PHARMACOKINETIC STUDY OF PROGESTERONE CAPSULES IN POST MENOPAUSAL FEMALES OF INDIA UNDER FASTING CONDITION

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Summary

The study was conducted to assess the pharmacokinetics of micronized progesterone administered in the capsule form. Ten post-menopausal females of India, with a mean age of 51.50 years, volunteered to participate in the study. The pharmacokinetics of 200 mg of progesterone evaluated by validated Liquid Chromatography Mass Sepectroscopy-Mass spectroscopy (LCMS-MS) method. After the initial administration of 200 mg, a mean serum Cmax of 3.59±0.66 was reached at a tmax of 3.50±0.59. The terminal half-life ($t^1/_2$) was 7.09±2.52. Micronized progesterone given as a capsule is well tolerated, safe and an easily administered.

Key Words: Pharmacokinetic, Progesterone, Post menopausal, females of India, LCMS-MS

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Introduction

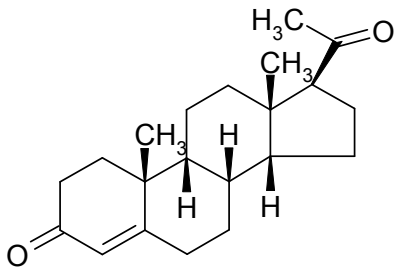

Figure 1: Chemical structure of Progesterone

In the recent years, hormonal therapy for postmenopausal female has evolved from unopposed synthetic compounds with oestrogenic activity to complex regimens containing bio-identical oestrogen plus a progestogen. Initially after its introduction, oestrogens alone were prescribed for postmenopausal females, until it was realized that this regimen was associated with a 5-10 fold increase in the risk of endometrial cancer. To reduce this risk, it was advised that a progestogen be added to the oestrogen. (1)

Synthetic oral progesterone has been used for a variety of gynecological conditions. However, androgenic activity inherent in the synthetic compound precludes its liberal use in assisted reproductive technology because of the threat of teratogenic effects; in hormonal replacement therapy (HRT) may partially reverse the oestrogenic benefits on the cardiovascular system and lipoprotein metabolism. Natural progesterone is devoid of any androgenic activity that might compromise lipoprotein metabolism or induce teratogenicity. Moreover, it probably has a direct beneficial effect on blood vessels. The major problem with natural progesterone is its route of administration. Oral intake is hampered by rapid and extensive intestinal and liver metabolism leading to poorly sustained serum concentrations and low bioavailability. Injection i.m. assures reliable absorption, but is related to low compliance. It is painful, can cause local irritation and cold abscesses, and must be administered by trained medical personnel. (2)

Orally administered progesterone has been associated with systematic adverse effects, e.g. drowsiness, flushing and nausea, Sedative and hypnotic effects or fluid retention have also been attributed to progesterone or its metabolites after oral ingestion.

Pharmacokinetic properties of orally administered progesterone are further influenced by food uptake or by the characteristics of progesterone preparation such as vehicle and particle size. After oral digestion, progesterone is rapidly absorbed, rapidly metabolized from the intestines and, during the first hepatic pass, cleared from the circulation. Micronization of natural progesterone improves its absorption and bioavailability. Nevertheless, even higher doses of oral micronized progesterone (200 or 300 mg/day) have failed to induce uniform secretory endometrial features in menopausal females. (3, 4, 5, 6)

A number of factors can influence the percutaneous absorption of a drug, eg. progesterone, from a vehicle such as a cream; they include progesterone concentration, physical and chemical properties of ingredients in the cream, solubility of progesterone in the cream, the extent to which the cream ingredients can change the integrity of the skin, and the site and surface area of cream application. Because progesterone creams can vary widely with respect to the types and characteristics of ingredients that they contain, and their site of application, the extent of progesterone absorption will also vary widely. The importance of differences in percutaneous progesterone absorption at different sites of application in females is evident in a study by Krause et al. (6) They showed a significant increase in serum progesterone levels 30 to 120 minutes after applying a progesterone ointment on the breast, but no increase was observed after application of the same ointment on other regions. (7)

Materials and Method;

Eligibility Assessment: The protocol was approved by the institutional review board for Clinical investigation. For the purpose of the study the following eligibility assessments were carried out before enrolment of any subject into the dosing / sampling phase of the study:

Screening: The screening was carried out after taking an initial informed consent from all the subjects for study screening procedure as per the regulatory and local guidelines and will include Demographic data, Medical and treatment history, relevant past medical history, family history, history of any allergy to food or drug, medication

history in the last three months. A Pap cervical smear test to exclude cancer of the cervix and mammography to exclude breast cancer. Complete physical examination including recording of vital signs and systemic examination, 12-lead ECG for heart rate, rhythm and specific finding, Chest X-ray.

