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Caution on the use of saliva measurements to monitor absorption of progesterone from transdermal creams in postmenopausal women

John G. Lewis [a,*], Helen McGill [b], Vivienne M. Patton [b], Peter A. Elder [a]

[a] *Steroid and Immunobiochemistry Laboratory, Canterbury Health Laboratories, P.O. Box 151, Christchurch, New Zealand*
[b] *Menopause and Women's Hormone Clinic, Haelan House, P.O. Box 25212, Christchurch, New Zealand*

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Abstract

Objectives: To determine the levels of progesterone in plasma, red cells and saliva as well as pregnanediol-3-glucuronide excretion in postmenopausal women using transdermal progesterone creams. *Methods:* A double-blind placebo controlled study was carried out using 24 postmenopausal women. Creams (placebo, 20 or 40 mg progesterone/g) were applied twice daily for 3 weeks followed by 1 week without before a further 3-week treatment. Morning samples were collected at 0, 1, 3, 4, 7 and 8 weeks for analysis. *Results:* There were small increases in plasma progesterone levels and pregnanediol-3-glucuronide excretion compared to the placebo group and red cell progesterone levels never exceeded plasma levels during progesterone cream use. Saliva progesterone levels were very high and variable in the progesterone cream groups compared to the placebo group and presented a paradox to the usual relationship observed between plasma and saliva progesterone in premenopausal women. *Conclusion:* The absorption of progesterone from transdermal creams is low and we caution against the use of saliva measurements to monitor progesterone absorption. The low systemic absorption of progesterone may not be due to peripheral conversion by 5α-reductase(s). We also conclude that the low level of progesterone associated with red cells suggests they are not important in the delivery of progesterone to target tissues.

Keywords: Progesterone; Postmenopause; Transdermal; Saliva; Hormone replacement therapy

1. Introduction

The use of transdermal natural progesterone creams by postmenopausal women, as alternative progestogen replacement is becoming widespread particularly as they are internationally available without prescription. Cooper et al. [1] showed that systemic progesterone absorption using Progest, a commonly available cream, is low and therefore not likely to be beneficial. There has been criticism of this study by Lee who suggests that saliva progesterone levels and red cell associated progesterone more accurately reflect transdermally delivered bioavailable progesterone [2].

* Corresponding author. Tel.: +64-3-3640-877; fax: +64-3-3640-889.
E-mail address: john.lewis@cdhb.govt.nz (J.G. Lewis).

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We investigated his controversial hypothesis because many laboratories use saliva progesterone testing to monitor the absorption of progesterone from transdermal creams. We carried out a double-blind placebo-controlled study and measured progesterone in saliva, red cells, plasma and urine pregnanediol in postmenopausal women using transdermal progesterone creams.

2. Materials and methods

2.1. Study design

Ethical approval was obtained and 24 consenting normal postmenopausal women were recruited (age range 43–66 years). They were non-smokers, at least 2 years menses free and had not taken HRT within the previous 3 months. They were randomly assigned into three treatment groups and used creams containing progesterone either 20 or 40 mg/g, or placebo. Creams were compounded by Pharmaceutical Compounding NZ Ltd., Auckland, NZ and supplied in lidded, coded glass jars together with a measuring device. The study lasted 2 months and participants were asked to apply 1/2 spoon morning and evening (approx. 1–2 g cream daily) for 3 weeks followed by 1 week without cream. Cream was then applied for a further 3 weeks and finally stopped. Participants applied the cream to areas of thinner skin, inner arms, thighs, upper chest and abdomen avoiding the neck and face.

2.2. Samples

Morning samples of blood, urine and saliva were collected at weeks 0 (basal), 1, 3, 4, 7 and 8. Sample containers and strict sampling instructions were provided for the collection of early morning urine and saliva samples. Samples were collected before cream application that morning, to minimize possible contamination. Blood (5 ml, EDTA) was drawn later the same morning. PCV was measured, plasma separated and plasma, unwashed red cells, urine and saliva stored at –20 °C.

2.3. Steroid analysis

Saliva, lysed red cells and plasma were assayed for progesterone following extraction in hexane. Progesterone was analyzed by ELISA using a monoclonal antibody [3]. Urinary pregnanediol-3-glucuronide analysis was by ELISA and creatinine was also determined to calculate excretion [4]. Analysis was in batches, each containing a complete set of samples from participants using the three creams as well as quality control material. After sample analysis the code was broken.

