Intra Subject Variability of Progesterone 200 mg Soft Capsules in Indian Healthy Adult Postmenopausal Female Subjects under Fasting 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 two period, crossover oral bioequivalence study was conducted in 12 healthy adult, human, post-menopausal female volunteers under fasting conditions. Subjects received progesterone 200 mg of either test or reference formulation with a washout period of 10 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 by a validated method using LC/MS/MS. Pharmacokinetic parameters C_{max}, T_{max}, AUC_{0-t}, AUC_{0-∞}, Kel and T_{1/2} were determined for both the formulations. The formulations were to be considered bioequivalent if the geometric least square mean ratio of test and reference of C_{max}, AUC_{0-t} and AUC_{0-∞} were within the predetermined bioequivalence range of 80% to 125%. A total of 12 subjects were enrolled. No significant differences were found based on analysis of variance. The 90% confidence interval of the geometric mean ratio (GMR) of AUC and C_{max} fall within 80-125% [1].

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 [2].

Distribution: Progesterone is approximately 96-99% bound to serum proteins, primarily to serum albumin (50-54%) and transcortin (43-48%) [2].

Excretion: The glucuronide and sulfate conjugates of pregnanediol and pregnanolone are excreted in the bile and urine. Progesterone

Table 1. Pharmacokinetic Parameters of Prometrium (progesterone) Capsules.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>100 mg</th>
<th>200 mg</th>
<th>300 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max} (ng/mL)</td>
<td>17.3 ± 21.9</td>
<td>38.1 ± 37.8</td>
<td>60.6 ± 72.5</td>
<td></td>
</tr>
<tr>
<td>T_{max} (hr)</td>
<td>1.5 ± 0.8</td>
<td>2.3 ± 1.4</td>
<td>1.7 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>AUC_{0-∞} (ng*hr/mL)</td>
<td>43.3 ± 30.8</td>
<td>101.2 ± 66.0</td>
<td>175.7 ± 170.3</td>
<td></td>
</tr>
</tbody>
</table>

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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 [2].

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 [2]. Progesterone is a steroid hormone which plays an important role in the preparation and maintenance of pregnancy. Under its influence the numerous minute glands line the uterine cavity are transformed into secreting glands. This alteration is a part of the change which is essential to provide for the implantation of a fertilized ovum and for the continuing development of the placenta. Progesterone is synthesized from cholesterol via pregnenalone, and then rapidly metabolized to pregenadiol, for the most part in the liver. The ovary and placenta are major production sites, but a small amount is also synthesized by the adrenal cortex in both men and women.

Circulating progesterone levels which are characteristically low during the follicular phase (<1.5 ng/mL), increase sharply during the lutéal phase of the menstrual cycle, reaching a maximum (>20 ng/mL) some 5 to 10 days after the mid cycle LH peak. Unless pregnancy occurs, a steep decline to follicular level sets in about 4 days prior to the next menstrual period [3].

The biologic effects of progesterone may be grouped as follows: (1) uterine endometrial cells are transformed in such a way that they may receive the early embryo and facilitate its implantation; (2) myometric activity is suppressed, aiding in retention of the embryo during implantation and growth prior to normal parturition; (3) numerous and varied metabolic parameters may be altered which may have no direct impact on maintenance and termination of pregnancy [3]. The aim of this study was to identify intra subject variability of progesterone soft capsules, to compare the rate and extent of absorption of Progesterone USP capsules 200 mg (Test) manufactured by Strides Arcolab 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 12 healthy, human, post menopausal female subjects under fasting conditions and to monitor the adverse events and to ensure the safety of the subjects.

Material And Methods

The tested treatment details are as given below for the conducted study.

Reference product (R)

Prometrium® (Progesterone USP) capsules 200 mg; each capsule contains 200 mg of progesterone micronized, USP, Marketed by Solvay Pharmaceuticals Inc., Marietta, GA.

Batch No: 510299
Exp. Date: 10/2009

Test product (T)

Progesterone Usp Capsules 200 mg; each capsule containing 200 mg of progesterone Micronized, USP, Manufactured By Aurobindo Pharma Limited, India.

Batch No: 7204798
Mfg. Date: 10/2007

Study design

This study was an open label, randomized, balanced, single-dose, two sequence, two period, crossover oral bioequivalence study was conducted in 12 healthy adult, human, post menopausal female volunteers under fasting conditions. All the subjects provided written informed consent to participate in the study prior to enrolment and were free to withdraw at any time during the study. The study was conducted in compliance with the ICH GCP, ICMR guidelines, and declaration of Helsinki at the research facility.

Screening

Volunteers aged from 40-55 years with a body mass index (BMI) in the range of 18-29.9 kg/m² 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, Chest X-ray (PA view) and Screening for infectious diseases including negative HIV-1 & -2, Hepatitis B, Hepatitis C, RPR 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.

Drug administration

After an over night fasting of at least 10 hours 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 fasting conditions.

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,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 ten (10) days was given between the any two periods.

Study conduct

A total of 12 subjects were enrolled in the study. All the 12 subjects were completed all the periods of the crossover.

Analytical method

Progesterone was analyzed using validated LC-MS/MS method. The run time is 3.4 minutes, polarity: +ve mode, column used is Zodiac Sili 120-3-C18, AQ 3.0 μ 4.6x100. Mobile Phase: 2 mM Ammonium Formate (pH 6.2): Methanol: Acetonitrile (ACN) in the ratio of
10:20:70. Flow rate was 1.0 mL, injection volume is 10mL, retention time for progesterone is 2.55 and for progesterone d9 is 2.50.