Clinical laboratory assessment: The approved normal laboratory values for the following tests was obtained from the Clinical laboratory performing these tests, prior to start of the study.
Complete blood count including hemoglobin, red blood cell count, leukocyte count, platelet count and ESR, Serum β-HCG except for those who have undergone bilateral oophorectomy.
Biochemistry including blood glucose, Serum albumin, Serum Chloride, Serum Potassium, Serum Sodium, Serum Calcium, Srum Urea, Serum total protein & Serum CRP.
Hepatic profile including SGOT, SGPT, Bilirubin (total, direct and indirect)& Serum Alkaline Phosphatase.
Renal profile including serum creatinine and BUN.
Urinalysis includes physical examinations, chemical examination, microscopic examination and *drugs of abuse (Benzodiazepines, opioids and amphetamine, cocaine, THC), *HIV, *HBS Ag and *HCV Ab was also evaluated.

Dose administration: Subjects were housed atleast 13 hours before dosing until 48 hours post dose sample. After overnight fasting (atleast 10 hours) the subjects were administered with one capsule of 200 mg Progesterone with 240 ml of water.

Blood sampling and Processing: With the help of an indwelling cannula the blood samples (1 X 7ml) were collected in the K_3 EDTA Vaccutainer at **0.0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16, 24, 36 & 48 hrs.** Then the samples were centrifuged at 5000 rpm (±100) for 5 minutes at 5 °C (±1) and the separated plasma was stored in the deep freezer (-86 °C) till the analysis.

Analysis of Progesterone: The progesterone was analyzed by validated LCMS-MS method. The required volume of plasma sample (0.25 ml) was taken out from the deep freezer and allowed them to thaw at room temperature. Vortexed the thawed samples to ensure complete mixing of contents. Added 25µL of internal standard dilution ~0.5 µg/ml and Vortexed for 10 sec. and add 4.0 ml Tertiary Methyl butyl ether (TBME), vortex for 5 minutes & centrifuge for 5 minute at

2000 rpm, transfer organic layer in evaporation test tube and evaporate the solvent under nitrogen flushing at 45 °C. Reconstitute the sample with 250 µl of Reconstitute Solution, transfer into vials and inject 30 µl in LCMS-MS.

Pharmacokinetic Evaluation: The following pharmacokinetic parameters were determined: Cmax (maximum serum levels) and tmax (time to reach Cmax) values were taken as determined. Areas under the serum level-time curve (AUC) were calculated according to the trapezoidal rule until the concentration point at time t, and until the last measured concentration point above the lower limit of quantitation at time tlast. The AUC value was calculated, if possible, by extrapolation to infinity according to the following equation (8):

$$AUC = AUC\ (0 - t) + C/\lambda z$$

with C as the last measured concentration point at time t.

The terminal rate constant λ, of the disposition of the drug in serum was calculated by means of regression analysis of the perceivable linear part of the curve in a semilogarithmic plot (λ = slope of the regression line). The corresponding terminal half-life was calculated by the following equation:

$$t^1/_2, = \ln 2/\lambda z$$

The mean residence time (MRT) in the central compartment was calculated by the ratio of the area under the moment curve (AUMC) and of the AUC value: MRT = AUMC/AUC. Clearance (CL) and the volumes of distribution (V, and Vss) were calculated according to:

$$CL = Dose/AUC$$
$$Vz, = CL/\ \lambda z,$$
$$Vss = CL \times MRT$$

Results

Thirty post-menopausal females volunteered to participate in the study. 10 of them were enrolled into the trial. Out of 30 subjects, 20 were found to be screen failure due to reasons like abnormal hemogram, volunteer withdrawal of consent, failure to adhere to protocol related procedures. 10 subjects were allocated with 200 mg micronized progesterone capsules. Table 1 to 5 summarizes the screening details of the enrolled post menopausal females.

The progesterone-containing capsules were well tolerated by all the females. The capsules were easily administered. All participating women experienced some degree of hot flushes, head ache, vaginal discharge, muscle pain, breast pain, vomiting, nausea, diarrhea, skin irritation, abdominal pain and discomfort, acidity. But no serious adverse event was reported. No subjects withdrew from the study because of side effects or adverse reaction to progesterone.