3. Results and discussion

Twenty-two of the participants completed the study, two using the 20 mg/g cream discontinued following the second and fourth samples. Participants using progesterone creams showed a small but significant increase in plasma progesterone during weeks of cream use compared to the placebo group (Table 1). There was also a small increase in pregnanediol-3-glucuronide excretion reaching significance at weeks 1 and 3 for the higher dose progesterone cream. Even though the daily doses of transdermal progesterone approximated daily peak luteal phase production we confirm previous findings that the systemic absorption of transdermal progesterone is low [1]. Progesterone absorption and excretion does not approach levels observed during the luteal phase of the cycle (>15 nmol/l for plasma progesterone and >6 μmol/24 h for pregnanediol-3-glucuronide) [4]. As such we also consider it unlikely that these doses of transdermal progesterone would offer any benefit in terms of endometrial protection or aid in the conservation of bone in postmenopausal women. This conclusion is also drawn by Wren [5] where administration of transdermal progesterone cream (up to 64 mg/day) for 14 days showed no induction of a secretory pattern in the endometrium and plasma progesterone levels were <3.2 nmol/l. Another study examining bone loss in postmenopausal women concluded that use of transdermal progesterone cream did not protect against bone loss although vasomotor symptoms improved [6]. Progesterone

absorption from transdermal creams was also studied by Burry et al. [7] who concluded that the application of 60 mg progesterone per day for 2 weeks following a lower daily progesterone dose raised plasma progesterone to a mean level of 7.3 nmol/l which they conclude reaches luteal phase levels. A more recent study by Carey et al. [8] in postmenopausal women using two different regimens of 40 mg transdermal progesterone/day showed a small rise in serum progesterone and urine pregnanediol.

Table 1
Progesterone levels in saliva, plasma and red cells and urine pregnanediol-3-glucuronide excretion normalized to a creatinine excretion of 10 mmol/24 h

Week	Cream 20 mg/g	Cream 40 mg/g	Placebo
Salivary progesterone (nmol/l)			
0	0.25 ± 0.12	0.45 ± 0.24	0.43 ± 0.21
1	**82.11 ± 104.52***	**59.71 ± 65.60***	**0.36 ± 0.21**
3	**36.80 ± 56.59***	**38.23 ± 38.41***	**0.36 ± 0.21**
4	2.07 ± 2.40*	6.05 ± 5.48*	0.34 ± 0.21
7	**34.77 ± 39.67***	**44.88 ± 38.63***	**0.36 ± 0.21**
8	11.75 ± 20.89	5.00 ± 5.56	0.38 ± 0.20
Plasma progesterone (nmol/l). Mean ± S.D.			
0	0.39 ± 0.43	0.59 ± 0.56	0.54 ± 0.42
1	**1.33 ± 0.85***	**3.53 ± 1.71***	**0.53 ± 0.38**
3	**1.38 ± 0.74***	**2.60 ± 1.20 #**	**0.43 ± 0.23**
4	0.81 ± 1.07	0.71 ± 0.62	0.35 ± 0.20
7	**1.77 ± 1.63***	**1.48 ± 0.87***	**0.34 ± 031**
8	0.32 ± 0.31	0.69 ± 0.94	0.39 ± 0.29
Urine pregnanediol-3-glucuronide (μmol/24 h). Mean ± S.D.			
0	0.66 ± 0.33	0.82 ± 0.49	0.89 ± 0.43
1	**1.48 ± 0.95**	**1.87 ± 0.89***	**1.03 ± 0.46**
3	**1.41 ± 0.59**	**1.89 ± 1.14***	**1.07 ± 0.19**
4	0.81 ± 0.62	1.34 ± 0.95	0.90 ± 0.26
7	**1.24 ± 0.54**	**1.55 ± 0.83**	**0.99 ± 0.37**
8	0.82 ± 0.44	1.15 ± 0.76	1.05 ± 0.30
Red blood cell progesterone (nmol/l). Mean ± S.D.			
0	0.55 ± 0.19	0.70 ± 0.52	0.53 ± 0.24
1	**0.80 ± 0.34**	**0.86 ± 0.25**	**0.54 ± 0.29**
3	**0.69 ± 0.25**	**0.43 ± 0.28**	**0.40 ± 0.24**
4	0.43 ± 0.24	0.56 ± 0.54	0.35 ± 0.22
7	**0.42 ± 0.12**	**0.56 ± 0.52**	**0.40 ± 0.29**
8	0.45 ± 0.21	0.80 ± 0.80	0.40 ± 0.17

Values are mean ± S.D. with units as indicated.

