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Pharmacokinetics of progesterone after its administration to ovariectomized rhesus monkeys by injection, infusion, or nasal spraying

(bioavailability/serum/cerebrospinal fluid)

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ABSTRACT **The pharmacokinetics of progesterone (dose: 10 μg per animal) were studied in blood and cerebrospinal fluid of adult ovariectomized rhesus monkeys after the administration of the steroid as an intravenous injection, intravenous infusion (duration of infusion: 10 min), or nasal spray. The bioavailability of progesterone, in terms of area under the time–concentration curve and the maximal concentration in the two body fluids, was significantly higher when the steroid was infused or sprayed intranasally than when it was injected intravenously. The clearance of the steroid from the serum, as estimated by its elimination rate constant, elimination half-life, and total body clearance, did not differ for the three methods of administration. These findings suggest that the bioavailability of progesterone is enhanced by extending the duration over which the steroid is delivered into the hemal circulation.**

Reports from our laboratories have shown that marked neuroendocrine effects leading to an impairment of ovarian (1) or testicular (2) functions occur after the intranasal spraying of progesterone in extremely low doses likely to be ineffective when administered by oral or systemic routes. It has been suggested (3) that these marked effects observed with low doses of the steroid administered by an unconventional route may be related to the rapid and preferential transfer of the steroid to the brain via the cerebrospinal fluid (CSF). This suggestion is based on the finding of much higher levels of progesterone in the CSF after its being sprayed intranasally in comparison with its systemic administration (4, 5).

Because the pharmacological effects of a drug are known to be related to its bioavailability to target tissues rather than to its administered dose, it was felt that a comparison of the pharmacokinetics of progesterone after its systemic administration and intranasal spraying would be useful in furthering our understanding of these unique, low-dose effects occurring upon administering the steroid by an unconventional method. The studies reported here were carried out to obtain such data with particular reference to the bioavailability of progesterone to tissues bathed by blood, CSF, or both after the administration of progesterone by three different methods: intravenous (i.v.) injection, i.v. infusion, and nasal spray (n.s.).

MATERIALS AND METHODS

Animals. Six healthy adult female rhesus monkeys of similar body weights (5–6.5 kg) and sizes (6) were selected from the Primate Research Facility of our institute (7). These animals consistently exhibited ovulatory menstrual cycles of normal duration (23–28 days) before they were ovariectomized. They were used 2 months after ovariectomy. The clinical condition of all the animals, with particular reference to their liver and renal functions, as evaluated by estimating blood or serum levels of proteins, albumin, bilirubin, alkaline phosphatase, glutamic–oxaloacetic transaminase, glutamic–pyruvic transaminase, urea, and creatinine were within normal values reported for this species (8). The animals remained in good health throughout the course of the present study.

Reagents. Analytical grade reagents, and doubly glass-distilled ethanol and water were used. Crystalline progesterone was obtained from Sigma and the progesterone antiserum was supplied by the World Health Organization under their Quality Control Programme for Radioimmunoassay of Reproductive Hormones (9).

Experimental Design. Progesterone was formulated in ethanol/propylene glycol/water, 3:3:4 (vol/vol), and administered at a dose of 10 μg by each of the following three routes: i.v. injection, i.v. infusion, or n.s. The infusion was carried out by using a model 351 syringe pump (Sage Instruments, Cambridge, MA) at a uniform rate of 1 μg of progesterone per minute for a total of 10 min. Five micrograms of progesterone was sprayed over a period of 10 sec into each nostril by using a commercially available glass atomizer as described and illustrated previously (3, 10).

The antecubital vein was used for the systemic administration of the steroid, and blood samples were drawn from the saphenous vein. Samples of clear free-flowing CSF were drawn from the cisterna magna as described (11).

All six of the animals were subjected to the three methods of progesterone administration in sequence (Table 1). The animals were anesthetized before progesterone was administered and they remained anesthetized throughout the period of sampling the body fluids. The animals were rested for 2 weeks between experiments and their diet was supplemented with hematinics.

Estimation of Progesterone. Progesterone was estimated in the two body fluids by specific radioimmunoassay as described (12, 13). The coefficients of variation within and between assays were, respectively, 7.12 ± 1.26% and 9.45 ± 1.04%.