Parameters	Range	Unit	Mean±SEM
Age	45 to 65	Years	51.50±1.82
Weight	-	Kg	52.80±2.10
Height	-	Cm	1.50±0.01
BMI	18 to 30	-	23.33±0.75

Table 1: Demographic details of the enrolled post menopausal females. Values are expressed as mean±SEM, Body mass index (BMI) was calculated as weight (in kg) divided by the square of height (in meters)

Parameters	Range	Unit	Mean±SEM
Hemoglobin	12.00-15.00	g%	10.57±0.64
RBC	3.80-4.80	million cell/mm^3	4.11±0.20
HCT	36.00-46.00	%	32.19±1.80
MCV	83.00-101.00	fL	78.37±1.97
MCH	27.00-32.00	pg	25.77±0.95
MCHC	31.50-34.50	%	32.78±0.54
TLC	4000-10000	cell/mm^3	8770±432.56
Neutrophil	40.00-80.00	%	58.42±3.03
Lymphocyte	20.00-40.00	%	29.00±2.67
Monocyte	2.00-10.00	%	5.39±0.29
Eosinophil	1.00-6.00	%	6.44±1.09
Basophil	0.00-2.00	%	0.83±0.12
Platelets	1.50-4.00	lacs cells /mm^3	2.56±0.15
ESR	20.00-50.00	mm/h	27.00±3.54

Table 2: Hematological details of the enrolled post menopausal females after screening.

Parameters	Range	Units	Mean±SEM
Plasma Glucose	60.00 - 140.00	mmol/L	95.79±3.28
Serum Cholesterol	Desirable : 200.00 Borderline High : 200.00 - 239.00 High: >=240.00	mmol/L	216.63±10.99
Serum Albumin	3.50 - 5.20	gm/L	4.47±0.07
Serum Chloride	98.00 - 107.00	mmol/L	99.85±0.56
Serum Potassium	3.50 - 5.10	mmol/L	4.73±0.19
Serum Sodium	136.00 - 145.00	mmol/L	133.02±0.66
Serum Calcium	8.10 - 10.40	mmol/L	9.80±0.12
Serum Urea	17.00 - 49.00	mmol/L	25.31±1.04
Serum Total Protein	6.00 - 7.80	gm/L	7.71±0.16
Serum C-Reactive Protein	0.5	mg/L	0.32±0.06

Table 3: Biochemical details of the enrolled post menopausal females after screening.

Parameters	Range	Units	Mean±SEM
Serum Alkaline Phosphatase	30.00 - 120.00	U/L	68.47±6.93
Serum Bilirubin - Direct	0.3	µmol/L	0.06±0.01
Serum Bilirubin - Indirect	0.10 - 1.00	µmol/L	0.32±0.03
Serum Bilirubin - Total	0.10 - 1.20	µmol/L	0.49±0.05
SGOT	8.00 - 33.00	IU/L	22.22±1.56
SGPT	4.00 - 36.00	IU/L	17.68±2.44

Table 4: Hepatic profile of the enrolled post menopausal females after screening.

Parameters	Range	Units	Mean±SEM
Blood Urea Nitrogen	8.00 - 23.00	µmol/L	6.37±0.43
Serum Creatinine	0.60 - 1.20	µmol/L	0.87±0.05

Table 5: Renal profile of the enrolled post menopausal females after screening.

A single dose application of 200 mg micronized progesterone capsules resulted in the rapid increase of plasma progesterone concentration (Figure 2). Mean peak plasma progesterone concentrations (Cmax) of 3.59±0.66 occurred at the tmax of 3.50±0.59 hr, with a mean $t^1/_2$ of 7.09±2.52 (Table 6).

