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Nasal delivery of progestational steroids in ovariectomized rabbits. I. Progesterone – comparison of pharmacokinetics with intravenous and oral administration

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Summary

Transnasal delivery of progesterone was studied in rabbits employing a nasal spray and a specially designed controlled delivery nasal device. The device is cylindrically shaped for better insertion into the nasal cavity, and is walled with a microporous membrane to permit controlled delivery of progesterone from a suspension formulation to the nasal mucosa. Ovariectomized New Zealand white rabbits were chosen as the animal model after preliminary studies which indicated that ovary-intact rabbits have significant fluctuations in endogenous progesterone levels. The pharmacokinetics of progesterone were evaluated in a cross-over study comparing i.v., oral, nasal device and nasal spray treatments. Using i.v. data as the reference, the systemic bioavailability of progesterone delivered by nasal device and nasal spray was calculated to be 72.4% and 82.5%, respectively. This was substantially greater than the oral bioavailability of 7.9%. The nasal device was observed to achieve a more prolonged and elevated plasma level of progesterone than the other routes of administration. The nasal spray and nasal delivery device will be employed as tools to study and to compare the effect of immediate and controlled drug release on nasal bioavailability of drugs.

Introduction

Oral bioavailability of steroidal drugs is reportedly extremely low due to extensive elimination by hepatic first pass metabolism (Maxon et al., 1985; Ottoson et al., 1984). Nasal absorption of steroids including progesterone (Hussain et al., 1981; David et al., 1981; Kumar et al., 1982; Ohman et al., 1978), 17-β estradiol (Hussain et al., 1982) and testosterone (Hussain et al., 1984), has resulted in a systemic bioavailability comparable with i.v. administration in rats and rhesus monkeys.

The objective of this series of studies is to investigate the effect of penetrant hydrophilicity on nasal and oral absorption, by studying and comparing the systemic bioavailability of progesterone and its hydroxy derivatives in rabbits. The relationship between nasal bioavailability and penetrant hydrophilicity will be examined for both an immediate release nasal spray and a controlled

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release nasal device. For the purposes of this investigation, the desired features for the controlled release nasal device are: to contain drug in solution form to minimize the complication of dissolution; to release progesterone and its hydroxy derivatives at the same controlled rate; to maintain intimate contact with the nasal mucosa; and to permit retrieval of the device for residual drug assay. The above criteria were met by fabricating a nasal device walled with a microporous membrane, which permits the controlled delivery of drug from a solution or suspension formulation.

In this first investigation, the in-vitro release kinetics of drugs from the nasal delivery device are described, and the pharmacokinetics of progesterone after nasal, oral and i.v. administrations are compared. The metabolism of progesterone to 20-dihydroprogesterone is assessed in both ovary-intact and ovariectomized rabbits. In addition the relationship between the in-vitro release and the in-vivo absorption from the nasal delivery device is explored.

Materials and Methods

Fabrication of nasal delivery device

The nasal delivery device was fabricated using a section (5 cm × 2 mm) of microporous membrane sleeve (Spectapor, 12,000-14,000 mol. wt. cutoff). A 6 cm length of a thin polyethylene tube (Intramedic, PE-60) was inserted 4.5 cm into the sleeve, and both ends were then sealed. The device was inserted into the rabbit nasal passage, filled with 0.35 ml of a donor drug suspension through the tubing opening, sealed, and left in place throughout the 6 h study. Once inflated with fluid, the device conforms to the rabbit nasal cavity, which maximizes the area in contact with the mucosa for drug absorption (Fig. 1).

The solvent used to prepare the drug suspension was an isotonic solution of 20% PEG 4000, buffered with 0.07 M Na_2HPO_4 and 0.07 M KH_2PO_4 to pH 8, the pH of rabbit nasal secretions. Polyethylene glycol (PEG) 4000 was incorporated to enhance the aqueous solubility of progesterone.

