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Assessment of bioavailability of oral micronized progesterone using a salivary progesterone enzymeimmunoassay

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Key words: MICRONIZED PROGESTERONE, BIOAVAILABILITY, SALIVARY STEROIDS, ENZYMEIMMUNOASSAY, POSTMENOPAUSE

ABSTRACT

Salivary progesterone was measured sequentially by enzymeimmunoassay following 1 month and 6 months of oral therapy with 100 mg of micronized progesterone (MOP) in 40 healthy estrogenized postmenopausal women (aged 40–68 years). MOP was administered for 23 days every month.

There were striking differences in the absorption of MOP between various subjects. Significant increases occurred in salivary progesterone concentrations over baseline and pretreatment levels and persisted for at least 10 h. Levels of salivary progesterone remained higher than pretreatment levels for at least 24 h after administration of MOP. Maximum mean concentrations of salivary progesterone of 827.2 and 888 pmol/l in the 1st and 6th months of therapy, respectively, were achieved within 2 h of administration and were above the 95th percentile of a control corridor which corresponds to the range found in the luteal phase. The areas under the salivary progesterone curve ($AUC_{0-24\,h}$, pmol/l) were 7177.75 and 7388.20 respectively, in the 1st and 6th months of therapy but the difference was not statistically significant. Serum and salivary progesterone peaked simultaneously and there was a significant correlation between the concentrations measured concurrently ($y = 233.08 + 35.575x$; $r = 0.89$, $p < 0.001$) thus supporting the current concept of a relatively rapid diffusion of steroids from plasma to saliva.

Results of this study confirm those of previous investigations which monitored the bioavailability of MOP with the use of serum progesterone measurements and showed that luteal phase progesterone concentrations can be attained easily. The use of non-invasive salivary sampling and a cost-effective, direct enzymeimmunoassay showed a considerable advantage in the present study, compared with previous ones. We conclude that 100 mg MOP should be given at least twice-daily to maintain a stable physiological luteal phase level of progesterone during clinical hormone replacement therapy.

INTRODUCTION

The oral route of administration of natural progesterone has not been practical because of its rapid hepatic metabolism, short biological half-life and poor bioavailability[1,2]. It is not surprising, therefore, that 19-nortestosterone and C-21 synthetic derivatives have been the only orally active progestational agents widely available.

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Interest in an orally active form of natural progesterone has increased recently in the hope that the problems experienced with synthetic progestogens may be avoided or reduced. Recent reports suggest that two galenic processes which have synergistic effects – micronization and addition of long-chain fatty acids[3] – enhance absorption and bioavailability of progesterone. Micronization of progesterone facilitates increased aqueous dissolution in the small intestine[1] which increases the percentage of steroid absorbed by the lymphatic capillary system and therefore decreases the influence of enzymic activity[1–4]. Addition of fatty acid stabilizes the resultant particle.

Previous studies of progesterone absorption used one or more of the following as an end-point: withdrawal bleeding in amenorrheic women, urinary level of pregnanediol, distribution of isotopically labelled progesterone or secretory transformation of proliferative endometrium. More recently, measurements of both free and bound plasma steroids have been used in studies of postmenopausal women[2,4,5]. These approaches have some disadvantages. The former are indirect measurements, whereas serial blood and endometrial sampling are invasive and stressful, and may be considered impractical for pharmacokinetic studies. We investigated an alternative approach of measuring progesterone in saliva. We previously described the development and clinical validation of a specific, sensitive, and rapid enzymeimmunoassay for salivary progesterone and estrone on microtiter plates[6–11]. The non-invasive and stress-free nature of saliva sampling facilitates multiple sampling and is therefore very suitable for pharmacokinetic studies. Here we describe the application of salivary analysis to monitor the bioavailability of micronized oral progesterone in estrogenized postmenopausal women.

