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Nasal delivery of progestational steroids in ovariectomized rabbits. II. Effect of penetrant hydrophilicity

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Summary

The influence of penetrant hydrophilicity on nasal pharmacokinetics and systemic bioavailability was studied in ovariectomized female New Zealand White rabbits. The systemic bioavailability of progesterone derivatives with one hydroxy (17-α-hydroxyprogesterone, HP), two hydroxy (cortexolone, CT), and three hydroxy (hydrocortisone, HC) groups were compared to progesterone, in separate crossover studies, following i.v., oral, and nasal administrations. Nasal administration was accomplished using an immediate-release nasal spray formulation and a controlled-release nasal device. The rank order of systemic bioavailability after nasal spray was HP > P > CT > HC (97.10, 82.52, 71.99 and 60.90%, respectively), which correlates in a hyperbolic pattern with the nasal mucosa partition coefficients of the drugs. The controlled-release nasal device achieved a more prolonged and steady plasma level of drug than the other routes of administration. The systemic bioavailabilities of progesterone and its hydroxy derivatives after nasal administration were all significantly greater ($P < 0.01$) than those after oral administration (P, 7.87%; HP, 5.93%; CT, 5.88%; HC, 2.67%). The results of this investigation demonstrate that the extent of nasal absorption is influenced by both the mode of nasal administration and the hydrophilicity of the penetrant, as expressed by the nasal mucosa partition coefficient.

Introduction

Transnasal absorption has been reported to enhance systemic bioavailability for a wide variety of drugs, including peptides like insulin (Hirai et al., 1978; Hirai et al., 1981a; Nagai et al., 1985) and leuprolide (Okada et al., 1982), and steroids like progesterone (Hussain et al., 1981; David et al., 1981; Kumar et al., 1982) and 17-β-estradiol (Hussain et al., 1982). Although several studies have explored the structure–function relationships of nasal absorption enhancers (Hirai et al., 1981b; Okada et al., 1982; Gordon et al., 1985; Duchateau et al., 1986), few studies have investigated the effect of structural modification of the drug molecule on nasal absorption.

The objective of this series of investigations is to study the effect of a variation in the hydrophilicity of the penetrant on nasal absorption. The influence of the mode of drug delivery on the relationship between nasal bioavailability and penetrant hydrophilicity is also investigated using

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an immediate release nasal spray and a controlled release nasal device. In the first investigation (Corbo et al., 1988), the pharmacokinetics of progesterone following administration via i.v. bolus injection, oral solution, nasal spray and controlled-release nasal device were described. In order to gain some insight into the mechanism of nasal absorption, in this study, a series of progesterone derivatives with a systematic variation in hydrophilicity were studied to evaluate the effect of hydrophilicity on transnasal absorption and pharmacokinetic profile. The pharmacokinetics and systemic bioavailability of a monohydroxy (17-α-hydroxyprogesterone, HP), dihydroxy (cortexolone, CT), and trihydroxy (hydrocortisone, HC) derivative of progesterone (P) after nasal administration is reported. Oral pharmacokinetics and bioavailability have also been investigated for comparison.

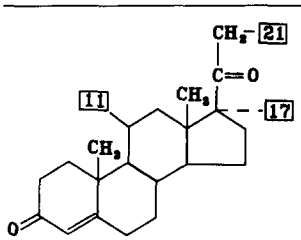
Experimental

Progesterone derivatives

A homologous series of P derivatives, differing in the number of hydroxy groups, was investigated (Table 1). P, HP, CT, and HC were obtained from Sigma Chemical Co. Literature values for the octanol–water partition coefficients of progesterone and its hydroxy derivatives, determined by high performance liquid chromatography (Caron et al., 1984), are shown in Table 2.