* $P<0.05$ compared to placebo group; # $P<0.0001$ compared to placebo group. Bold figures denote samples obtained during cream use. All analysis Student's t test.

More importantly, in our study, saliva levels show very high and variable progesterone levels and present a paradox to the normal relationship between saliva and plasma progesterone documented in pre-menopausal women [9]. According to Lee [2] the high saliva levels in postmenopausal women using transdermal progesterone creams reflect the bioavailable hormone and the lipophilic membrane of red blood cells is the primary progesterone transport mechanism to target tissues. This would appear unlikely. Red cell progesterone levels are negligibly low in all treatment groups and never exceed plasma levels during progesterone cream use. In vitro studies using supra-physiological doses of progesterone show that up to 15% remains associated with the red cells even following cellular washing [10]. The authors equate this similarly to the role of albumin in steroid transport mechanisms. In our study the low levels detected in the red cell fraction cannot account for the high saliva levels. The low plasma progesterone and even lower red cell levels together with low urine pregnanediol-3-glucuronide excretion present problems in accounting for the high saliva levels. We attempted to address this question using small pilot studies in one of us. Fig. 1 shows the time course of saliva, plasma, red cell progesterone in following the application of 40 mg of transdermal progesterone followed by a further 40 mg after 12 h. Salivary progesterone peaked 10 h after the first dose and rapidly declined to basal levels, even after the second transdermal dose. The absence of a second dose saliva progesterone peak or maintenance of the high saliva progesterone levels is of interest and could indicate spurious progesterone entry during previous saliva sampling. This is in contrast to plasma levels that rose to 4 nmol/l and did not decline. Red cells had even lower progesterone levels and pregnanediol-3-glucuronide excretion was similarly low. These results are consistent with Table 1. In this time course experiment stringent attempts were taken to minimize spurious entry of progesterone into saliva. Cream was applied using two layers of surgical gloves. Saliva sampling was also carried out using freshly gloved hands. However, we can not rule out the possibility of spurious entry unless an aspiration

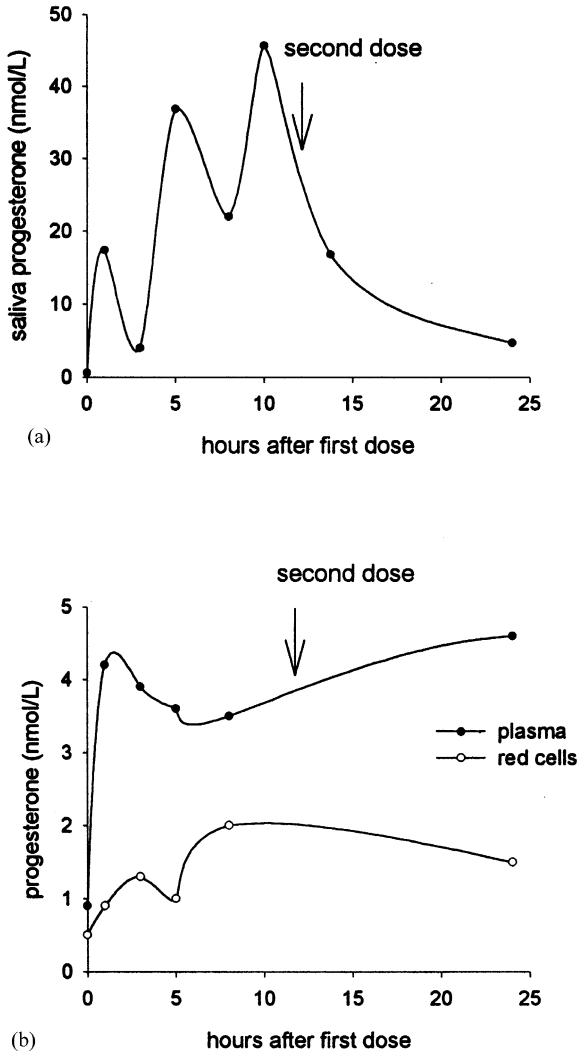


Fig. 1. Progesterone levels in saliva (a) and plasma and red cells (b) after two doses each of transdermal progesterone (40 mg) at 0 and 12 h.

