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Percutaneous progesterone delivery via cream or gel application in postmenopausal women: a randomized cross-over study of progesterone levels in serum, whole blood, saliva, and capillary blood

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Abstract

Objective: This study aims to investigate the distribution of progesterone in venous whole blood, venous serum, fingertip capillary blood, and saliva after its topical application in both cream and gel formulations.

Methods: Ten postmenopausal women were randomized to receive 80 mg of progesterone cream or gel applied daily for 14 days, crossing over after a 14-day washout. On the last day of each treatment period, venous blood, fingertip capillary blood, and saliva were sampled frequently for 24 hours after the final application.

Results: After progesterone cream or gel application, serum progesterone levels rose gradually, reaching a peak at 9 and 8 hours, respectively; $AUC_{0\text{-}24\ h}$ was significantly higher with cream (12.39 vs 8.32 ng h mL^{-1}, $P = 0.0391$). Whole venous blood levels followed a pattern similar to that of serum but were considerably lower. Saliva progesterone showed a peak at 1 and 6 hours after cream and gel application, respectively, and C_{max} was comparable with cream and gel. Saliva $AUC_{0\text{-}24\ h}$ was substantially higher than the corresponding area under the curve for serum or whole blood but did not differ significantly by delivery method (39.02 and 58.37 ng h mL^{-1}, $P = 0.69$). In capillary blood, C_{max} was reached at the same time (8 h) and was similar with both formulations; $AUC_{0\text{-}24\ h}$ was also similar with both formulations (1,056 ng h mL^{-1} for cream and 999 ng h mL^{-1} for gel) but was dramatically higher than the corresponding areas under the curve for venous serum and whole blood.

Conclusions: After application of topical progesterone, saliva and capillary blood levels are approximately 10-fold and 100-fold greater, respectively, than those seen in serum or whole blood. High capillary blood and saliva levels indicate high absorption and transport of progesterone to tissues. Reliance on serum levels of progesterone for monitoring topical dose could lead to underestimation of tissue levels and consequent overdose.

Key Words: Progesterone – Serum – Saliva – Blood – Cream – Gel.

Progestogens are commonly prescribed for postmenopausal women who use estrogen therapy to alleviate menopausal symptoms such as hot flashes and vaginal dryness, as well as to preserve bone health. The progestogen component of such treatment is required in women with an intact uterus to prevent estrogen-induced endometrial hyperplasia and endometrial cancer.[1] Synthetic progestogens have adverse effects (eg, reversal of the beneficial effects of estrogen on serum lipids, weight gain, bleeding, and mood changes) and are associated with an increased risk of breast cancer.[2,3] For this reason, many women turn to the natural progestogen progesterone to treat menopausal symptoms. Oral, transvaginal, and nasal administration of progesterone have been shown to be effective and well tolerated.[4-7] However, controversy exists as to the effectiveness of progesterone applied as cream or gel to the skin, even though the lipid solubility of progesterone predisposes it toward a percutaneous method of administration.[8]

The effectiveness of topically administered progesterone cream in endometrial protection has been questioned because sufficiently elevated serum levels of progesterone are not achieved even at relatively high progesterone doses. It is a widely held assumption that serum progesterone levels higher than 5 ng/mL are needed to inhibit estrogen-stimulated endometrial cell proliferation. Several studies have shown that topical administration of progesterone at physiological to

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supraphysiological doses ranging from 16 to 80 mg/day for 1.4 to 6 weeks does not increase serum progesterone levels above 3.5 ng/mL.[9-14]

Antiproliferative effects of topical progesterone on the endometrium have been demonstrated by some,[15] but not by others.[12,13] Leonetti et al[15] randomized 32 postmenopausal women to receive orally 0.625 mg of conjugated equine estrogens plus twice-daily dosing of 15 mg of progesterone cream, 40 mg of progesterone cream, or placebo progesterone cream for 28 days, and showed an antiproliferative effect on the endometrium even though serum progesterone levels were low. A similar antiproliferative effect on the endometrium was observed with nasal administration of progesterone. Cicinelli et al[16] randomized 10 postmenopausal women to receive oral conjugated equine estrogens 1.25 mg/day for 3 to 4 weeks plus progesterone 34 mg/day given by nasal spray for the last 5 to 7 days, or progesterone alone for 6 days; endometrial samples showed secretory changes in the endometrium after progesterone treatment in women pretreated with estrogens, whereas samples taken just before the progesterone treatment showed proliferative changes.