MATERIALS AND METHODS

Subjects

Forty healthy postmenopausal women (aged 40–68 years), as judged by amenorrhea of at least 1 year, and who required hormone replacement therapy for relief of symptoms or for prophylactic purposes, were recruited from the Climacteric Research Clinic of University College Hospital, Galway. Serum plasma levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol reflected their postmenopausal status (i.e. FSH > 40 mIU/l, FSH : LH ratio > 1 and estradiol < 40 pmol/l). Mean age and mean body mass index (BMI) were 53.5 and 25.6 years, respectively. One male patient (aged 34 years) and two women with regular periods (aged 30 and 31 years) were included for comparison. Exclusion criteria eliminated patients with a known sensitivity to progesterone, 75 years of age or older, previous hysterectomy, history of breast cancer or other hormone-sensitive neoplasia, severe hypertension and submucosal fibroids. All patients had normal liver function test results and normal hematological profiles including serum folate and vitamin B_{12} levels, thus excluding gross malabsorption problems.

Thirty-seven patients had never received any hormone replacement therapy but three patients had received treatment for less than 6 months and each had had a 3–6-month wash-out period prior to enrolment in the study. Approval for the study was obtained from the National Drugs Advisory Board (Ireland) and The Ethics Committee of University College Hospital, Galway. All patients gave written informed consent. All investigations conformed with the ethical standards laid down in the Helsinki Declaration (1964) as revised at Tokyo (1975).

Hormone therapy

All postmenopausal women received a combination of estrogen and micronized progesterone. The estrogen preparation comprised 0.625 mg of conjugated equine estrogen (Premarin®; Wyeth Laboratories, Dublin, Ireland) containing estrone (50%), equilin (25%), and 17α-dihydroequilin (15%) and miscellaneous estrogenic conjugates (10%), all as sulfates, administered orally, daily, for 365 consecutive days. Micronized oral progesterone (MOP; Utrogestan®, Laboratoire Besins Iscovesco, Paris), 100 mg daily, was administered for the first 23 days of every calendar month, in addition to estrogen, and both tablets were ingested in the evening before patients retired to bed. The active component of the progesterone consists of 100 mg micronized progesterone with a mean particle diameter of 10 μm and excipient (soya bean lecithin and arachis oil), encapsulated in gelatin. Minimal drug migration occurs into this

capsule[12]. The premenopausal and male patients received only one dose of 100 mg MOP. Results of studies on the clinical efficacy of this novel regimen will be published separately.

PROTOCOL

Each patient had pretreatment pelvic ultrasonography and provided serum samples for LH and FSH determination. Saliva was collected for 3 consecutive days for progesterone and estrone measurements. Serum LH and FSH measurements were repeated at the 6th, 9th and 12th months and salivary progesterone and estrone after the 1st and 6th months of treatment.

Saliva and serum sampling

Every patient received written and verbal instructions explaining the salivary sampling procedure. After an overnight fast and between 07.00 and 10.00 each volunteer rinsed her mouth with water, rested for 5 min and collected 2–5 ml of unstimulated saliva into a 5-ml polystyrene tube over a period of about 10–15 min. Salivary samples were obtained for progesterone and estrone assays at the end of the progesterone treatment (day 22 or 23) in cycles 1 and 6, i.e. after 1 and 6 months of treatment, respectively. On these occasions, subjects were instructed to delay taking both of the aforementioned tablets from the evening of the second day before the visit to the clinic until the following morning (a day before the clinic visit). A series of appropriately labelled polystyrene bottles (50 × 44 mm) were provided and after an overnight sleep, patients collected saliva samples in their homes immediately before ingestion of their tablets and at the following times before ingestion: 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 h (Figure 1). A subset of 15 patients produced samples 24 h after ingestion of tablets. These volunteers were fasting at the onset of the study but were allowed to eat 4 h after the administration of progesterone and conjugated equine estrogen. Saliva samples were obtained at least 30 min after any eating or drinking episode to avoid contamination of samples by food constituents. The patients were ambulatory but were not allowed to partake in any strenuous exercise during the course of sampling. Regularly cycling women had their test treatment on the 6th day of their menstrual cycle. Saliva samples were immediately stored in the subject's domestic freezers until brought to the hospital, where samples were stored at −20 °C.