technique is used for saliva collection thereby avoiding any lip contact with the sampling container. We addressed this issue in another study where a single dose of progesterone cream (40 mg progesterone) was applied by another person and saliva sampling was carried out by an aspiration technique and using freshly gloved hands for all procedures. Despite these precautions saliva progesterone rose to levels observed previously suggesting that spurious entry is unlikely (Fig. 2). However, the actual mechanism of transdermal progesterone entry into saliva is still unknown and is the subject of future investigation. Our study rules out spurious entry as well as red cell transport. A recent investigation [11] found high saliva progesterone levels following a single dose of progesterone cream in postmenopausal women with no significant changes in serum progesterone and urine pregnanediol and suggests, although unproven, that progesterone may enter saliva via the lymph.

The paradox between high and variable saliva progesterone levels and low systemic absorption necessitates caution on the use of saliva progesterone assays for monitoring transdermal progesterone cream therapy. It is known that the metabolic activity of saliva is low [12] and it would therefore seem unlikely that conversion to a unique cross-reacting metabolite could account for the high progesterone levels in saliva. Furthermore, as the same antibody has been used for all the progesterone assays it would be expected that other cross-reacting substances should be equally recognized. Like others [11], we also confirmed by HPLC, in selected representative saliva samples, that levels of progesterone measured by our ELISA corroborated with an independent

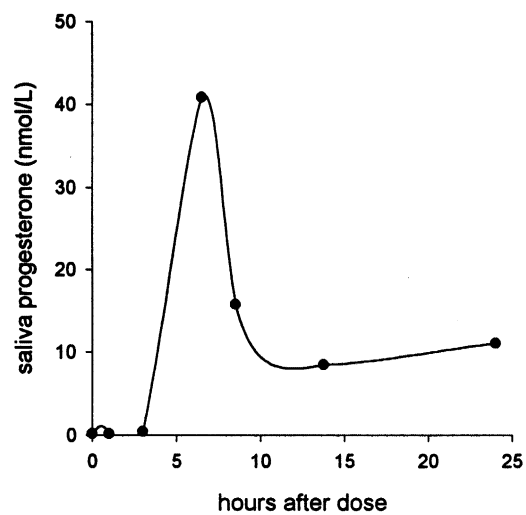
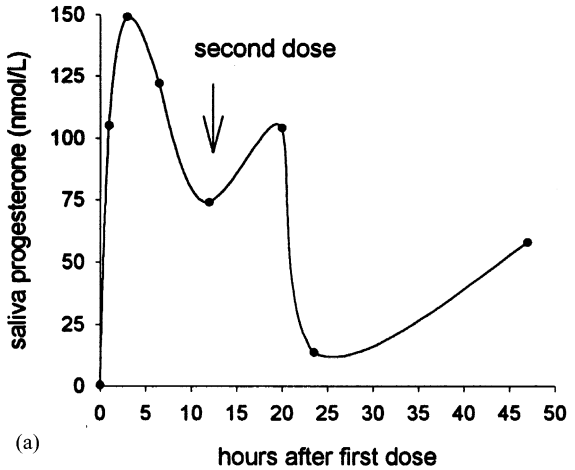
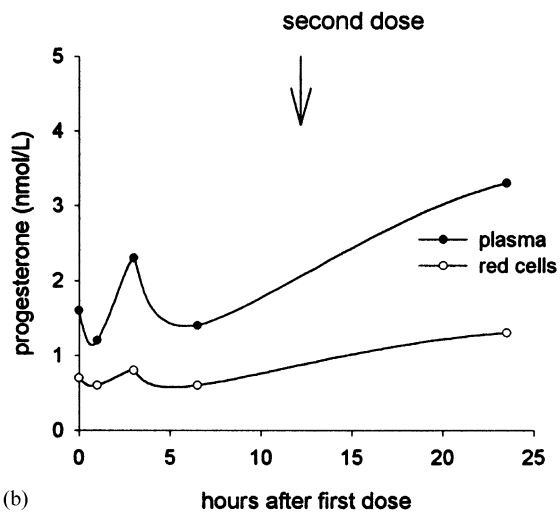


Fig. 2. Progesterone levels in saliva after a single dose of transdermal progesterone (40 mg) at 0 h. Saliva sampling was by aspiration.