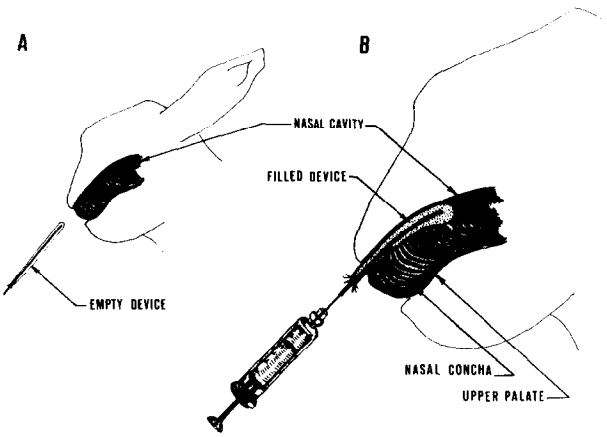

Fig. 1. Diagrammatic illustration of the nasal delivery device prior to insertion into the rabbit nasal passage (A), and the filling of the nasal delivery device with the progesterone formulation in the nasal cavity (B).

Solubility studies

The aqueous solubility of progesterone was determined in isotonic phosphate buffer solution (pH 8), with and without 20% PEG 4000. Solubility studies were conducted at 36 °C (the temperature on the nasal mucosa surface of the rabbit) by equilibrating an excess amount of progesterone in the medium with shaking for 48 h, and then filtering the drug solution. The concentration of progesterone in the filtrate was assayed by UV spectrophotometry (Perkin Elmer Model 559A) at a wavelength of 254 nm.

In-vitro studies

The in-vitro release kinetics of progesterone from nasal delivery devices containing a solution (45 μg/ml) and suspensions (100 and 170 μg/ml) were determined in triplicate. Each device was filled and placed in a test tube containing 4.5 ml of isotonic phosphate buffer (0.07 M Na_2HPO_4 and 0.07 M KH_2PO_4, at pH 8), screw-capped, and shaken gently (1 oscillation per second) in a water bath (Fisher model 127 shaking water bath) at 36 °C. Samples were taken at regular intervals and the concentration of progesterone in the samples was assayed by an HPLC method with UV detection at 254 nm (Waters Model 590 solvent delivery system, WISP 710B, Waters Model 440 detector), using a 15 cm C-18 μ-Bondapak column (Waters)

with a mobile phase of methanol–water (3 : 1) at a flow rate of 2 ml/min.

In-vivo studies

In-vivo studies were performed in female New Zealand white rabbits (3–4 kg). A randomized cross-over study design was used with a one week washout period between treatments. Blood samples (0.8 ml) were withdrawn from the central ear artery via a catheter (22 gauge) at appropriate time intervals, and were collected in heparin-treated tubes. The tubes were immediately centrifuged, and the plasma was separated and frozen (at −5 °C) until assay. The plasma concentrations of progesterone and 20-dihydroprogesterone were assayed using commercially available radioimmunoassay kits (InterSci Diagnostics and ICN Immunobiologicals, respectively).

Initial studies were conducted in 4 conscious ovary-intact female rabbits. Crossover treatments consisted of an i.v. bolus injection (60 μg/kg), a 3-h i.v. infusion (1 μg/kg/min), a nasal delivery device (60 μg/kg), and an oral administration (60 μg/kg). A 10% ethanolic solution of progesterone in normal saline was used for the i.v. and oral administrations, while isotonic 20% PEG 4000 in phosphate buffer was used for the nasal delivery device. Animals were fasted for 12 h prior to study and, for oral treatments, the solution was administered via a gastric tube. The amount of progesterone remaining in the nasal device at the end of the 6-h study period was assayed by UV spectrophotometry (Perkin Elmer Model 559A) to determine the exact dose delivered.