Surprisingly, despite the low serum progesterone levels achieved with progesterone creams, salivary progesterone levels are very high, suggesting that progesterone levels in serum may not reflect those in tissues.[13,14,17] Unpublished results from D.T.Z.'s laboratory have demonstrated that capillary blood derived from the fingertip contains much higher levels of progesterone than venous blood (serum or whole blood) after topical progesterone delivery, again supporting the notion that venous blood may underestimate the delivery of progesterone to tissues. In addition, in one study[18] investigating the effectiveness of topical progesterone in the breast, it was shown that progesterone accumulates to high levels in breast tissue and inhibits breast cell proliferation stimulated by estradiol but does not significantly raise the level of serum progesterone beyond the placebo-treated group. The physiological mechanism whereby salivary, capillary blood, and tissue (eg, breast)[18,19] levels of progesterone rise dramatically to luteal levels and higher with physiological topical progesterone dosing—whereas venous serum levels change very little—remains unsolved.

If topically administered progesterone indeed delivers higher levels of progesterone to tissues than is apparent from venous serum testing of progesterone, this could potentially lead to excessive dosing in a futile attempt to achieve physiological serum progesterone levels (>5 ng/mL). To investigate this paradox in more detail, we designed a study to compare progesterone levels at various time points in venous serum, venous whole blood, capillary whole blood, and saliva after topical delivery of progesterone to a group of healthy women. Our goal in this study was to identify a window period when absorption of progesterone administered as cream or gel is at steady state. Using these data, we plan to determine, in a future study, if using progesterone cream or gel continuously with estrogen can protect the endometrium. Our secondary goal was to directly compare the absorption rates of progesterone cream and gel in a randomized cross-over study.

METHODS

A prospective, randomized, cross-over clinical trial was conducted from March 2010 to July 2010 at the Clinical Trials Unit of the Los Angeles County/University of Southern California Medical Center. The University of Southern California Health Sciences Institutional Review Board approved the study protocol, and all study participants submitted a written informed consent form.

Participants

We recruited 10 postmenopausal women who were at least 1 year postmenopausal and had serum follicle-stimulating hormone levels higher than 40 mIU/mL, estradiol levels lower than 30 pg/mL, serum progesterone levels within the reference range, no history of hormone use within the last month, and an intact uterus. Women were excluded if their body mass index was 35 kg/m^2 or higher; if they had breast cancer, endometrial cancer, or any active heart, lung, liver, kidney, or endocrine disorders; or if they were unable to apply the study drug to their inner thigh.

Treatment and sampling

Eligible women (after providing their consent) were randomly assigned, through a computer-generated randomization scheme, to 80 mg of progesterone cream or gel applied daily with nitrile gloves to the inner thigh for 14 days, with 14 days of washout period during which no hormone was applied. They were subsequently crossed over to the other vehicle for the last 14 days of the study. Metered doses of custom-compounded progesterone cream or gel were prepared by Rox San Compounding Pharmacy (Beverly Hills, CA). The cream consisted of 80 mg of progesterone, water, glycerine, stearyl alcohol, cetearyl alcohol, macadamia oil, vitamin E, panthenol, sodium hydroxide, and citric acid. The gel consisted of 80 mg of progesterone, water, and carbomel. The dose of progesterone in the cream and gel was confirmed by liquid chromatography–mass spectrometry.

On the last day of each 14-day treatment period, study participants, after applying the designated progesterone cream or gel, came into the Clinical Trials Unit for 24 hours of frequent sampling to obtain whole blood, serum, capillary blood, and saliva. Blood and saliva samples were taken just before drug application and at 1, 2, 3, 4, 6, 8, 10, 12, 16, and 24 hours after application of the drug. The blood was processed to obtain whole blood and serum. Whole blood was collected in tubes containing sodium heparin, whereas serum was collected in serum separator tubes. After the mouth had been rinsed with cool water, saliva samples were collected by passive flow into clean plastic collection tubes and taken at least 45 minutes apart from any food intake. Capillary blood spot samples were obtained via fingerstick method using contact-activated lancets (Fisher Scientific) just before drug application and at 3, 4, 6, 8, 12, and 24 hours after treatment, and spotted onto specialized filter papers (Ahlstrom grade 226; ID Biological Systems). The papers were air dried at room temperature for at least 1 hour and subsequently stored at −70°C.