Fifty milliliter of internal standard was taken (500 ng/mL) which was mixed with 0.4 mL of plasma and then 0.4 mL 2% OPA solution was added and vortexed for 10 min. Extracted the solution with Solid Phase Extraction using HCB Barry/ICC and conditioned with 1 mL methanol and 1 mL of milliQ water. The plasma sample was loaded and washed with 1 mL of 0.2% ammonia solution. Then 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 is 1.00 ng/mL to 80.00 ng/mL.

### Pharmacokinetic and statistical analysis

Calculation of pharmacokinetic parameters was done for progesterone baseline-corrected data and progesterone baseline-uncorrected 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 of SAS® release 9.1.3 (SAS Institute Inc., USA) to assess the comparative oral bioavailability of progesterone baseline-corrected data and progesterone baseline-uncorrected data. Descriptive statistics, ANOVA, nominal 90% confidence intervals, intra-subject variability for reference product was computed and reported for primary and secondary pharmacokinetic parameters of progesterone baseline-corrected data and progesterone baseline-uncorrected data [4].

Pharmacokinetic parameters of the formulations, based on the following primary and secondary pharmacokinetic parameters were assessed:

For Progesterone baseline-corrected data the following pharmacokinetic parameters were estimated:

- **C<sub>max</sub>**: Maximum measured plasma concentration over the time span specified.
- **AUC<sub>0-t</sub>**: The area under the plasma concentration versus time curve, from time 0 to the last measurable concentration, as calculated by the linear trapezoidal method.
- **AUC<sub>0-∞</sub>**: The area under the plasma concentration versus time curve, from time 0 to the last measurable concentration, as calculated by the linear trapezoidal method.
- **T<sub>max</sub>**: Time of the maximum measured plasma concentration. If the maximum value occurs at more than one time point, T<sub>max</sub> is defined as the first time point with this value.
- **K<sub>e</sub>**: Apparent first order elimination rate constant calculated from a semi-log plot of plasma concentration versus time point. The parameter was calculated by linear square regression analysis using the last 3 (or more) non-zero plasma concentrations.
- **T<sub>1/2</sub>**: The elimination or terminal half-life was calculated as 0.693/\(K_e\).

For Progesterone baseline-uncorrected data the following pharmacokinetic parameters were estimated:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test Product (T)</th>
<th>Reference Product (R)</th>
<th>Mean ± SD (Un-transformed data)</th>
<th>90% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC&lt;sub&gt;0-t&lt;/sub&gt; (hr. ng/mL)</td>
<td>68.3649 ± 51.62570</td>
<td>70.6821 ± 57.54212</td>
<td>75.6% 64.6%</td>
<td>52.10-148.80 68.2</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (hr. ng/mL)</td>
<td>70.5905 ± 51.74866</td>
<td>73.0414 ± 57.78191</td>
<td>92.50 56.05-152.68 64.6</td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (hr)</td>
<td>1.50 (1.50-5.00)</td>
<td>2.50 (1.00-4.00)</td>
<td>52.10-148.80 68.2</td>
<td></td>
</tr>
<tr>
<td>K&lt;sub&gt;e&lt;/sub&gt; (hr&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.19783 ± 0.143029</td>
<td>0.10196 ± 0.102788</td>
<td>52.10-148.80 68.2</td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>7.516 ± 10.3630</td>
<td>11.508 ± 6.9087</td>
<td>52.10-148.80 68.2</td>
<td></td>
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</tbody>
</table>

**Results And Discussion**

The descriptive statistics, ANOVA, nominal 90% confidence intervals, intra-subject variability were computed for the pharmacokinetic parameters of progesterone baseline-corrected data were as mentioned below (Table 1). The log-transformed pharmacokinetic parameters, C<sub>max</sub>, AUC<sub>0-t</sub> and AUC<sub>0-∞</sub> of baseline corrected data for progesterone were subjected to analysis of variance (ANOVA) with the main effects of sequence, treatment, and period at 5% level of significance. The obtained intra-subject variability for C<sub>max</sub>, AUC<sub>0-t</sub> and AUC<sub>0-∞</sub> were found to be 68.2%, 75.6% and 64.6% respectively. The obtained geometric least squares means for test product of C<sub>max</sub>, AUC<sub>0-t</sub> and AUC<sub>0-∞</sub> were found to be 18.3805, 56.3681 and 59.9745. The T/R ratio for were found to be 88.05%, 93.17% and 92.50% respectively. The 90% confidence intervals for C<sub>max</sub>, AUC<sub>0-t</sub> and AUC<sub>0-∞</sub> were found to be 18.3805, 56.3681 and 59.9745. The T/R ratio for were found to be 88.05%, 93.17% and 92.50% respectively. The obtained geometric least squares means for reference product of C<sub>max</sub>, AUC<sub>0-t</sub> and AUC<sub>0-∞</sub> were found to be 18.3805, 56.3681 and 59.9745.

**Conclusions**

Based on the results obtained using average bioequivalence criterion,
the test product fails to prove the bioequivalence in comparison with that of innovator. The global variance and the within subject standard deviation of reference product was greater than at least 60% for Cmax, AUC₀₉ and AUC₀₋∞ under fasting conditions. Based on these results it has been concluded that oral progesterone was found to be highly variable and exhibits erratic absorption pattern from the formulation.
Baseline values are also not detected indicated that a sensitive LOQ of 5 pg/mL is required for appropriate detection baseline concentrations.

References