The second study was conducted using a randomized cross-over design in ovariectomized female rabbits. Ovariectomies were performed under sterile surgical conditions. A 3 cm lateral incision was made approximately 8 cm below the diaphragm. The ovaries were isolated, the ovarian artery and vein were ligated with 4-0 silk (Ethicon Inc.), and the ovaries were then removed. Abdominal muscles and skin were sutured using 2-0 chromic and 3-0 silk suture, respectively. Animals were permitted a two week recovery period before the initiation of the studies. Rabbits were anesthetized with Ketamine (35 mg/kg) and Xylazine (4 mg/kg) (Butler Veterinary Supply Co.) during surgery and all treatments. Cross-over treatments consisted of an i.v. bolus injection (60 μg/kg), an oral administration (60 μg/kg), and nasal administrations by nasal device (60 μg/kg) and nasal spray (2 μg/kg). The nasal spray was administered by two actuations of a 100 μl metered-dose pump (BLM Packaging Inc.) of a 10% ethanolic solution of progesterone in normal saline.

Data analysis

The progesterone plasma concentration–time data for individual rabbits were analyzed by a first-order kinetic plot, and the following pharmacokinetic parameters were determined:

$$\text{Elimination half-life } (t_{1/2}) = 0.693/k_e$$

$$\text{Absolute bioavailability } (F) = (AUC/\text{Dose})_{\text{route}}/(AUC/\text{Dose})_{\text{i.v.}}$$

$$\text{Systemic clearance } (Cl) = (\text{Dose}/AUC)_{\text{i.v.}}$$

$$\text{Apparent volume of distribution } (V_d) = Cl/k_e$$

The individual elimination rate constant (k_e) was calculated by linear regressional analysis of the data points on the terminal log–linear segment of the plasma concentration–time plot. The area under the plasma concentration time curve from time zero to infinity (AUC) was calculated by the linear trapezoidal method up to time t (AUC_t), and then by extrapolation to infinity, i.e.,

$$AUC = AUC_t + Cp^t/k_e$$

in which Cp^t is the progesterone plasma concentration observed at time t, the last sampling time with a measurable Cp.

All of the data are expressed as the mean ± the standard error. Individual means were compared using a t-test.

Results and Discussion

Solubility studies

The solubility of progesterone in the donor solution for the nasal delivery device and in the

TABLE 1

Aqueous solubility of progesterone in the vehicles used

Vehicle	Solubility (±S.D.) (μg/ml)
Phosphate buffer	15.3 (±1.1)
20% PEG 4000 in phosphate buffer	65.9 (±3.2)

receptor solution for the in-vitro release studies was determined, and the results are shown in Table 1. The data suggests that the addition of 20% PEG 4000 enhances the aqueous solubility of progesterone by more than 4 times, so a higher drug concentration can be incorporated in the nasal delivery device for in-vivo studies. The receptor solution solubility is sufficient to maintain a sink condition in the receptor during the in-vitro study.

In-vitro studies

The in-vitro release profiles, shown in Fig. 2, indicate that progesterone releases from the nasal delivery device with linear (zero order) kinetics when the amount of progesterone in the donor solution exceeds its solubility (65.9 ± 3.2 μg/ml). The release rate from devices containing a 100 μg/ml suspension of progesterone was 86 (±7) ng/min (Fig. 2B). Increasing the amount of progesterone in the suspension to 170 μg/ml resulted in a longer duration of linear release, but did not increase the release rate (Fig. 2C). The in-vitro release profile for a 45 μg/ml solution, shown in Fig. 2A, deviated considerably from the theoretical zero-order release. However, a semilogarithmic relationship was observed between the percent of dose remaining in the device and time (Fig. 3), indicating that first-order release occurs at progesterone concentrations below the saturation solubility.

During the in-vitro drug release studies, the volume of fluid inside the device was noted to increase as much as 71% within 1 h of immersion in the receptor solution. The increase in the volume may be due to the osmotic pressure gradient resulting from the presence of PEG 4000 in the donor solution. The influxing fluid could act against the flux of drug release through the pores in the microporous membrane, and thus decrease the apparent release rate of progesterone from the device.