To ensure that blood spot samples were not contaminated by the applied cream or gel, we instructed study participants to wear nitrile gloves when applying the cream or gel to the inner thighs. Before blood collection from the finger, the women thoroughly washed their hands and wiped the finger with an alcohol swab.

Study participants recorded any adverse reactions or postmenopausal bleeding in a study diary.

Assays

Progesterone in serum and whole blood was measured by radioimmunoassay (RIA) at the laboratory of F.Z.S. as described previously,[20] with the exception that a chromatographic step was added before RIA. Separate 0.5-mL aliquots of serum or whole blood were taken for each assay, and approximately 800 dpm of [^{3}H]progesterone, with high specific activity, was added to each aliquot to follow procedural losses. The steroids were extracted with ethyl acetate/hexane (3:2) to remove conjugated steroids; Celite column partition chromatography, with ethylene glycol as stationary phase, was used to eliminate potentially interfering unconjugated progesterone metabolites. Progesterone was eluted off the column with isooctane. After evaporation of the solvent, the residue was dissolved in assay buffer, which was aliquoted to determine procedural losses and for RIA. The sensitivity of the progesterone RIA was 20 pg/mL, and the interassay coefficient of variation, on average, ranged from 9% to 13%.

Progesterone was assayed in dried capillary blood spots by a method described previously, with modifications.[21] Progesterone standard was prepared by mixing the highest progesterone standard from an enzyme immunoassay (EIA) kit (DRG International Inc, Springfield, NJ) with washed human red blood cells (1:1; Red Cross, Pacific Northwest Regional Blood Services, Portland, OR). For blood spot controls, commercial control serum levels 1, 2, and 3 (BioRad Laboratories, Anaheim, CA) were mixed with washed red blood cells (1:1). Seventy-five microliters of prepared high standard and controls was spotted onto the filter paper, dried overnight at room temperature, and stored in Ziploc plastic bags with desiccant packets at −70°C. Six 6.0-mm blood spot disks from each of the filter papers containing the high standard, controls, and study samples were punched into deep 96-well plates using an automated blood spot puncher (Wallac MultiPuncher; PerkinElmer, Wellesley, MA). Five hundred microliters of methanol was added to each well, and the plates were sonicated for 3 minutes and incubated on a microplate shaker for 1 hour at 37°C to extract steroids. The methanol was transferred to another deep 96-well plate. This step was repeated with another 300 μL of methanol, which was subsequently added to the same deep 96-well plate and dried under nitrogen. Dried extract was reconstituted by adding 600 μL of T-buffer (phosphate-buffered saline containing 0.1% T904 detergent and 0.05% Proclin antimicrobial), sonicated for 3 minutes, and shaken for 1 minute. A progesterone standard curve was created by serially diluting the extracted high standard with T-buffer. One hundred microliters of extracted standard, controls, and samples was used to quantify progesterone following a modified immunoassay procedure using progesterone EIA kits (DRG International Inc). Final absorbance was read at 450 nm using the Wallac Victor2 1420 Multilabel Counter (PerkinElmer). The sensitivity of the dried blood spot assay was 0.1 ng/mL. The intraassay and interassay coefficients of variation ranged from 9.1% to 19.2% at 0.7 to 28.2 ng/mL. The established progesterone reference ranges for capillary dried blood were 0.8 ng/mL or lower (postmenopausal and premenopausal follicular phase) and 3.3 to 22.5 ng/mL (premenopausal luteal phase).