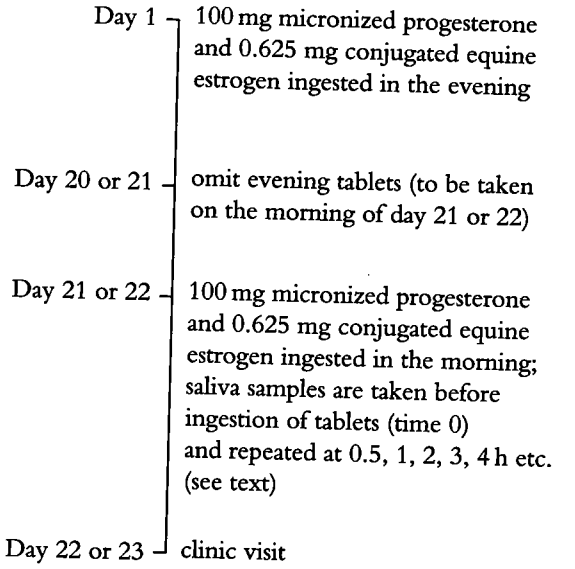

Figure 1 Flow chart for the administration of trial drugs and timing of saliva sampling

A subset of five patients provided timed, matched serum/saliva samples over the aforementioned time sequence in the 1st month of treatment in the hospital. An 18-gauge intravenous cannula (Intraflon 2, Trocart catheter I.V. Teflon, Vygon, Medical Produkte Aachen, Germany) was inserted into the antecubital vein at the onset and kept *in situ* for venipuncture until the completion of the sampling process. On each occasion 10 ml blood samples were collected in plain bottles and centrifuged immediately. Serum and saliva samples were stored at −20 °C until analyzed.

LABORATORY INVESTIGATIONS

Biochemical analysis

LH and FSH were measured with commercial kits (Delfia™, LKB Wallac, Croydon, UK) by solid phase, two-site fluoroimmunoassay based on the direct sandwich technique using a dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA). Intra- and inter-assay coefficients of variation were within the standard curve, with values of 3–5% and 5.5–7%, respectively.

HORMONE ASSAYS

Salivary progesterone was measured by solid-phase enzymeimmunoassay on microtiter plates using horseradish peroxidase–progesterone conjugate[6,8,10,11]. Serum progesterone was also measured by solid-phase enzymeimmunoassay after extraction with petroleum ether. Salivary estrone was measured after solvent extraction by solid-phase enzymeimmunoassay on microtiter plates using horse radish peroxidase conjugate[7,11].

Serum estradiol was assayed by a double antibody ^{125}I radioimmunoassay supplied in kit form by Diagnostic Product Corporation, Los Angeles, CA. Between-batch coefficients of variation were < 8% at levels of 70 pmol/l and < 6% at levels of 290 and 830 pmol/l. Serum estradiol levels of < 40 pmol/l constituted one of the entry criteria.

Progesterone analysis was given priority and estrone was analyzed only when sufficient saliva (at least 2 ml) was available. Salivary sampling was complete (i.e. samples in the 1st and 6th months of the treatment cycle) in 30 patients but samples from only 25 of these postmenopausal women were considered in the analysis. Salivary progesterone measurement from five patients were not included because of inappropriate time-intervals during serial sampling or suspected contamination. The five subjects who were excluded provided saliva samples 15–30 min outside the range of the specified time-intervals. One patient continued sampling into the 3rd day and the saliva from another was colored, probably because of dissolution of the tablets in her mouth after sampling. The level of progesterone in this latter subject exceeded 10^6 pmol/l. The mean of the three pretreatment determinations of salivary progesterone and estrone was used for analysis and only one salivary sample was used subsequently.

Control corridor for salivary progesterone concentrations

Normal ranges of salivary progesterone were previously determined by us in 41 regularly cycling females, aged between 20 and 39 years, who provided daily saliva samples for one complete single ovulatory cycle[8]. The day of maximum preovulatory follicular diameter (19.5 ± 2.0 mm, mean ± SD), determined ultrasonographically, was designated as day zero. This defined the onset of the luteal phase and all data were then normalized around this day. A nonparametric approach was adopted in the establishment of normal ranges and the 5th and 95th percentiles were extracted from these data. Each of the completed salivary progesterone profiles in the present study was assessed against the normal range of the menstrual cycle as described. Assessment of the mean concentration of progesterone of the subjects in the present study was made by comparison with the normal luteal phase-range of daily progesterone concentrations corresponding to the 5th and 95th percentiles of the control population[10,11].

