% upper confidence bound was found to be less than zero (0.0000) for C_{max} and AUC_{0-t} whereas for $AUC_{0-\infty}$ was found to be greater than zero (0.0000) (Table 3)[8].

Based on the obtained results test product fails to prove bioequivalence with that of innovator using scaled average bioequivalence criterion approach as indicated in Table 3.

The linear and semi-log mean plasma concentration versus time profiles of progesterone (ng/mL) under fed conditions were provided in Figure 1 and 2. Based on the profiles it was observed that progesterone exhibits a high variability mainly in its absorption pattern and is found to highly be erratic. Suggested sample size for future studies on progesterone to produce ANDA (Abbreviated New Drug Application) would be at least 80 subjects for fasting using partial replicate design and 150 postmenopausal female subjects using partial replicate design or at least 100 postmenopausal female subjects using full replicate design for fed study would be a appropriate strategy to be followed. Based on the obtained within subject standard deviation of reference product (>100% for C_{max}), recruitment of approximately 80 to 100 postmenopausal subjects may be a nightmare to produce ANDA of progesterone 200 mg capsules by Indian generic players, though the USA (United States of America) is the largest market for Indian generic drug makers, followed by the United Kingdom[7] due to poor turnout of literate postmenopausal female subjects considering the Indian culture and habits. In general bioequivalence studies are conducted in a single group or a maximum two groups, but in case of progesterone capsules, bioequivalence studies may have to be conducted in several groups.

CONCLUSIONS

Based on the results obtained using scaled average bioequivalence criteria, the test product fails to prove the bioequivalence in comparison with that of innovator. Within subject standard deviation of reference product was greater than at least 70% for AUC_{0-t} and $AUC_{0-\infty}$ whereas it was found to be 113% (>100%) for C_{max} under fed conditions. Based on these results it has been concluded that oral progesterone was found to be highly variable in both under fasting[9] and fed conditions and exhibits erratic absorption pattern from the formulation. Baseline values are also not detected indicated that a sensitive LOQ (Lower Limit of Quantitation) of 5 pg/mL is required for appropriate detection baseline concentrations. The observed significantly high intra subject variability for progesterone under fed conditions might be due to following factors including low apparent absolute and relative bioavailability of progesterone less than 10%, lack of aqueous solubility of progesterone, poor absorption when taken orally unless micronized in oil, fluctuation in endogenous levels of progesterone through various factors (e.g. stress, mood changes etc), high intra-subject variability within the reference formulation and lack of sensitive bioanalytical method.

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