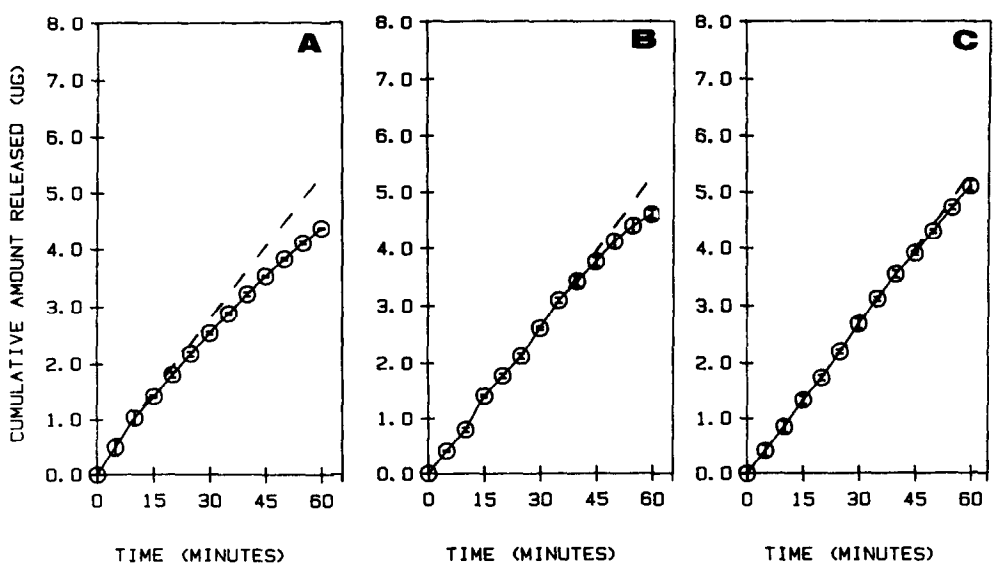

Fig. 2. In-vitro release profile of progesterone from nasal devices ($n = 3$) containing progesterone in a 45 μg/ml solution (A), a 100 μg/ml suspension (B) and a 170 μg/ml suspension (C).

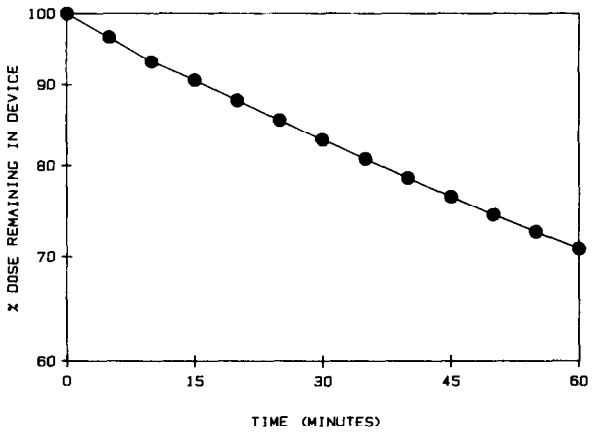

Fig. 3. Percent of dose remaining in the device as a function of time for nasal delivery devices containing a 45 μg/ml solution of progesterone ($n = 3$).

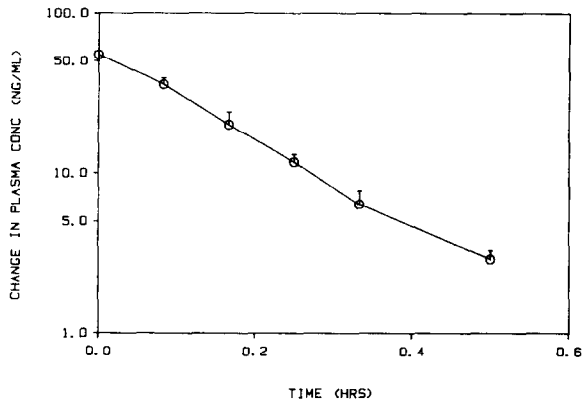

Fig. 4. Time course for the change in the plasma concentration of progesterone after intravenous bolus injection of progesterone in ovariectomized rabbits ($n = 4$).