(a)



(b)

Fig. 3. The effect of prior and continued exposure to finasteride (5 mg/day over 9 days) on progesterone levels in saliva (a) and plasma and red cells (b) after two doses each of transdermal progesterone (40 mg) at 0 and 12 h.

method. We would suggest that if non-invasive sampling is deemed necessary then pregnanediol-3-glucuronide excretion may seem the preferred option although higher dose progesterone creams or alternative delivery systems would presumably be needed to achieve useful luteal phase levels.

Table 2
Urine pregnanediol-3-glucuronide excretion based on 24-h collection on a volunteer before and following topical progesterone cream (approx. 80 mg/day) and before and following finasteride

No finasteride		Finasteride	
Hours	P-3-G (μmol/24 h)	Hours	P-3-G (μmol/24h)
0 basal	0.71	0 basal	0.65
24	1.01	24	2.11
48	1.36	48	2.09

It is possible that transdermally delivered progesterone is a substrate for peripheral 5α-reductase [13] and conversion to 5α reduced progestins may be a significant factor contributing to low systemic progesterone levels and pregnanediol-3-glucuronide excretion. This question could be resolved by the use of 5α-reductase inhibitors, such as finasteride, prior to transdermal cream use. To investigate this hypothesis one of us ingested finasteride (5 mg daily) for 9 days. Progesterone cream (40 mg/g) was applied twice on day 8 (80 mg progesterone) using the protocol described. Salivary progesterone peaked after 1 h but both plasma and red cell progesterone remained low throughout (Fig. 3). This together with low urine pregnanediol-glucuronide excretion suggests that conversion of progesterone by 5α-reductase(s) is an unlikely mechanism to account for low systemic progesterone levels (Table 2).

In conclusion we confirm that the systemic absorption of transdermal progesterone is low, using common dosage regimens, and suggest that low absorption may not be due to peripheral conversion by 5α-reductase(s). We also caution against the use of salivary progesterone measurements to monitor such therapy and show that red cell progesterone levels and spurious entry are unlikely to account for the high and variable levels of progesterone measured in saliva samples during cream use. In view of these findings we would therefore exercise caution on the use of saliva progesterone assays for monitoring transdermal progesterone cream therapy.

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References

[1] Cooper A, Spencer C, Whitehead MI, et al. Systemic absorption of progesterone from Progest cream in postmenopausal women. Lancet 1998;351:1255–6.
[2] Lee JR. Use of Pro-gest cream in postmenopausal women. Lancet 1998;352:905.
[3] Elder PA, Gaynor PT, Lewis JG, et al. Generation of monoclonal progesterone antibodies by electrofusion techniques. Bioelectrochem Bioenerg 1997;43:35–40.
[4] Lewis JG, Manley L, Whitlow JC, et al. Re-examining steroid hormone metabolites as ovulation markers using monoclonal antibodies. Steroids 1994;59:288–91.
[5] Wren BG, McFarland K, Edwards L. Micronised transdermal progesterone and endometrial response. Lancet 1999;354:1447–8.
[6] Leonetti HB, Longo S, Anasti JN. Transdermal progesterone cream for vasomotor symptoms and postmenopausal bone loss. Obstet Gynecol 1999;94:225–8.
[7] Burry KA, Patton PE, Hermsmeyer K. Percutaneous absorption of progesterone in postmenopausal women treated with transdermal estrogen. Am J Obstet Gynecol 1999;180:1504–11.
[8] Carey BJ, Carey AH, Sanjaykumar P, et al. A study to evaluate serum and urinary hormone levels following short and long term administration of two regimens of progesterone cream in postmenopausal women. Br J Obstet Gynaecol 2000;107:722–6.
[9] Wang DY, Knyba RE. Salivary progesterone: Relation to total and non-protein-bound blood levels. J Steroid Biochem 1985;23:975–9.
[10] Koefoed P, Brahm J. The permeability of the human red cell membrane to steroid hormones. Biochim Biophys Acta 1994;1195:55–62.
[11] O'Leary P, Feddema P, Chan K, et al. Salivary, but not serum or urinary levels of progesterone are elevated after topical application of progesterone cream to pre- and postmenopausal women. Clin Endocrinol 2000;53:615–20.
[12] Laine MA, Ojanotko AO. Progesterone metabolism in human saliva in vitro. J Steroid Biochem Mol Biol 1999;70:109–13.
[13] Li X, Chen C, Singh SM, et al. The enzyme and inhibitors of 4-ene-3-oxosteroid 5α-oxidoreductase. Steroids 1995;60:430–41.