Progesterone in saliva was measured by a previously described method.[22] After collection, all samples were stored at −70°C before analysis. Samples were thawed, and particulate matter was removed by centrifugation. Five hundred microliters of each sample was transferred to a 96-well polypropylene block along with 50 μL of phosphate-buffered saline buffer. Saliva was transferred from each block onto assay plates for progesterone (DRG Salivary Progesterone EIA). Each 96-well block of samples included two blanks and 11 other control samples. One hundred microliters of sample and conjugate was incubated at room temperature for 1 hour before four washes with 300 μL of wash buffer; 200 μL of substrate was added, followed by 0.1 M sulfuric acid 30 minutes later. The plates were read on a spectrophotometer at 450 nm, and progesterone levels were calculated from the standard curve generated using known standards in the same assay. The sensitivity of the saliva assay was 5 pg/mL. The intraassay and interassay coefficients of variation ranged from 7% to 10% at 24 to 1,867 pg/mL.

Statistical analysis

The maximal concentration (C_{max}), the time at which it occurred (T_{max}), and the area under the curve (AUC) of serial measurements of progesterone in serum, whole blood, saliva, and capillary blood over time were calculated for each participant while she was using cream and gel. AUC were calculated with the trapezoidal formula, using either interpolation or substitution of the measurement closest in time when missing values occurred. These pharmacokinetic parameters were summarized as median, 25th percentile, and 75th percentile for each medium and each application method. After cream and gel application, within-woman paired comparisons of pharmacokinetic parameters were performed using Wilcoxon signed rank test. Repeated-measures analysis of variance was used to investigate the effects of delivery method, time, and their interaction on log-transformed progesterone levels in serum, whole blood, saliva, and capillary blood. Pairwise comparisons of levels at various times were evaluated, with adjustment for multiple comparisons when a significant time effect was found. Statistical tests were two-sided at a 0.05 level of significance using SAS/STAT® software.

RESULTS

Of the 10 women who completed an informed consent form, eight completed the entire study. One of the eight women

only had whole blood and serum collected by venipuncture. Two women completed only half the study because of inability to complete another 24-hour sample collection. The final participants were aged 51 to 61 years (median, 58.5 y), and their body mass indexes ranged from 20 to 30 kg/m^2 (median, 24.5 kg/m^2). Saliva and capillary blood results from two women after cream application were excluded because the 0-hour sampling time (just before progesterone application) showed extremely high progesterone levels; these were attributed to sample contamination during collection because such high levels were not observed in any of the women within the 24-hour sampling time.

Mean progesterone levels in serum (venipuncture), whole blood (venipuncture), saliva, and capillary blood (fingerstick) during the 24-hour sampling period after progesterone cream and gel application are depicted in Figure 1A to D, and the corresponding pharmacokinetic values of progesterone are shown in Table 1. Large interindividual variability was observed in progesterone levels and pharmacokinetic parameters for both cream and gel, as evident in the interquartile ranges shown for the pharmacokinetic parameters. Baseline serum progesterone levels in the eight study participants ranged from 0.039 to 0.092 ng/mL (median, 0.059 ng/mL). After progesterone cream or gel application, serum progesterone levels rose gradually; C_{max} was a little higher but was attained at a slightly later time with cream versus gel (0.71 ng/mL at 9 h vs 0.59 ng/mL at 8 h, respectively), and $AUC_{0\text{-}24\ h}$ was significantly higher with cream (12.39 vs 8.32 ng h mL^{-1}, P = 0.0391). Repeated-measures analysis found serum progesterone to be significantly higher overall (P = 0.0001) after cream versus gel application but to have no effect on time (P = 0.30) or on the interaction between delivery method and time (P = 0.99). Progesterone levels in whole venous blood showed a profile similar to that observed in serum for both cream and gel, but were lower. The levels fluctuated widely for both delivery methods; however, the fluctuations after cream application were more pronounced. C_{max} and $AUC_{0\text{-}24\ h}$ were significantly higher (P = 0.0391 and P = 0.0156, respectively) and T_{max} was 1 hour longer with cream, although this difference was not significant. In comparison with serum, the values for C_{max} and $AUC_{0\text{-}24\ h}$ were substantially lower. Progesterone levels in whole blood were significantly higher overall