In-vivo studies

Studies conducted in conscious ovary-intact rabbits using a placebo formulation indicated that the endogenous levels of progesterone could fluctuate by as much as 2 ng/ml during the 6-h study period. This fluctuation in the baseline levels of progesterone was observed to affect the pharmacokinetic profiles of progesterone after i.v., oral and nasal administration. Since the ovariectomy resulted in a much lower and more stable endogenous baseline level of progesterone (0.2–0.5 ng/ml), the ovariectomized rabbit was chosen for the subsequent studies.

As can be seen in Fig. 4, the pharmacokinetic profile of progesterone in the ovariectomized rabbits after i.v. administration is well approximated by a one-compartment model. The pharmacokinetic parameters calculated for i.v. bolus injection are summarized in Table 2. The results indicate that in ovariectomized rabbits, progesterone is eliminated with a first-order rate constant of 6.77 ($\pm$0.16) h^{-1}, and has a volume of distribution of 2.80 ($\pm$0.23) liter and a systemic clearance of 18.91 ($\pm$1.52) liter/h.

The plasma profiles of progesterone after nasal administration by two different delivery systems, nasal spray and nasal device, are compared in Fig. 5. The nasal spray led to a rapid attainment of the peak plasma level of progesterone ($t_{max} < 2$ min), followed by an elimination with a rate similar to that seen after i.v. bolus (6.18 h^{-1} vs. 6.77 h^{-1}), indicating a rapid absorption of progesterone by the nasal mucosa. In contrast, the nasal adminis-

TABLE 2

Pharmacokinetic parameters of progesterone in ovariectomized rabbits following various routes of administration

Parameter	i.v.	Oral	Nasal spray	Nasal device
Dose (μg/kg)	60	60	2	60
k_e(1/h)	6.77 ($\pm$0.16)	6.81 ($\pm$0.70)	6.18 ($\pm$0.10)	5.94 ($\pm$0.60)
$t_{1/2}$(h)	0.10 ($\pm$0.01)	0.10 ($\pm$0.02)	0.11 ($\pm$0.01)	0.11 ($\pm$0.02)
V_d (liter)	2.80 ($\pm$0.23)	–	–	–
Cl (liter/h)	18.91 ($\pm$1.52)	–	–	–
AUC (ng*h/ml)	10.47 ($\pm$0.19)	0.83 ($\pm$0.16)	0.21 ($\pm$0.04)	8.04 ($\pm$3.71)
AUC/dose $\times 10^{-3}$ (h/liter)	53.17 ($\pm$2.70)	4.67 ($\pm$0.90)	42.0 ($\pm$5.41)	37.5 ($\pm$14.8)
F(%)	100	7.87 ($\pm$1.59)	82.52 ($\pm$13.50)	72.37 ($\pm$25.71)

Values in parentheses are $\pm$S.E.M.

tration of progesterone by the nasal delivery device led to a gradual increase in the plasma levels of progesterone, which reached a plateau within 30 min and remained elevated throughout the 6-h study. A significant prolongation of the plasma progesterone level was achieved by the controlled delivery of progesterone from the nasal device as compared to the immediate drug release from the nasal spray (Fig. 5). The systemic bioavailability after nasal delivery was calculated to be 82.5 (±13.5)% for nasal spray and 72.4 (±25.7)% for nasal device (Table 2), which are not significantly different ($P < 0.05$).

The data in Fig. 5 suggest that the plasma concentration profile of progesterone delivered by nasal device peaks at approximately 1 h, and then gradually declines. The observed decline in the plasma concentration after the peak level may be attributed to the decrease in the solution volume inside the device with time. When initially filled, the shape of the device conforms to the nasal passage, maximizing the area in intimate contact with the nasal mucosa for efficient drug absorption. As the solution volume of the device gradually declines, the area in direct contact with the mucosa should decrease, resulting in a reduced efficiency in drug absorption. Experimentally, it was noted that at the end of the 6-h study period, the volume of solution inside the device was negligibly small, and 20–35% of the administered