TABLE 1

Chemical structure of P and derivatives



Drug	Hydroxy group position
Progesterone (P)	–
17-α-OH progesterone (HP)	17
Cortexolone (CT)	17, 21
Hydrocortisone (HC)	11, 17, 21

TABLE 2

Log P values of progesterone derivatives

Compound	Log P *
Progesterone (P)	3.26
17-α-Hydroxyprogesterone (HP)	2.74
Cortexolone (CT)	2.04
Hydrocortisone (HC)	1.50

Data from Caron and Shroot (1984). * Log P: log of octanol–water partition coefficient.

Controlled release nasal device

The controlled release nasal device was fabricated using the method previously described (Corbo et al., 1988). Briefly, a thin-wall polyethylene tube (Intramedic, PE-60) was inserted into a 5 cm × 2 mm microporous membrane sleeve (Spectrapor, 12,000–14,000 mol. w. cutoff), and both ends of the sleeve were then sealed. As shown in Fig. 1, the device was inserted into the rabbit nasal passage, filled with 0.35 ml of drug suspension, and left in place throughout the course of the 6-h study. The filled device conformed to the contour of the nasal cavity (confirmed by surgical observation), which maximized the area in contact with the mucosa for drug absorption.

An isotonic solution of 20% PEG 4000 was used to prepare the drug suspension, which was buffered to the pH of the rabbit nasal secretions (pH 8) using 0.07 M Na_2HPO_4 and 0.07 M KH_2PO_4. Previous studies indicated that PEG 4000 is released from the device, but is not adsorbed through the rabbit nasal mucosa.

Nasal mucosa partitioning study

Nasal mucosa was excised from the rabbit nasal septum and sliced into 20 mg sections. The nasal mucosa slices obtained were incubated in a 1 μg/ml drug solution ($n = 3$) in isotonic phosphate buffer (0.07 M, pH 8) at 36 °C for 4 h. Solutions containing drug without mucosa and mucosa

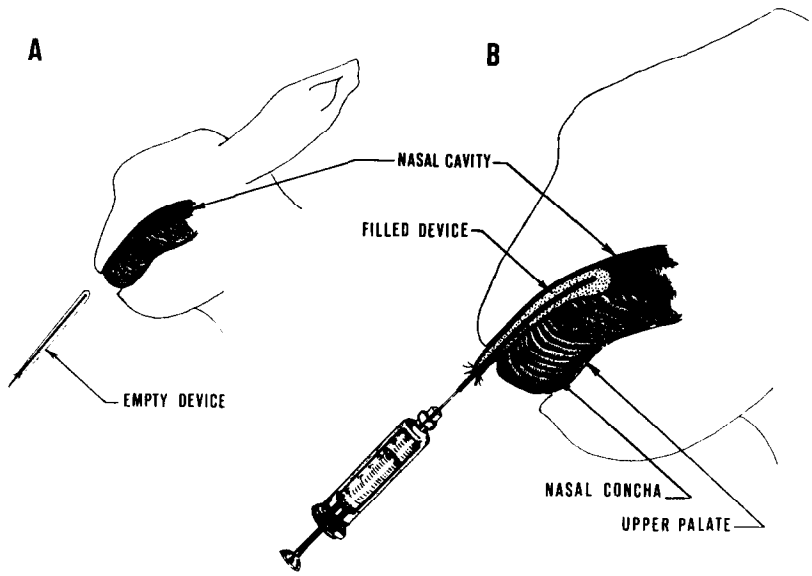


Fig. 1. Diagrammatic illustration of the controlled-release nasal device prior to insertion into the rabbit nasal passage (A), and the in-situ filling of the device with drug formulation in the nasal cavity (B).

without drug were also incubated as controls. After the incubation period, the mucosa was removed from the incubation solution and extracted with 3 ml of chloroform twice for 15 min. The extracts were evaporated to dryness under a stream of nitrogen, and reconstituted with 0.2 ml of methanol.