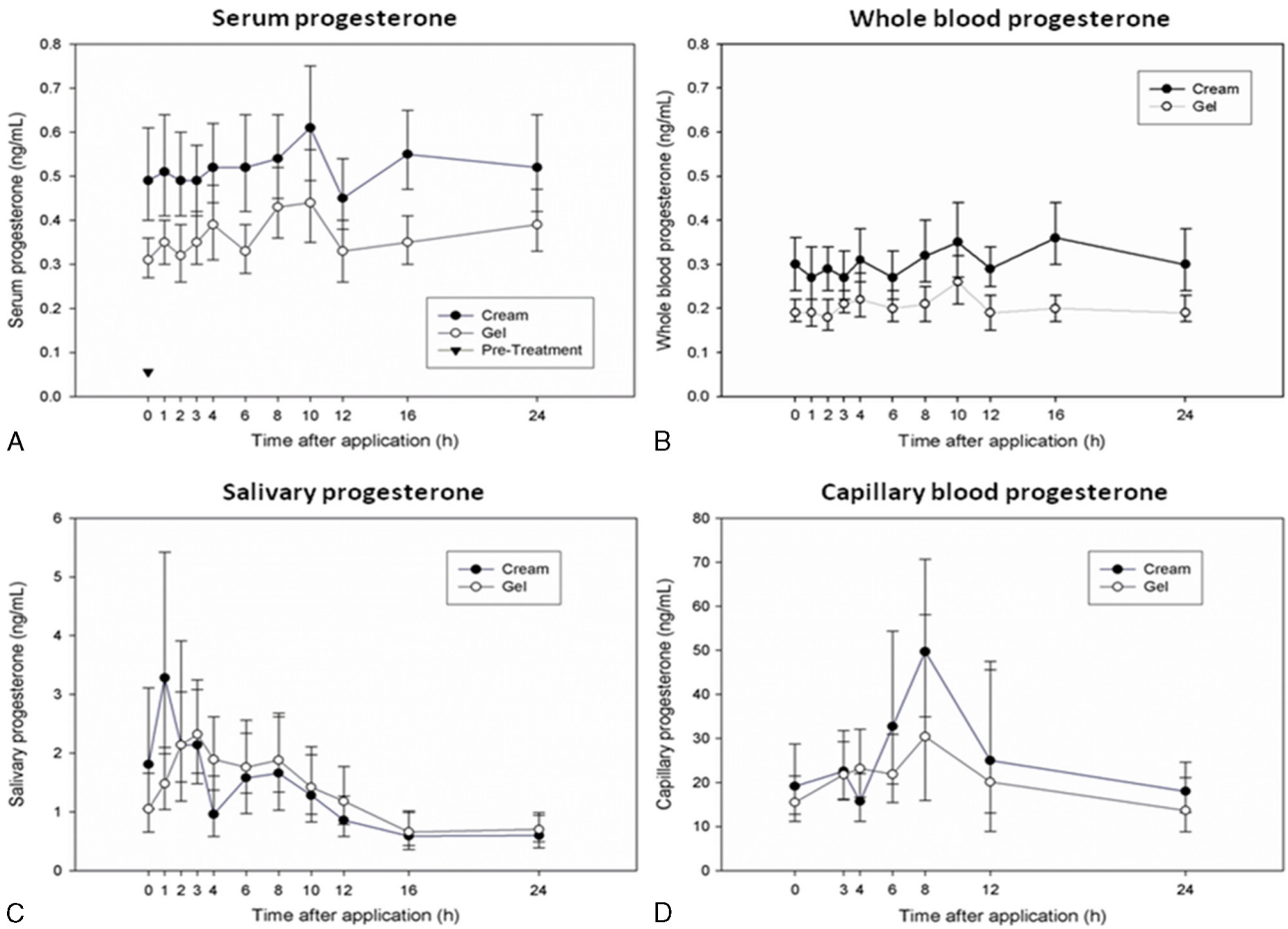

FIG. 1. Progesterone levels in serum (**A**), whole blood (**B**), and saliva (**C**) before and at 1, 2, 3, 4, 6, 8, 10, 12, 16, and 24 hours after the application of 80 mg of progesterone as cream and gel formulations. **D:** Progesterone levels in capillary blood before and at 3, 4, 6, 8, 12, and 24 hours after the application of cream and gel. Values plotted are geometric means with asymmetrical error bars, obtained by exponentiating the mean of log-transformed values (SEM). Progesterone levels in capillary blood were 100-fold higher than those seen in serum or whole blood and 10-fold higher than those seen in saliva.