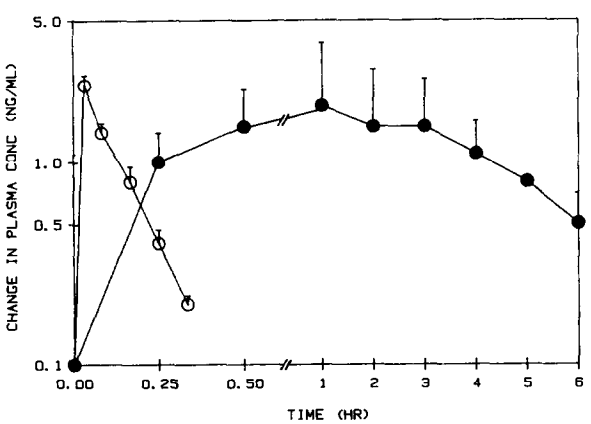

Fig. 5. Time course for the change in the plasma concentration of progesterone after nasal administration of progesterone in ovariectomized rabbits ($n = 3$) by (○) nasal spray (2 μg/kg) and (●) nasal device (60 μg/kg).

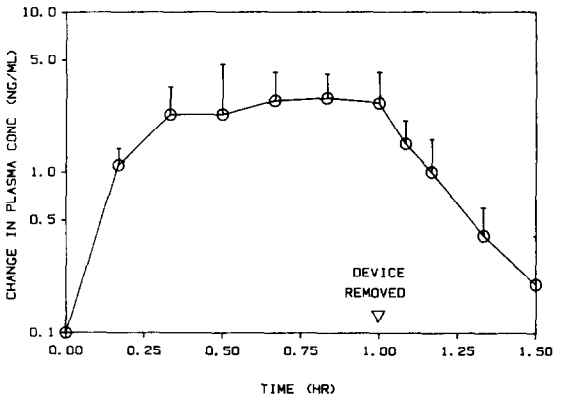

Fig. 6. Time course for the change in the plasma concentration of progesterone after administration of progesterone by nasal device in ovariectomized rabbits ($n = 3$) for 1 h.

dose could be recovered from the device. This problem of reduced volume could be minimized, and the peak plasma level could be further sustained if one used a formulation with a vehicle unable to diffuse through the microporous membrane.

Further investigation using the nasal delivery device filled with tritiated PEG 4000 demonstrated that although the PEG is released from the device, it is not absorbed through the nasal mucosa. This observation differs from the findings by Kotani et al. (1983) who reported that PEG 4000 is absorbed nasally; however, he used a turbidity detection method.

The reservoir function of the rabbit nasal mucosa for progesterone was also investigated by withdrawing the nasal device after 1 h of application to terminate the drug delivery (Fig. 6). The results indicated that following device removal, the plasma concentration of progesterone in the ovariectomized rabbits declines with a rate constant (5.94 h^{-1}) which is not statistically different from that seen after nasal spray (6.18 h^{-1}) and i.v. bolus (6.77 h^{-1}) (Table 2), indicating that the nasal mucosa has no significant reservoir capacity for progesterone.

To evaluate the extent of hepatogastrointestinal elimination, oral administration was also carried out using a progesterone solution (60 μg/kg). A peak plasma concentration of 3.2 ng/ml was achieved within 10–15 min after dosing, and the

elimination rate constant was similar to i.v. bolus injection (6.81 h^{-1} vs. 6.77 h^{-1}). Oral systemic bioavailability was approximately 7.9%, which is significantly less than the bioavailability achieved by the nasal route of administration (72.4–82.5%). The results demonstrate that nasal delivery can result in as much as a 10-fold reduction in the hepatogastrointestinal first pass elimination of progesterone.