Drug concentrations in the incubation and extraction solutions were assayed by a HPLC method with UV detection at 240 nm (Waters Model 590 solvent delivery system, WISP 710B, Kratos Spectroflow 783 detector), using a 15 cm μBondapak C-18 column (Waters Associates). The mobile phase consisted of methanol : water (2 : 1 for P and HP, 1 : 1 for CT and HC), at a flow rate of 1.5 ml/min. The coefficients of variation for the HPLC assays were less than 4% for all derivatives. No interfering peaks were observed in the control solution containing mucosa without drug.

Since the control solution containing drug without mucosa showed no change in drug concentration, the amount of drug partitioning into the mucosa was calculated by the drug loss from the incubation solution. The amount of drug extracted from the mucosa was also determined for verification.

In-vivo studies

As in the P study, the in-vivo evaluations of the hydroxy derivatives of P were performed in the ovariectomized female New Zealand White rabbit (3–4 kg). Animals were anesthetized with Ketamine (35 mg/kg) and Xylazine (4 mg/kg) (Butler Veterinary Supply Co.) before surgery and during all treatments. Ovariectomies were performed under sterile surgical conditions, and animals were permitted a two-week recovery period before the initiation of a study. The mean baseline plasma concentrations of the derivatives after the ovariectomy were as follows: P, 0.3 ng/ml; HP, 2.1 ng/ml; CT, 0.7 ng/ml; and HC, 13.2 ng/ml.

Each of the steroids were investigated in a separate randomized cross-over study. There were 4 rabbits in each of the four cross-over studies, and 16 rabbits in total. The cross-over treatments consisted of an i.v. bolus injection (60 μg/kg), an oral administration (60 μg/kg), and nasal administrations by controlled release nasal device (60 μg/kg) and nasal spray (2 μg/kg). Animals were fasted for 12 h prior to drug administration, and a one-week washout period was allowed between treatments.

To confirm that linear pharmacokinetics ex-

isted in the dosage range studied, an i.v. infusion of each steroid was performed in another group of 4 rabbits (16 rabbits total). The steady-state plasma concentration was assessed at 3 different infusion rates (0.5, 1 and 1.5 μg/kg/min).

A 10% ethanolic solution of drug in normal saline was used for i.v. infusion, oral and nasal spray administrations, while isotonic 20% PEG 4000 in phosphate buffer was used for the nasal device. The oral solution was delivered via a gastric tube, and the nasal spray was administered by two actuations of a 100 μl-metered dose pump (BLM Packaging Co.). The exact dose of drug delivered by the nasal device was determined by assaying the amount of drug remaining in the device at the end of the 6-h study period, with UV spectrophotometry (Perkin Elmer Model 559A).

Blood samples for pharmacokinetic analysis were withdrawn via a catheter (22 gauge) from the central ear artery at predetermined time intervals, and collected in heparin-treated tubes. Samples were immediately centrifuged, and the plasma was separated and stored at −5 °C until assay. The plasma concentrations of P and HP were assayed using commercially available radioimmunoassay kits (InterSci Diagnostics) with high selectivity and sensitivity (coefficient of variation: P, 10.4; HP, 6.2). Plasma levels of CT and HC were analyzed using highly specific commercially available antisera (ICN Immunobiologicals; coefficient of variation: CT, 11.2; HC, 9.7). Tritium-labelled CT and HC were obtained from New England Nuclear Products. Samples were counted in a liquid scintillation spectrometer (LKB Wallac Model 1214 Rackbeta).

Data analysis

Plasma–drug concentration–time profiles for individual rabbits were analyzed using a one-compartment pharmacokinetic model. The value of the elimination rate constant (k_e) was determined from linear regressional analysis of the terminal log-linear segment of the plasma concentration–time profile. The area under the plasma concentration–time curve from time zero to time t (AUC_t) was calculated using the linear trapezoidal method. The area under the curve to time infinity (AUC) was then extrapolated by the relationship:

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

where Cp^t is the drug concentration at the last sampling time (t) with a measurable Cp. For the controlled release nasal device, the slope of the elimination phase following the nasal spray administration was used to calculate AUC to infinity, since it has been shown that the nasal mucosa does not have a significant reservoir capacity for these drugs.