Pharmacokinetic Parameters. A two-compartment kinetic model with first-order absorption and clearance (14) was assumed to determine the following parameters (15). Each of these parameters was obtained separately for individual monkeys and the data are expressed as geometric mean values with 95% confidence limits for each of the treatment groups.

Area under time–concentration curve (AUC). AUC was cal-

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Abbreviations: CSF, cerebrospinal fluid; i.v., intravenous; inf, infusion; n.s., nasal spray; AUC, area under curve.

Table 1. Distribution of six ovariectomized monkeys into three groups of three animals each for sequential administration of progesterone by diverse methods

Group	Monkey nos.	Sequence of methods of administration 1	2	3
A	912, 981, 986	i.v.	inf	n.s.
B	886, 1005, 1030	n.s.	i.v.	inf
C*	912, 981, 986	inf	n.s.	i.v.

i.v., Intravenous injection; inf, intravenous infusion.
* Only six animals of similar body weights were available for the present study. Monkey nos. 912, 981, and 986 were used twice. However, analyses of data based only on groups A and B or B and C provided the same statistical significance.

culated by using the trapezoidal rule (16) according to the following formula (17):

$$\text{AUC} = \tfrac{1}{2} \sum_{i=1}^{n-1} \{y(i+1) + y(i)\} \cdot \{t(i+1) - t(i)\},$$

in which n is the number of experimental points, $y(i)$ is the ith serum/CSF sample, and $t(i)$ is time of the ith sample.

AUC for serum and CSF was calculated between 0 and 60 min. The AUC for serum between 0 and ∞ min was calculated by using the formula

$$\text{AUC} \int_0^{\infty} = \text{AUC} \int_0^{60} + \frac{C_{60}}{\beta},$$

in which C_{60} is the concentration of progesterone at 60 min and β is the elimination rate constant.

$\text{AUC}\int_0^{\infty}$ could not be calculated for CSF because progesterone levels in the CSF obtained at the different time intervals in the present study did not show a progressive decline in all the individuals for estimating the elimination rate constant.

Concentration of progesterone. The maximal concentration (C_{max}) and the time of maximal concentration (T_{max}) were estimated from a graph in which mean concentrations of progesterone in the serum or CSF were plotted against time.

Elimination rate constant (β). β was determined by linear regression analysis of the serum decay curve after the i.v. injection. The last three data points in 15, 30, and 60 min showed a linear decline and hence these points were taken for calculating β.

Elimination half-life ($t_{1/2}\beta$). This was calculated by using the relationship

$$t_{1/2}\,\beta = \frac{0.693}{\beta}.$$

Apparent volume of distribution (V_d). V_d was estimated from the relationship

$$V_d = \frac{\text{i.v. dose}}{C_0},$$

in which C_0 is concentration of progesterone in serum at 0 min obtained by extending the regression line to the y axis for data obtained after i.v. injection of progesterone.

Apparent total body clearance (Cl_{TB}). Cl_{TB} was calculated from the relationship

$$Cl_{TB} = V_d\beta.$$

Calculations. The pharmacokinetic data were subjected to analysis of variance by using a model 700 Wang programmable calculator. This analysis was followed by the Newman–Keul multiple range test of significance (18).

RESULTS

General. In marked contrast to the systemic administration of progesterone, in which the entire amount of the predetermined dose of the steroid was delivered directly into the blood circulation, spraying progesterone into one of the nostrils with a glass atomizer resulted in the unavoidable loss of some of the steroid contained in the atomized spray escaping through the other nostril. Thus, it was not possible to precisely estimate the actual amount of the steroid actually deposited in the nasal cavities. Because the predetermined dose of 10 μg of progesterone was delivered into the nostrils it is most likely that the actual amount of progesterone deposited within the nasal cavities was much less than 10 μg.

Concentrations of Progesterone in the Serum. Progesterone concentrations declined progressively in samples taken at different intervals after the i.v. or n.s. administration of progesterone. After i.v. infusion, progesterone concentrations showed a marked increase in the second sample (9 min) as compared with the initial sample (3 min), but the concentrations declined progressively in samples taken after the cessation of the infusion at 10 min.