Statistics

To identify the appropriate statistical analysis, the distribution of estrone and progesterone concentrations (with and without log transformation) for every time-point in the 1st and 6th treatment cycles was tested for normality using the Kolmogorov-Smirnov one-sample test for goodness of fit with use of a standard normal distribution (Table 1). Before transformation, the distribution significantly deviated from a normal or Gaussian distribution ($p < 0.05$, two-tailed test at the 5% significance level) on 3 out of the total 10 time-points in the first and second set of progesterone data (Table 1). Deviation occurred in three and one time-points in the first and second estrone dataset, respectively (data not shown). Log transformation improved the normality of the distributions on all the data for progesterone concentration but did not improve the distribution for estrone. This transformation has made it possible to use parametric statistics for the analysis of progesterone data and a nonparametric approach for estrone data analysis. The handling of these data was facilitated by SYSTAT and CRICKET 1.2 Apple Macintosh® computer applications.

Conventional methods were used to calculate the logarithm of the mean, standard deviation and standard error of the mean (SEM). The corresponding antilogarithms, which are equivalent to geometric mean, geometric standard deviation and geometric SEM, were calculated and used for analysis of results. The mean salivary progesterone concentrations presented in the results section therefore represent geometrical means. The parametric data were analyzed with Student's *t*-test and Pearson's correlation analysis. The data for estrone

Table 1 Salivary progesterone concentrations (pmol/l) at the end of the first treatment cycle after oral administration of 100 mg micronized progesterone and 0.625 mg conjugated equine estrogen. Means and standard deviations (SDs) are given before and after log transformation of data. The distribution of the data was tested for normality by the Kolmogorov–Smirnov test for goodness of fit (with Lilliefors modification) both before and after transformation. For each case, the two-tailed level of significance (p value) is shown, which is the probability of obtaining such a sample from a population having a Gaussian (normal) distribution. A p value of < 0.05 is evidence that the population is abnormal

		Untransformed data										*Log-transformed data*				
Time			*Percentiles*				*Mean*	*Skew-*		*KS*†		*Mean*	*Skew-*		*KS*†	
(h)	*No.**	*Median*	*5th*	*10th*	*90th*	*95th*	(SD)	*ness*	*Kurtosis*	*statistic*	*p*	(SD)	*ness*	*Kurtosis*	*statistic*	*p*
0.0	25	182	75	102	538	972	395 (651)	3.746	14.106	0.308	< 0.001	5.468 (0.886)	0.979	1.048	0.137	0.256
0.5	26	782	156	177	1630	2756	994 (861)	1.420	1.678	0.178	0.034	6.514 (0.981)	−0.483	−0.456	0.113	0.530
1.0	24	963	189	213	2007	2478	1141 (912)	0.985	0.550	0.135	0.308	6.654 (1.003)	−0.565	−0.601	0.112	0.619
2.0	26	1011	208	305	1784	2516	1111 (895)	1.737	3.776	0.149	0.141	6.718 (0.816)	−0.210	−0.796	0.120	0.424
3.0	24	699	129	211	1349	1972	814 (631)	1.344	1.758	0.135	0.311	6.406 (0.883)	−0.318	−0.747	0.099	0.856
4.0	25	572	87	100	1300	1399	637 (449)	0.517	−0.859	0.123	0.423	6.116 (0.953)	−0.652	−0.854	0.203	0.010
6.0	26	389	96	102	792	1498	472 (392)	1.337	1.359	0.148	0.148	5.811 (0.903)	−0.277	−0.909	0.136	0.240
8.0	26	325	79	103	648	1088	388 (290)	0.972	0.337	0.146	0.161	5.654 (0.868)	−0.455	−0.594	0.147	0.155
10.0	26	308	81	83	644	908	345 (261)	1.048	0.135	0.165	0.066	5.555 (0.812)	−0.225	−0.834	0.121	0.414
12.0	25	234	64	88	618	879	304 (298)	2.018	3.734	0.294	< 0.001	5.384 (0.804)	0.348	−0.400	0.141	0.222