The remaining pharmacokinetic parameters were calculated using the following relationships:

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

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

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

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

All of the data are expressed as the mean value ± S.E.M. Statistical analysis was performed using an analysis of variance (X-Stat, Wiley Professional Software).

Results and Discussion

The relationships between the steady-state concentrations of P and its hydroxy derivatives and the corresponding i.v. infusion rates are shown in Fig. 2. The linear relationships obtained indicated that linear pharmacokinetics existed in the dosage range studied, which allowed comparison between treatments with different drug dosages.

The plasma concentration profiles of P and its hydroxy derivatives after i.v. administration are shown in Fig. 3. The exponential decline observed in the plasma concentrations suggested that the systemic elimination of all of the steroids could be described by a one-compartment pharmacokinetic model. The pharmacokinetic parameters calculated for progesterone and its hydroxy derivatives

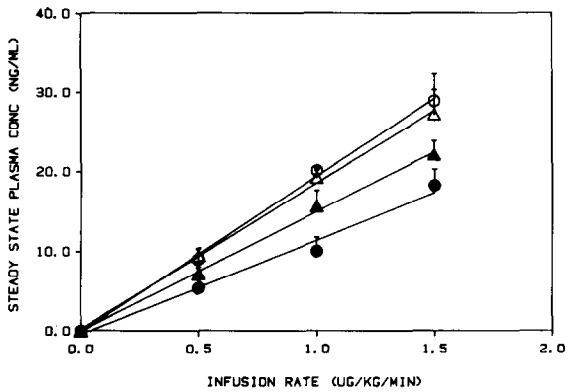


Fig. 2. Linear relationship between steady-state plasma concentrations and corresponding i.v. infusion rates for P (○), HP (●), CT (△), HC (▲) ($n = 4$).

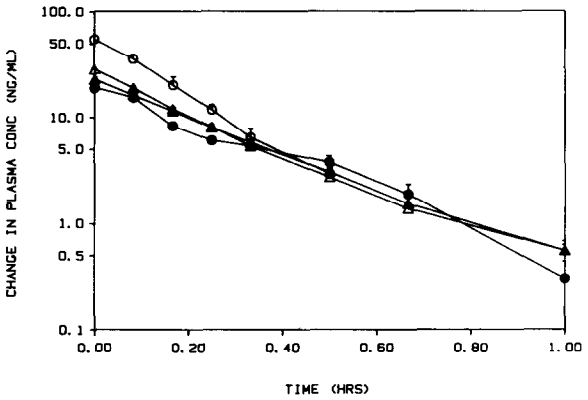


Fig. 3. Time course for the change in plasma concentrations following the i.v. injection (60 μg/kg) of P (○), HP (●), CT (△), and HC (▲) ($n = 4$).

after i.v. bolus administration are summarized in Table 3.

The plasma concentration–time profiles following oral administration are compared in Fig. 4. The time to reach the peak plasma concentration (t_{max}) was approximately 15 min for P and HP, and 10 min for CT and HC. The first-order rate constants of elimination for the P derivatives were similar to those observed after their i.v. bolus injection. The oral bioavailabilities for P and its hydroxy derivatives were very low, and appeared to decrease as the hydrophilicity of the steroid was increased (P, 7.87%; HP, 5.93%; CT, 5.88%; HC, 2.67% (Table 4).

As can be seen in Fig. 5, administration of P and its hydroxy derivatives by nasal spray led to a rapid attainment of peak plasma levels. The time to reach peak concentration (t_{max}) was less than 2 min for P, less than 5 min for HP and CT, and 5–10 minutes for HC. The absorption of drug after the nasal spray was too rapid to permit estimation of in-vivo absorption rate. Using an ex-vivo method in rats, Gibson and Olanoff (1987) found that the more lipophilic progesterone had a faster rate of nasal absorption than the more hydrophilic hydrocortisone.