Progesterone concentrations in serum samples taken at 3 min were not significantly different for the three methods of administration. However, in all subsequent samples concentrations of progesterone were significantly higher after i.v. infusion and n.s. as compared with its i.v. injection; serum concentrations of progesterone did not differ significantly between i.v. infusion and n.s. (Table 2).

Concentrations of Progesterone in the CSF. Progesterone concentrations after its i.v. injection showed a progressive decline in samples taken at different intervals. After i.v. infusion

Table 2. Progesterone concentrations in serum samples taken at different intervals after administration by diverse methods

Time, min	Progesterone, nM i.v.	inf	n.s.	Analysis of variance† i.v. vs. inf	i.v. vs. n.s.	inf vs. n.s.
3	4.79 (3.98–5.76)	5.43 (4.33–6.82)	5.45 (4.41–6.73)	NS	NS	NS
9	3.83 (2.77–5.30)	7.15 (5.96–8.58)	5.31 (4.71–5.98)	**	*	NS
15	2.59 (2.01–3.33)	4.41 (3.79–5.11)	4.59 (3.88–5.42)	**	**	NS
30	2.05 (1.47–2.87)	3.37 (2.80–4.05)	3.16 (2.67–3.73)	*	*	NS
60	1.55 (1.10–2.24)	2.41 (1.86–2.55)	2.17 (1.86–2.55)	*	*	NS

Data are presented as geometric mean concentrations with 95% confidence limits in parentheses ($n = 9$). Values obtained in samples taken from individuals at 0 min—i.e., just prior to the administration of the steroid—have been subtracted from the values obtained for the same individual at different time intervals of sampling serum both here and in subsequent calculations. The 0 min values showed a range between 0.64 and 1.70 nM.
† One-way analysis of variance followed by multiple range test (18). NS, not significant; *, $P < 0.05$; **, $P < 0.01$.

Table 3. Progesterone concentrations in CSF samples taken at different intervals after administration by diverse methods

Time, min	Progesterone, nM			Analysis of variance†		
	i.v.	inf	n.s.	i.v. vs. inf	i.v. vs. n.s.	inf vs. n.s.
3	0.19 (0.15–0.24)	0.25 (0.17–0.35)	0.18 (0.11–0.28)	NS	NS	NS
9	0.17 (0.12–0.24)	0.42 (0.26–0.67)	0.37 (0.28–0.48)	**	**	NS
15	0.17 (0.13–0.22)	0.58 (0.38–0.88)	0.35 (0.24–0.51)	***	**	NS
30	0.10 (0.05–0.18)	0.34 (0.23–0.51)	0.23 (0.10–0.51)	*	NS	NS
60	0.04 (0.01–0.12)	0.23 (0.13–0.41)	0.18 (0.11–0.28)	*	*	NS

Data are presented as geometric mean concentrations with 95% confidence limits in parentheses ($n = 9$). Values have been corrected as described for Table 2 both here and in subsequent calculations. The 0 min values showed a range between 0.06 and 0.24 nM.

† One-way analysis of variance followed by multiple range test (18). NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

the concentrations of progesterone increased in the first two samples, taken at 3 and 9 min, and thereafter the levels declined progressively. When progesterone was sprayed intranasally, its concentrations in the second and third CSF samples (9 and 15 min) were higher as compared with the first samples (3 min). The levels of the steroid declined progressively in the fourth and fifth samples (30 and 60 min) (Table 3).

Progesterone concentrations in CSF samples taken at 3 min were not significantly different for the three methods of administration. In all subsequent samples, however, progesterone concentrations were significantly higher after i.v. infusion and n.s. as compared with i.v. injection. CSF concentrations of progesterone did not differ significantly between i.v. infusion and n.s. (Table 3).

Pharmacokinetics of Progesterone in the Serum. Data obtained for the various pharmacokinetic parameters and the levels of statistical significance of the differences in these parameters between the three methods of administering progesterone are shown in Table 4.

A comparison of the data obtained for $AUC\int_0^{60}$, $AUC\int_0^{\infty}$, C_{max}, and T_{max} among the three methods of administering the steroid clearly indicates that the values obtained for i.v. infusion were the highest, followed by those obtained by n.s. and i.v. injection.