*Number of patients (from total of 30) from whom data were derived; †KS = Kolmogorov–Smirnov statistic

were expressed as nonparametric statistics for 'central tendency' (median), variability (percentiles) and analyzed for significance by the Mann–Whitney U test. The difference was considered significant where $p < 0.05$. The area under the curve was calculated by the trapezoidal method. This method approximates the curve to a set of straight lines connecting the data points, then sums the areas of the trapezoids formed. The calculation was facilitated by MULTIFIT 2.0 (Milton, Cambridge, UK) curve-fitting software for the Apple Macintosh computer.

RESULTS

Mean pretreatment concentration of serum FSH and LH decreased by 35 and 15% respectively during the 12 months of treatment. A similar degree of gonadotropin suppression by hormone replacement therapy was described previously[13,14] and confirms compliance with treatment in the present study.

Salivary progesterone profiles from three postmenopausal women after 1 month's treatment with MOP and conjugated equine estrogen, and from a regularly cycling female and a male volunteer following only one course of MOP are shown in Figure 2. Wide interpatient differences in absorption of progesterone are shown by the variation in the level of maximal concentration (C_{max}) and time taken to achieve this level (T_{max}) following administration of tablets. One patient attained her peak after 1 h, two after 2 h and all five patients 3 h after administration. Values for C_{max} were 2631 pmol/l in the male volunteer (IIB), 1213 pmol/l in the regularly cycling woman (SSB) and ranged from 963 to 4312 pmol/l in the three postmenopausal women who received concomitant estrogen

therapy. Similar interpatient variability patterns were observed for salivary estrone. Similar differences in the rate of absorption of estrone and progesterone were detected in the aforementioned subjects using the same dose at different treatment cycles. In some instances, however, there was good reproducibility between the profiles at the 1st and 6th months of treatment.

All subjects exhibited a statistically significant elevation of progesterone over the baseline concentrations which persisted for 8 and 10 h at 1 and 6 months' treatment, respectively (Table 2) and which returned to baseline 24 h after administration. After 1 month's therapy the baseline concentration was 237 pmol/l, a rise of more than 50% from the pretreatment level of 105.6 pmol/l. The peak concentration was 827.2 pmol/l, which is about a fourfold increase from the baseline concentration, and an eightfold increase from the pretreatment level. This increase was achieved 2 h (T_{max}) after administration. The concentration decreased to 134 pmol/l after 24 h, which is lower than the baseline but significantly higher than the pretreatment level (Table 2). After 6 months' therapy the mean peak concentration increased to 888 pmol/l, from a baseline of 186 pmol/l, and decreased