Metabolite studies

Plasma levels of 20-dihydroprogesterone, a biologically active metabolite which is reportedly formed in the target tissues in humans (Padwich et al., 1986; Morville et al., 1982; Whitehead et al., 1980), was also determined in this investigation following progesterone administration in both ovary-intact and ovariectomized rabbits. The plasma concentration-time profiles of both progesterone and 20-dihydroprogesterone after oral administration of progesterone to ovary-intact rabbits are shown in Fig. 7. While the rate constant of formation of 20-dihydroprogesterone from progesterone cannot be determined from this data, the elimination half-life of 20-dihydroprogesterone was calculated to be approximately 3 min in a separate study with i.v. administration of 20-dihydroprogesterone (60 μg/kg) to 4 ovary-intact rabbits. This is slightly shorter than the elimination half-life of progesterone (6 ± 1.2 min).

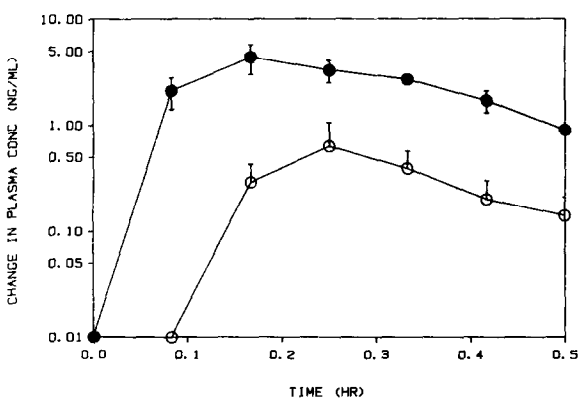

Fig. 7. Time course for the change in the plasma concentration of progesterone (●) and its metabolite, 20-dihydroprogesterone (○) following oral administration of progesterone to ovary-intact rabbits ($n = 4$).

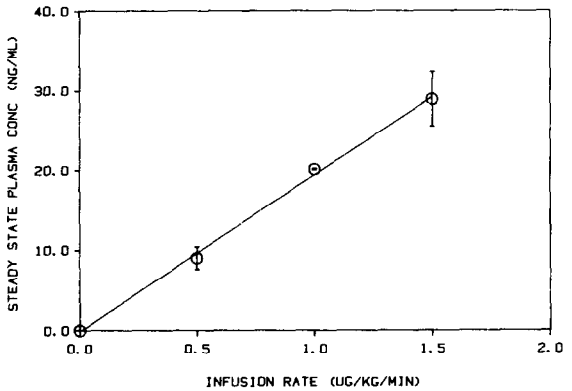

Fig. 8. Linear relationship between the steady-state plasma concentration and the correspondent i.v. infusion rate of progesterone in ovariectomized rabbits ($n = 4$).

Plasma concentrations of 20-dihydroprogesterone in the ovariectomized rabbit were undetectable following various routes of progesterone administration. This indicates that, in the rabbit, this metabolite is largely formed in the ovary. However, it is surprising to note that the calculated systemic clearance of progesterone in the ovariectomized rabbits after its oral administration was not significantly different from the ovary-intact rabbits (18.20 ± 2.57 liters/h vs. 17.55 ± 2.02 liters/h). Apparently, there are alternative metabolic sites and pathways (Senciall et al., 1981; Senciall et al., 1978), so that ovary removal does not affect the systemic clearance of progesterone.

In-vitro / in-vivo correlation

To estimate the transnasal delivery rate of progesterone by the nasal delivery device, the steady-state plasma level of progesterone was investigated at 3 different i.v. infusion rates (Fig. 8). A linear relationship was obtained which indicates that progesterone follows linear pharmacokinetics in the dosage range studied. Based on this linear relationship, the steady-state plasma progesterone level (1.5 ng/ml) observed following the administration of the nasal device corresponds to an average input rate of 0.104 μg/kg/min of progesterone. For a 3.5 kg rabbit, this represents a transnasal delivery rate of 364 ng/min, or a total dosage of 131 μg delivered nasally over 6 h. This

estimated in-vivo rate of nasal delivery of 364 ng/min is considerably greater than the release rate of 86 ng/min determined from the in-vitro studies (Fig. 2B, C). This difference could result from the in-vitro release conditions (4.5 ml of receptor fluid) not simulating those in-vivo. As discussed earlier, during the in-vitro studies, inflow of the elution medium via the device pores could affect the outflow diffusion of the drug from the nasal device, and therefore decrease the apparent in-vitro release rate of progesterone. Thus, although the in-vitro data is valuable in assessing the drug release kinetic profile, the in-vitro release rate obtained may not be used to predict the in-vivo rate.