The results in Table 5 indicate that the systemic bioavailability following nasal spray administration was greater for HP (97.10%) than for P, CT and HC (82.52, 71.99 and 60.90%, respectively). While these differences were not statistically significant, the addition of one hydroxy group at the 17-position appeared to enhance the nasal bioavailability of P. This result cannot be predicted from the magnitude of the octanol–water partition coefficients of P and its hydroxy derivatives (Table 2). Other investigators have noted a lack of correlation with octanol–water partition coefficients in the relative nasal absorption of various drugs (Huang et al., 1985a; Huang et al., 1985b),

TABLE 3

Pharmacokinetic parameters of P and its hydroxy derivatives following i.v. administration in ovariectomized rabbits

Parameter	P	Hp	CT	HC
k_e (1/h)	6.77(±0.16)	4.05(±0.45)	5.05(±0.14)	4.14(±0.24)
$t_{1/2}$ (h)	0.10(±0.01)	0.18(±0.02)	0.14(±0.01)	0.17(±0.01)
V_d (L)	2.80(±0.23)	13.60(±0.35)	7.94(±0.48)	9.17(±1.00)
Cl (L/h)	18.91(±1.52)	58.19(±2.21)	40.20(±2.90)	37.24(±3.36)
AUC (ng*h/ml)	10.47(±0.19)	5.10(±0.01)	6.04(±0.20)	5.71(±0.11)
AUC/Dose × 10^3 (h/liter)	53.17(±2.70)	20.05(±0.39)	27.58(±1.75)	28.80(±2.41)

Values are mean (±S.E.M.).

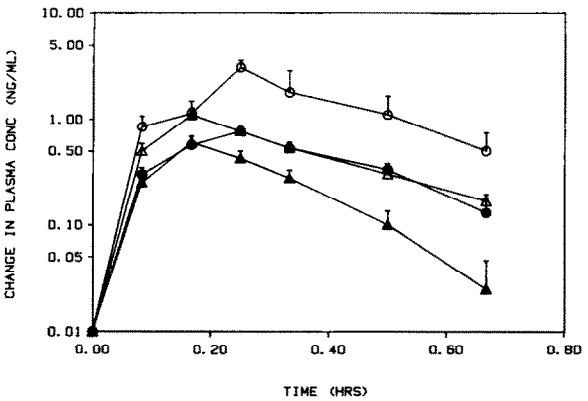


Fig. 4. Time course for the change in plasma concentrations after oral administration (60 μg/kg) of P (○), HP (●), CT (△) and HC (▲) ($n = 4$).

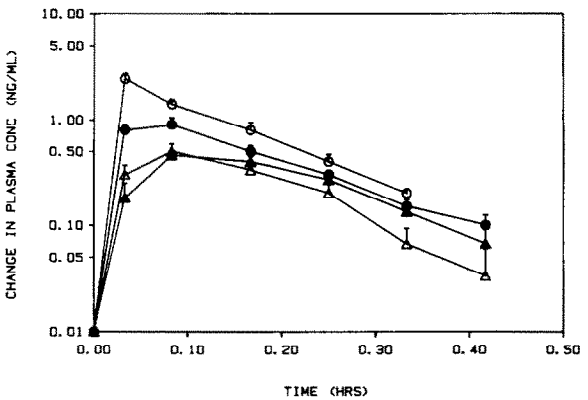


Fig. 5. Time course for the change in the plasma concentrations after intranasal administration (2 μg/kg), using nasal spray, of P (○), HP (●), CT (△), and HC (▲) ($n = 4$).

and in the skin permeability of phenolic compounds (Jetzer et al., 1986).