A statistical comparison of the $AUC\int_0^{60}$ and $AUC\int_0^{\infty}$ between i.v. injection and i.v. infusion or between i.v. injection and n.s. showed that the higher values obtained for the i.v. infusion and n.s. were significantly different from the value observed for i.v. injection. The slight differences observed in the AUCs between the i.v. infusion and n.s. were not statistically significant.

The C_{max} values for i.v. injection and i.v. infusion were significantly different, whereas the C_{max} values for i.v. injection and n.s. or for i.v. infusion and n.s. were not.

T_{max} values were significantly different for i.v. injection and i.v. infusion but were not significantly different for comparisons of i.v. infusion and n.s. or i.v. injection and n.s.

Although the β, $t_{1/2}\beta$, and Cl_{TB} values showed slight differences among the three routes of administering progesterone, these differences were not statistically significant.

Pharmacokinetics of Progesterone in CSF. The values obtained for the $AUC\int_0^{60}$, C_{max}, and T_{max} were highest after i.v. infusion of progesterone, followed by those obtained after n.s. and i.v. injection. A comparison of all these parameters among the three methods of progesterone administration showed that statistically significant differences were evident between i.v. injection and i.v. infusion as well as between i.v. injection and n.s. but were not evident between i.v. infusion and n.s. (Table 5).

Linear Regression Analysis. In a linear regression analysis of progesterone concentrations in serum vs. those in CSF after diverse methods of administering the steroid, a highly significant correlation coefficient was obtained with the i.v. injection; the correlation coefficients obtained with i.v. infusion and n.s. were not statistically significant (Table 6).

DISCUSSION

The pharmacokinetics of endogenously produced progesterone have been extensively investigated and reviewed (19–21), but comparable data for exogenously administered progesterone are not available. The present study has thrown some light on the pharmacokinetics of progesterone after its systemic administration. Besides providing such information, the present studies have shown that the detectable levels of progesterone in serum

Table 4. Pharmacokinetics of progesterone in the serum after its administration by diverse methods to ovariectomized monkeys

Pharmacokinetic parameter	i.v.	inf	n.s.	Analysis of variance†		
				i.v. vs. inf	i.v. vs. n.s.	inf vs. n.s.
$AUC\int_0^{60}$, nM·min	144.74 (112.32–186.53)	230.30 (203.97–260.03)	213.45 (186.00–244.95)	**	**	NS
$AUC\int_0^{\infty}$, nM·min	203.33 (179.52–230.30)	408.13 (337.51–493.53)	371.19 (313.40–439.64)	**	**	NS
C_{max}, nM	5.05 (3.97–6.42)	7.57 (6.55–8.75)	6.00 (5.13–7.02)	*	NS	NS
T_{max}, min	3.83 (2.79–5.25)	8.92 (6.58–12.09)	5.52 (3.78–8.06)	**	NS	NS
β, min^{-1}	0.196 (0.129–0.0299)	0.0135 (0.0104–0.0175)	0.152 (0.0114–0.0205)	NS	NS	NS
$t_{1/2}\beta$, min	35.21 (23.11–53.65)	50.94 (39.19–66.12)	45.27 (33.79–60.66)	NS	NS	NS
Cl_{TB}, liters·min^{-1}	0.15 (0.10–0.23)	0.11 (0.08–0.15)	0.13 (0.08–0.19)	NS	NS	NS
V_d, liters	8.14 (6.34–10.45)	—	—	—	—	—

Data are presented as geometric mean values with 95% confidence limits in parentheses ($n = 9$).

† Analysis of variance followed by multiple range test (18). NS, not significant; *, $P < 0.05$; **, $P < 0.01$.