In summary, administration by the nasal route has resulted in a systemic bioavailability which is significantly greater than by oral administration. Although there is no significant difference in nasal bioavailability via spray or device, the nasal delivery device has significantly prolonged the elevated plasma level of progesterone, as compared to nasal spray. In subsequent studies, which are currently underway, the nasal spray and nasal device are also being used to provide the immediate and controlled delivery of the hydroxy derivatives of progesterone, and to study the effect of hydrophilicity on nasal absorption and systemic bioavailability.

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References

David, G.F.X., Puri, C.P. and Kumar, T.C.A., Bioavailability of progesterone enhanced by intranasal spraying. *Experientia,* 37 (1981) 533–534.

Hussain, A.A., Hirai, S. and Bawarshi, R., Administering natural female sex hormones. *US patent 4, 315, 925,* Feb. 16, 1982.

Hussain, A.A., Hirai, S. and Bawarshi, R., Nasal absorption of natural contraceptive steroids in rats – Progesterone absorption. *J. Pharm. Sci.,* 70 (1981) 466–467.

Hussain, A.A., Kimura, R. and Huang, C.H., Nasal absorption of testosterone in rats. *J. Pharm. Sci.,* 73 (1984) 1300–1301.

Kotani, A., Hayashi, M. and Awazu, S., Selection of volume indicator for the study of nasal drug absorption. *Chem. Pharm. Bull.*, 31 (1983) 1097–1100.

Kumar, T.C.A., David, G.F.X., Sankaranarayanan, A., Puri, V. and Sundram, K.R. Pharmacokinetics of progesterone after its administration to ovariectomized rhesus monkeys by injection, infusion, or nasal spraying. *Proc. Natl. Acad. Sci. U.S.A.,* 79 (1982) 4185–4189.

Maxon, W.S. and Hargrove, J.T., Bioavailability of oral micronized progesterone. *Fertil. Steril.,* 44 (1985) 622–626.

Morville, R., Dray, F., Reynier, J. and Barrat, J., Bioavailability of natural progesterone given by mouth. *J. Gynecol. Obstet. Biol. Reprod.,* 11 (1982) 355–363.

Ohman, L., Hahnenberger, R. and Johansson, E.D.B., Topical administration of progestational steroids in the eye and nose. A rapid absorption to the blood. *Contraception,* 18 (1978) 171–179.

Ottoson, U.B., Carlstrom K., Damber, J.E. and VonSchoultz, B., Serum levels of progesterone and some of its metabolites including deoxycorticosterone after oral and parenteral administration. *Br. J. Obstet. Gynecol.,* 91 (1984) 1111–1119.

Padwich, M.L., Endacott, J., Matson, C. and Whitehead, M.I., Absorption and metabolism of oral progesterone when administered twice daily. *Fertil. Steril.,* 46 (1986) 402–407.

Senciall, I.R. and Dey, A.C., Acidic steroid metabolites: Tritium transfer and the in-vitro formation of 4-pregnene-3,20-dione-21-oic acid by rabbit and rat hepatic and extrahepatic tissues. *J. Steroid Biochem.,* 9 (1978) 1093–1097.

Senciall, I.R., Ian, R., Dey, A.C. and Rahal, S., C-21 and C-6 hydroxylation of progesterone by detergent solubilized hepatic microsomal fraction. *J. Steroid Biochem.,* 14 (1981) 281–284.

Whitehead, M.I., Townsend, R.T., Gill, D.K., Collins, W.P. and Campbell, S., Absorption and metabolism of oral progesterone. *Br. Med. J.,* 22 (1980) 825–827.