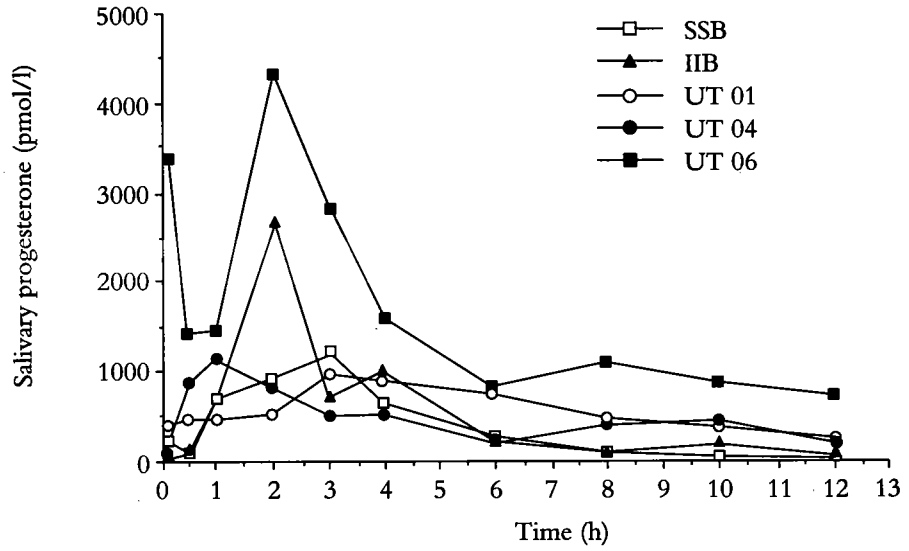

Figure 2 Progesterone concentration after oral administration of micronized progesterone at time zero in one male volunteer (IIB), one female premenopausal (SSB) and three estrogenized postmenopausal women (UTs)

Table 2 Salivary progesterone concentrations (pmol/l, geometric mean values) after oral administration of progesterone in estrogenized postmenopausal women. The baseline was compared with the pretreatment progesterone concentration and concentrations at other time-intervals after ingestion of tablets

	1st month of treatment			*6th month of treatment*		
Time (h)	*Progesterone*	*t**	*p*	*Progesterone*	*t**	*p*
Pretreatment	105.6	—	—	105.6	—	—
0	236.9	−3.349	0.005	185.7	−1.882	0.092
0.5	674.5	−5.014	< 0.001	618.9	−7.372	< 0.001
1	775.8	−5.288	< 0.001	868.7	−7.155	< 0.001
2	827.1	−6.625	< 0.001	888.0	−7.707	< 0.001
3	605.4	−5.564	< 0.001	656.6	−7.003	< 0.001
4	453.9	−3.229	< 0.001	504.7	−5.770	< 0.001
6	333.9	−1.507	0.145	361.4	−4.549	< 0.001
8	285.4	−0.766	0.451	349.7	−4.640	< 0.001
10	258.5	−0.183	0.856	280.3	−2.764	0.012
12	217.9	1.005	0.325	188.1	−0.013	0.990
24	134.2	1.473	0.179	130.3	2.336	0.052

* = Student's *t* statistic

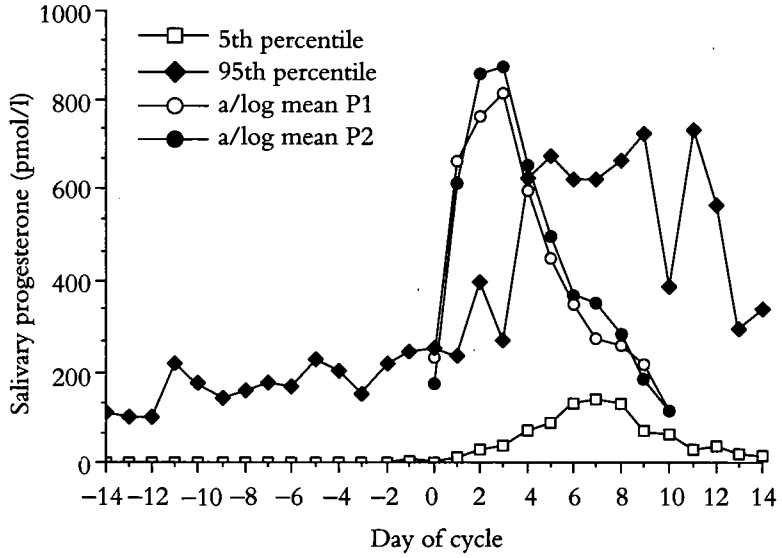

Figure 3 Salivary progesterone profiles during hormonal replacement therapy fitted into the normal range of salivary progesterone levels based on nonparametric statistics. The 5th to 95th percentile limits describe the normal control corridor for the local population. Geometric mean P1 = geometric mean of salivary progesterone in the 1st month of therapy; geometric mean P2 = geometric mean of salivary progesterone in the 6th month of therapy

to 130 pmol/l, which is below the baseline but above the pretreatment 24 h after administration. A lower mean peak progesterone concentration of 1123 ± 370 pmol/l was observed in ten randomly selected patients with a body mass index (BMI) ≥ 30, compared with 1656 ± 389 pmol/l in those patients similarly selected but with BMI ≤ 20 (data not shown). However, this difference did not attain any level of significance (Mann-Whitney U statistic = 70.0, significance = 0.455)