TABLE 1. *Paired comparisons of cream versus gel for median pharmacokinetic parameter values of progesterone in serum, whole blood, saliva, and capillary blood*

	Cream			Gel			Cream/gel paired difference[a]	
	Median	25% percentile	75% percentile	Median	25% percentile	75% percentile	Median	*P*
Serum (n = 8)								
C_{max}, ng/mL	0.71	0.53	1.42	0.59	0.42	0.93	0.331	0.0781
T_{max}, ng/mL	9.00	5.00	14.00	8.00	3.50	10.00	1.00	0.6250
$AUC_{0\text{-}24\ h}$, ng h mL^{-1}	12.39	10.31	19.68	8.32	6.92	12.98	5.00	**0.0391**
Whole blood (n = 8)								
C_{max}, ng/mL	0.57	0.38	0.96	0.29	0.22	0.52	0.306	**0.0391**
T_{max}, ng/mL	8.00	5.00	16.00	7.00	3.00	10.00	1.50	0.2969
$AUC_{0\text{-}24\ h}$, ng h mL^{-1}	7.51	6.40	13.07	4.41	3.66	7.19	3.88	**0.0156**
Saliva (n = 7)								
C_{max}, ng/mL	8.71	4.13	12.07	7.36	2.42	8.14	2.63	0.4688
T_{max}, ng/mL	1.00	1.00	2.00	6.00	3.00	8.00	−4.00	0.0938
$AUC_{0\text{-}24\ h}$, ng h mL^{-1}	39.02	23.64	51.78	58.37	14.66	73.86	−11.97	0.6875
Capillary (n = 7)								
C_{max}, ng/mL	65.10	61.60	95.60	58.70	28.70	84.40	11.20	0.8125
T_{max}, ng/mL	8.00	1.00	24.00	8.00	4.00	12.00	2.00	0.4688
$AUC_{0\text{-}24\ h}$, ng h mL^{-1}	1,056	585	1,260	999	219	1,250	260	0.6875

[a]Values in bold show significant difference.

($P < 0.0001$) after cream versus gel application, but repeated-measures analysis found no effect of time ($P = 0.48$) or the interaction between delivery method and time ($P = 0.96$). In saliva, there was a pronounced early peak (at 1 h) of progesterone levels after cream application. A similar profile, with progesterone levels a little lower and occurring later, was observed with gel. The progesterone C_{max} was a little higher with cream than with gel (8.71 vs 7.36 ng/mL, respectively), but this difference was not significant. Thus, C_{max} was reached very early after cream application, as compared not only with gel application but also with serum and whole blood with both delivery methods. Salivary progesterone levels differed significantly over time ($P < 0.0001$), but not by delivery method ($P = 0.65$) or by the interaction between delivery method and time ($P = 0.57$). Post hoc paired comparisons between the different sampling times found progesterone levels at 16 and 24 hours to be significantly lower than progesterone levels at 1, 2, 3, and 8 hours (adjusted $P < 0.05$ for all).

Progesterone levels in capillary blood were very high, with a similar profile for cream and gel. C_{max} was reached at the same time (8 h) with both delivery methods. C_{max} was not significantly different with cream versus gel (65.1 vs 58.7 ng/mL, respectively). Although $AUC_{0\text{-}24\ h}$ was not significantly different between cream and gel (1,056 vs 999 ng h mL^{-1}, respectively), capillary blood AUC was dramatically higher than serum, whole blood, and saliva AUC with both delivery methods. Repeated-measures analysis showed nonsignificant effects for delivery method ($P = 0.22$), time ($P = 0.33$), and their interaction ($P = 0.93$).