Table 5. Pharmacokinetics of progesterone in CSF after its administration by diverse methods to ovariectomized monkeys

Pharmacokinetic parameter	i.v.	inf	n.s.	Analysis of variance† i.v. vs. inf	i.v. vs. n.s.	inf vs. n.s.
AUC$\int_0^{60}$, nM·min	8.48 (6.51–10.95)	22.41 (15.59–32.19)	16.30 (10.28–25.84)	**	*	NS
C_{max}, nM	0.2285 (0.1653–0.3158)	0.6579 (0.447–0.9669)	0.5336 (0.3546–0.8027)	**	**	NS
T_{max}, min	5.91 (3.33–10.49)	13.66 (10.63–17.57)	10.20 (6.82–15.25)	*	*	NS

Data are presented as geometric mean values with 95% confidence limits in parentheses ($n = 9$).
† Analysis of variance followed by multiple range test (18). NS, not significant; *, $P < 0.05$; **, $P < 0.01$.

and CSF as well as its bioavailability in terms of AUC and C_{max} in both the body fluids differ markedly depending on the method of administering the compound.

The AUC and C_{max} of progesterone in the two body fluids were significantly enhanced when the steroid was delivered over an extended period of time as an i.v. infusion in comparison with a comparable dose being almost instantaneously delivered as a bolus into the systemic circulation by i.v. injection. Interestingly, the clearance of the steroid from serum as estimated by determining β, $t_{1/2}\beta$, and Cl_{TB} did not differ significantly between i.v. injection and i.v. infusion. These findings suggest that the enhancement in the levels of progesterone as well as the amplification of its bioavailability in the body fluids may be related to the duration over which the steroid enters the blood circulation rather than to differences in its clearance rates among different methods of its administration. More studies are, however, necessary to define in precise mathematical terms the relationship between the duration of administering into the systemic circulation and the amplification of its bioavailability.

The values obtained for the concentrations of progesterone and its bioavailability in the body fluids after the intranasal spraying of the compound were significantly higher than those observed after i.v. injection. Though the values obtained after n.s. were lower than those obtained after i.v. infusion, these values were not significantly different. It must, however, be noted that the actual amount of the steroid deposited into the nasal cavity was much less than that administered by the systemic routes.

Because progesterone sprayed into the nostrils would be deposited onto the nasal mucosa the question arises as to how can such "topically" administered steroid show bioavailability values that are much higher than the values observed after its direct injection into the venous blood. Studies involving radionuclide imaging techniques have shown that most of the substances sprayed into the nostrils are deposited on the olfactory mucosa (10). Electron microscopic studies of colloidal gold particles sprayed onto the olfactory mucosa have shown that these particles are pinocytosed by both the olfactory dendrites and the adjoining supporting cells of the olfactory epithelium. Substances entering each of these two cell types are transported separately to different destinations. Particles entering the olfactory dendrites are transported across the olfactory neuron into the fila olfactoria. It has been suggested (10) that diffusable substances, such as steroids, could traverse the olfactory nerves and enter the CSF present in the perineural space surrounding these nerves; this CSF is in communication with the CSF present in the subarchnoid spaces. Substances pinocytosed by the supporting cells are transported directly into the subepithelial blood capillaries (10).

Table 6. Linear regression of progesterone concentrations in serum (x axis) and CSF (y axis) after administration of the steroid by diverse methods (45 xy pairs)

Regression parameter	i.v.	inf	n.s.
Slope	0.027	0.010	0.023
y intercept	0.070	0.394	0.244
Correlation coefficient	0.551	0.066	0.104
t	4.329*	0.434	0.685

*, $P < 0.001$.

These morphological studies have traced the pathways by which particulate materials are transported into the body fluids across the olfactory mucosa, but the question as to whether lipophilic substances, such as progesterone in solution, also enter the olfactory mucosa by pinocytosis or by simple diffusion needs to be determined.

Be that as it may, progesterone entering the olfactory mucosa by pinocytosis or by simple diffusion will enter the body fluids at a much slower rate than progesterone injected as a bolus directly into the systemic circulation. It is therefore not surprising that the levels and bioavailability of progesterone in the body fluids after its being administered by n.s. is greater than those observed after i.v. injection, as has been observed when the steroid is infused over an extended period of time.

Thus, the present studies have shown that the concentrations of progesterone and its bioavailability differ markedly with different methods of administration. These studies also offer an explanation for the marked pharmacological effects observed after the intranasal spraying of extremely low doses of progesterone (22, 23). The present studies also indicate the possibility of administering physiological doses of progesterone as nasal sprays for replacement therapy or contraceptive purposes.

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