The areas under the time–salivary progesterone curve ($AUC_{0-24\,h}$), pmol/l) were 7177.75 and 7388.20 in the 1st and 6th months of therapy, respectively. This difference was not statistically significant ($p = 0.05$). The mean peak salivary progesterone concentrations were above the 95th percentile of our control corridor which corresponds to the range found in the luteal phase in the two therapy phases of the investigation (Figure 3).

Serum and salivary progesterone peaked simultaneously and there was a significantly positive correlation between the serum and salivary progesterone concentrations measured concurrently ($y = 233.08 + 35.57x$; $r = 0.89$, $p < 0.001$) (Figure 4).

All patients exhibited a non-significant elevation of estrone over the pretreatment and baseline levels, in part because of the large variability (10th–90th percentile) in the estrone levels. The profiles

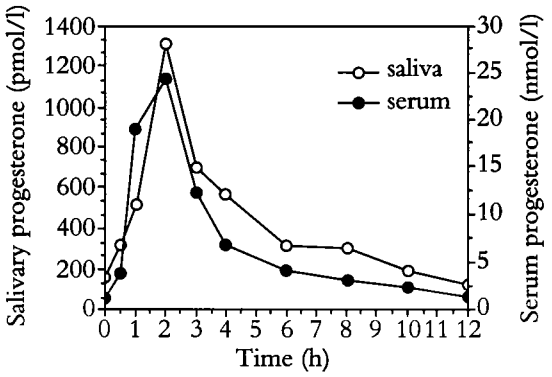

Figure 4 Mean serum/salivary progesterone concentrations in five postmenopausal women who provided matched samples after oral administration of micronized progesterone and conjugated equine estrogen

showed a triphasic pattern in the 1st and 6th months of treatment (Figure 5). The areas under the time–salivary estrone curve ($AUC_{0-24\,h}$, pmol/l) were 719.75 and 747.2 in the 1st and 6th months, respectively, but the difference was not statistically significant.

DISCUSSION

This study evaluated the bioavailability of MOP after 1 and 6 months of therapy using non-invasive salivary analysis. To our knowledge, the bioavailability of progesterone has not previously been

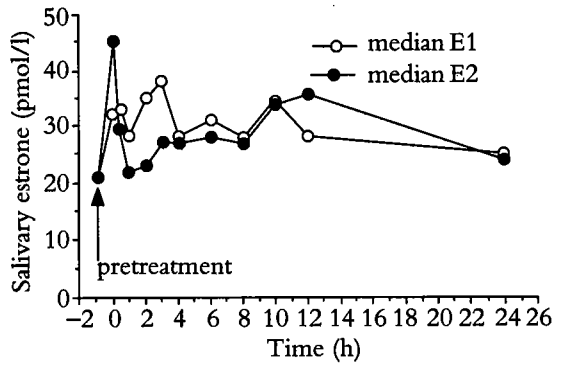

Figure 5 Serial estrone concentrations expressed as medians after oral administration of micronized progesterone and conjugated equine estrogen in the first (E1) and the sixth (E2) months of treatment

studied beyond 2 weeks of treatment, in more than 20 patients or by salivary analysis. Only one of ten relatively recent publications on the measurement of serum progesterone concentrations after administration of oral progesterone provided data for 8 days of treatment on 14 young women with regular cycles[15]. The majority of the other studies involved the single-dose treatment of ten or fewer subjects[3,4,16].

The mean mass of progesterone produced daily by the ovary during the mid-luteal phase of the ovarian cycle is 25 mg[17], which is 25% of the mass of micronized progesterone administered daily in this study. The peak salivary concentrations attained in this study, however, were within the range previously found in the luteal phase[8], indicating that at least 25% of the orally administered progesterone appeared in saliva.