DISCUSSION

In this study, we investigated the pharmacokinetics of the distribution of progesterone in venous blood, capillary blood, and saliva after topical application of progesterone. Our results show that, after the treatment of postmenopausal women with either progesterone cream or gel, venous serum and whole blood progesterone levels increased slightly in all women but remained below 1 ng/ml. This is consistent with findings in several studies where postmenopausal women were treated with progesterone cream at doses of 30 to 80 mg.[8,13,14,23] In these studies, serum or plasma progesterone levels were maximal at less than 1 ng/mL. Similarly, we found that progesterone levels were generally well below 1 ng/mL; the median maximal level was only 0.71 ng/mL. Other studies[9] have observed slightly higher serum progesterone levels, but these are well below the range considered as luteal (5-25 ng/mL) and are not able to counter estrogenic stimulation of target tissues. The slightly lower serum progesterone levels in our study are probably attributable to the fact that we used two purification steps to remove progesterone metabolites interfering with most commercial serum progesterone immunoassays.[24] It is well recognized that immunoassays that do not include appropriate purification steps can overestimate true values.

The serum progesterone levels achieved with the gel were slightly lower than those obtained with the cream, probably attributable to differences in absorption kinetics. In a previous preliminary study in which postmenopausal women were treated with a progesterone gel containing 100 mg of progesterone, serum progesterone levels of 5.9 to 8.0 ng/mL were observed at 2 to 3 hours after dosing.[25] The discrepancy in the findings can most probably be attributed to differences in progesterone formulations. The formulations were prepared by two different compounding pharmacies; the notable difference is that, in our previous study, alcohol was added to the carbomel (gelling substance) used to prepare the progesterone gel, whereas only water was used with carbomel in the present study.

Whole venous blood progesterone levels in women after both cream and gel application were substantially lower than the corresponding venous serum progesterone levels; the median $AUC_{0\text{-}24\ h}$ values were 7.51 and 4.41 ng h mL^{-1},

respectively, as compared with 12.39 and 8.32 ng h mL^{-1}, respectively. Our findings are consistent with those reported in another study in which whole blood progesterone levels were substantially lower than in plasma during two 3-week periods in postmenopausal women treated with 20 or 40 mg of progesterone cream twice daily.[14] This observation would suggest that progesterone carried in the bloodstream is concentrated in the plasma fraction—not the cellular fraction—of the blood. Indeed, Lewis et al[14] noted that progesterone was not present in red blood cells prepared from venipuncture serum.

The wide interindividual variability in venous serum and whole blood progesterone levels could also indicate a considerable variation in skin absorption kinetics between women. We attempted to eliminate as much of this variability as possible by having the women apply the cream or gel to the inner thigh at the same time of day using gloves. However, it is possible that differences in the use of lipophilic skin products or in bathing habits might have affected progesterone absorption and retention in the skin. Because progesterone is very lipophilic, interindividual differences in the amount of subcutaneous fat may have also affected the rate of progesterone absorption and clearance.

In contrast to the low progesterone levels found in serum and whole blood with both treatment methods, salivary progesterone levels were high and capillary blood progesterone levels were even much higher. The salivary progesterone levels observed in this study are much higher than physiological saliva levels, which range from about 75 to 270 pg/mL during the luteal phase of the menstrual cycle and from about 12 to 50 pg/mL in postmenopausal women (ZRT Laboratory reference range data). These results are consistent with those from several other studies, which showed that saliva levels increase dramatically despite serum and plasma levels changing very little after topical progesterone application.[13,14,17] Salivary levels of progesterone that exceed physiological levels after physiological or supraphysiological topical progesterone dosing have been consistently observed by multiple laboratories, and this has been interpreted to indicate that it is caused by the unique physiology of the salivary gland. Indeed, there is more blood flow to the salivary glands than to most other tissues (eg, about 10 times higher than in exercising muscle). Furthermore, there are two capillary beds in the salivary glands: one at the level of the salivary duct and the other at the acinar glandular tissue, which could result in higher transport of progesterone into saliva. The very high levels of progesterone in capillary blood are consistent with the administration of the supraphysiological doses (80 mg) of progesterone used in our study. In the laboratory of D.T.Z., clinical test results of more than 10,000 women have shown that topical administration of progesterone at physiological doses (10-30 mg) results in a physiological luteal level of capillary progesterone ranging from about 20 to 30 ng/mL. These results with clinical samples are consistent with the present study, where the median progesterone C_{max} values were 65.1 and 58.7 ng/mL after the application of 80 mg of cream and gel, respectively—100-fold higher than in venous blood.