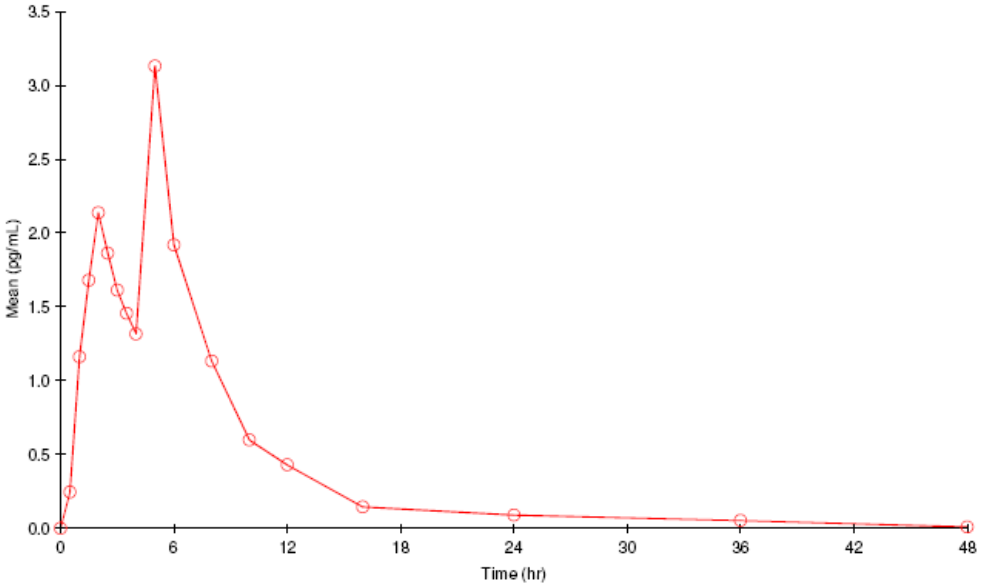

Figure 2: Plasma progesterone concentrations after administration of 200 mg of micronized progesterone capsules.

PK parameters	n	Mean±SEM
Cmax (pg/mL)	10	3.59±0.66
AUC_{0-t} (hr*pg/mL)	10	16.62±3.24
AUC∞ (hr*pg/mL)	10	18.58±3.69
tmax (hr)	10	3.50±0.59
Kel (1/hr)	10	0.26±0.06
$t^1/_2$ (hr)	10	7.09±2.52

Table 6: Pharmacokinetic parameters of progesterone capsules

Discussion

Human chorionic gonadotrophin (HCG) and progesterone are ordinarily used as luteal phase support in IVF. It has been suggested that HCG might be superior to progesterone in gonadotrophin releasing hormone (GnRH) agonist cycles for IVF, but its application has also been associated with ovarian hyperstimulation syndrome (OHSS). Since natural progesterone is rapidly metabolized after oral administration, synthetic progestins have been designed which resist enzymatic degradation.

However, synthetic progesterone derivatives have been associated with a number of undesirable effects, notably on lipids or with psychological effects that may be severe enough to limit their use. In addition, synthetic progestogens, mainly those with androgenic properties, have been connected with an increased risk of fetal congenital malformations. Natural progesterone has no adverse effects on high density lipoproteins (HDL), no known teratogenicity and is more effective in inducing secretory endometrial features than progesterone derivatives, eg. dehydrogesterone. Different routes of progesterone administration have been analysed, such as the intranasal, sublingual and rectal routes. However, oral, i.m. and vaginal routes have been the most frequently investigated. As natural progesterone is rapidly metabolized after oral ingestion, a number of techniques have been developed in order to improve its pharmacokinetic properties. Micronization of progesterone reduces particle size and increases progesterone absorption and bioavailability. Furthermore the combination of micronized progesterone with polycarbophil gel results in a sustained-release vaginal formulation. (9, 10)

This study was started with the aim of pharmacokinetics of micronized progesterone administered by the present formulation in post menopausal females of India, in order achieve the behavior of the drug in Indian females. For this extensive review of articles were carried out and no such data were available

The pharmacokinetic results indicated that for assisted reproduction at least 200 mg of progesterone should be administered. This dosage resulted in a mean Cmax of 3.59 ±0.66 pg/mL. As per the previous studies, serum progesterone concentrations above 3.3 ng/ml (10.5 nmol/l, SI conversion factor 3.18) indicate that ovulation has occurred, whereas mid-luteal phase values of 10 ng/ml (31.8 nmol/l) and above demonstrate adequate corpus luteum function.

Conclusion

In conclusion, micronized progesterone given in the present formulation is a well-tolerated and safe product. Reliable release and absorption were also obtained. Adequate plasma concentrations were achieved with the 200-mg dose. The easy administration and lack of side effects assure good patient

compliance. Further pharmacokinetic studies are suggested to fully understand the drug behavior in Indian post menopausal females and also the study to be conducted in the combination of estradiols, as well as their effect on endometrial histology.

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