This study also showed that the absorption and clearance of oral progesterone are relatively rapid with a peak concentration being achieved within 2 h. Peak mean factorial increases of 8 and 8.5 occurred in the first and second phases of the investigation, repectively. Salivary progesterone concentration increased to within the control corridor and was sustained for 12 h after administration. Although the concentration at 24 h was below the baseline, it was significantly elevated from pretreatment levels. Continuous therapy did not interfere with bioavailability because the area under the time–salivary progesterone curve was unchanged between the 1st and the 6th month of therapy. Peak plasma and salivary progesterone concentrations were attained simultaneously at 2 h, suggesting that the passive intracellular diffusion of progesterone from serum to saliva is rapid. This relatively rapid increase in salivary progesterone concentrations indicates that the gastrointestinal mucosal tract is an effective site for absorption and delivery of MOP to the circulation. The present results on the time-course of progesterone absorption and clearance are similar to those in other studies following oral[4,5,18], rectal and vaginal[5,19,20] administration and oral ingestion by men[1,4].

The increase (above the 95th percentile) in salivary progesterone concentrations in this study, and the duration of this increase, probably reflect plasma progesterone concentrations which are sufficiently high to influence target tissues for progesterone. Orally administered progesterone is physiologically active and a significant increase in progesterone occurred in tissues with progesterone receptors such as myometrium, endometrium and breast[14,21]. Histological and biochemical changes were detected in endometrium following 10 or more days of administration of oral progesterone to estrogenized postmenopausal women[22–24].

The interpatient variability in progesterone concentration which we found in this study has been reported previously after oral[4], rectal, vaginal and intramuscular[19] progesterone administration. It was suggested that this variability may be due to individual differences in the site and rate of absorption, clearance rates, and extent of absorption of progesterone into fatty tissues[4]. We found a non-significant lower mean peak progesterone concentration in women with a high BMI compared with those with a low BMI, which might be due to a 'depot effect' of adipose tissue. The disappearance curve in the former is likely to be slower as progesterone deposited in adipose tissue diffuses back into the circulation when plasma concentrations decline. This variability in absorption could have a significant impact on the induction of end-organ response and clinical efficacy of oral progesterone treatment. The intra- and interpatient variability may account for the established observation that the same dose may produce either overdosage effects, such as nausea, or underdosage effects such as breakthrough bleeding or a suboptimal progesterone effect on the endometrium. It also suggests that attempts at 'fine-tuning' the dosage for a desired effect[25], or to minimize adverse effects, may be more beneficial than the administration of a fixed dose for all patients.

Villanueva and colleagues[20] found that the most rapid absorption of progesterone occurred in those postmenopausal women who were receiving estrogen, and suggested that anatomical and metabolic differences in the estrogenized women were responsible for improved progesterone absorption. This would be an advantage to the patients who receive the hormone replacement therapy regimen used in this study. We are unable to confirm their proposed explanation or compare our results with previous studies because, to our knowledge, this present study is the first to use salivary analysis to monitor MOP therapy. An accurate comparison of the present salivary progesterone results with serum progesterone levels in other studies was not possible because of differences in assay procedures and the use of antibodies with different cross-reactivities.

The pharmacokinetics of orally administered conjugated equine estrogen are complicated and the results reported here may not reflect its true bioavailability. This is because many different compounds are administered, such as estrone sulfate, equilin sulfate, 17α-dihydroequilin and other estrogenic conjugates which may undergo further metabolic conversions in the gastrointestinal tract. The separation and identification of these compounds from blood or saliva samples is tedious and assays for steroids, such as equilin, were not available in this study.

We conclude that the monitoring of the bioavailability of MOP by serial salivary progesterone sampling was acceptable to all our patients who readily complied with the regimen and found the resultant profiles educational. The present pharmacokinetic results are consistent with the requirements of general substitutional hormone replacement and they justify the use of MOP, provided the amount given is modified according to the individual tolerance, need and response. In view of the present results, a daily dosage of 100 mg progesterone for 23 days every month should be considered a low dosage. Because the elevated level of progesterone persisted for only 12 h, it is essential to administer the dose at least every 12 h in order to maintain a stable physiological luteal phase level during clinical treatment.

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