An additional factor contributing to the high levels of progesterone in saliva after transdermal application, as observed in this study and other studies,[13,14,17] is the avoidance of first-pass metabolism by the liver. Lewis et al[14] also observed no increase in urinary progesterone or progesterone metabolites after transdermal application, suggesting that progesterone delivered transdermally has relatively little exposure to the digestive system, liver, or kidneys, and is therefore unlikely to be affected by nutritional factors and liver metabolism. In contrast, orally administered progesterone is extensively metabolized in the gut and liver, resulting in very high levels of progesterone metabolites in venous serum and urine.[24]

From a clinical perspective, there would be a very different interpretation of the serum, saliva, and capillary blood progesterone data obtained in the present study. The serum results would suggest that progesterone absorbs poorly through the skin when applied as cream or gel and that this is not a viable delivery method. In contrast, the salivary and capillary blood results show high levels of progesterone, suggesting that serum levels significantly underestimate the delivery of progesterone to tissues.

The much higher levels of progesterone observed in capillary whole blood taken from the fingertips compared with serum or venous whole blood, coupled with the high saliva concentrations, raise some questions regarding the mechanisms by which progesterone might be transported through the body after topical administration. Based on the results that progesterone could not be found in red blood cells, Lewis et al[14] suggested the possibility that progesterone may travel to the saliva via the lymphatic system. The lymphatics parallel the vascular system in blood transport, and both systems are present in the capillary beds of tissues, such as the salivary glands and fingertips. In tissue capillary beds, small molecules with molecular weight less than 600, such as steroid hormones, glucose, and oxygen, transfer by passive diffusion between capillary blood vessels, the interstitial fluid surrounding target cells, and the lymphatic vessels nearby, and are carried through the lymphatic system to other tissues, such as the salivary glands, endometrium, and breasts. Magness and Ford[26] studied estrogen and progesterone concentrations in uterine lymph and systemic blood during the estrous cycle in pigs and found that the steroid levels correlated positively in the two fluids throughout the cycle; they noted that the small molecular size of the steroids allows them to freely exchange between the two fluids via the intercellular gaps between lymphatic capillaries in the uterus. Blood taken from the fingertip consists of components from the lymphatics, the blood vasculature, and the interstitial fluid bathing these vessels, and is probably representative of fluids present in most other tissues in the body. Progesterone levels in the capillary blood that are 100-fold those seen in venous blood and peak at around 8 hours after topical progesterone administration offer support to the hypothesis that progesterone may be transported via the lymphatic system after topical administration. It is equally plausible that progesterone weakly binds to red blood cells as it enters the arterial circulation and is quickly delivered to the interstitial

fluid, where it can equilibrate with target tissues, the blood vasculature, and lymphatics.

A limitation of this study is the relatively small sample size. However, this was a randomized cross-over study, and based on the results (medians, 25th percentiles, and 75th percentiles) shown in Table 1, the number of women is sufficient to meet the objectives of the study. As stated previously, our objective was to identify a window period when absorption of progesterone, administered as cream or gel, is at steady state. Using the present data, we plan to determine in a future study whether using progesterone cream or gel with estrogen can protect the endometrium.

CONCLUSIONS

Whatever the mechanism of transport, we suggest that progesterone absorption is much more efficient than has been reported based solely on venous serum or plasma levels measured after topical application in postmenopausal women. Our findings that salivary and capillary blood levels of progesterone increase dramatically, in addition to published observations of the direct therapeutic effects of progesterone on the endometrium with topical progesterone application, confirm the distribution of progesterone to tissues despite low serum levels. Although the mechanisms for progesterone absorption, entry into the blood vasculature or lymphatics, and delivery to tissues remain unclear, we can conclude that the use of conventional venipuncture serum or plasma results in a gross underestimation of the tissue exposure and clinical efficacy of topical progesterone. We believe that topical progesterone is well absorbed and should be reconsidered as an effective means of treating clinical conditions that respond favorably to progestogens.

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