In an attempt to gain a better understanding of the role of partitioning in the nasal absorption of P and its hydroxy derivatives, a partition study of these compounds in the rabbit nasal mucosa was conducted. The results, calculated using drug loss from solution, are shown in Table 6. Partition coefficients calculated from the mucosa extraction method were similar, but slightly lower, possibly due to drug binding. There were no HPLC peaks attributable to metabolites observed in the chromatograph after incubation of the mucosa in the drug solution.

As shown in Table 6, the experimental value for the nasal mucosa partition coefficient for the 17-hydroxy derivative of P was 2.5 times that of the parent compound. The results indicated that the octanol–water partition coefficient does not reflect the hydrophilicity of P and its hydroxy

TABLE 4

Pharmacokinetic parameters of P and its hydroxy derivatives following oral administration in ovariectomized rabbits

Parameter	P	HP	CT	HC
k_e (1/h)	6.81(±0.70)	3.63(±0.25)	4.31(±0.22)	5.54(±0.44)
$t_{1/2}$ (h)	0.10(±0.02)	0.19(±0.01)	0.18(±0.01)	0.13(±0.01)
AUC (ng*h/ml)	0.83(±0.16)	0.30(±0.03)	0.36(±0.05)	0.14(±0.04)
AUC/Dose × 10^3 (h/liter)	4.67(±0.90)	1.16(±0.11)	1.70(±0.31)	0.72(±0.15)
F (%)	7.87(±1.59)	5.93(±0.56)	5.88(±0.67)	2.67(±0.68)

Values are mean (±S.E.M.).

TABLE 5

Pharmacokinetic parameters of P and its hydroxy derivatives following intranasal administration by nasal spray in ovariectomized rabbits

Parameter	P	HP	CT	HC
k_e (1/h)	6.18(±0.10)	7.79(±0.36)	5.97(±0.38)	5.04(±0.66)
$t_{1/2}$ (h)	0.11(±0.01)	0.09(±0.01)	0.12(±0.01)	0.14(±0.01)
AUC (ng*h/ml)	0.21(±0.04)	0.12(±0.03)	0.11(±0.01)	0.11(±0.01)
AUC/Dose × 10^3 (h/liter)	42.00(±5.41)	19.42(±2.90)	18.42(±1.89)	18.07(±2.05)
F (%)	82.52(±13.50)	97.10(±12.13)	71.99(±12.52)	60.90(±5.75)

Values are mean (±S.E.M.).

TABLE 6

Nasal mucosa partition coefficients for P and its hydroxy derivatives

Compound	Partition coefficient
Progesterone (P)	180.2 (±26.0)
17-α-Hydroxyprogesterone (HP)	441.7 (±176.8)
Cortexolone (CT)	99.6 (±46.9)
Hydrocortisone (HC)	6.3 (±1.2)

derivatives with respect to partitioning behavior in the nasal mucosa. This lack of correlation between octanol–water partition coefficient and nasal mucosa partition coefficient is not surprising in view of the complex nature of the nasal mucosa.

The relationship between systemic bioavailability by nasal spray and nasal mucosa partition coefficient is shown in Fig. 6. It was observed that nasal bioavailability increased linearly with increasing nasal mucosa partition coefficients ($r = 0.995$), and approached a plateau at partition coefficients above 200. The correlation between nasal bioavailability and nasal mucosa partition coefficient may be a result of the competition between the rate of absorption and the rate of mucociliary clearance. The derivatives with higher partition coefficient have higher absorption rates (Gibson et al., 1987), which may result in reduced mucociliary drug clearance and greater extents of absorption.

The plasma concentration profiles of P and its hydroxy derivatives after intranasal administration via the controlled release nasal device are shown in Fig. 7. As with P, the nasal device produced a prolonged, steady plasma level of the hydroxy derivatives. The systemic bioavailability of HP after nasal device administration (87.97%) was greater than that of P, CT and HC (72.37, 80.87% and 77.72%, respectively), but the difference was not statistically significant (Table 7).

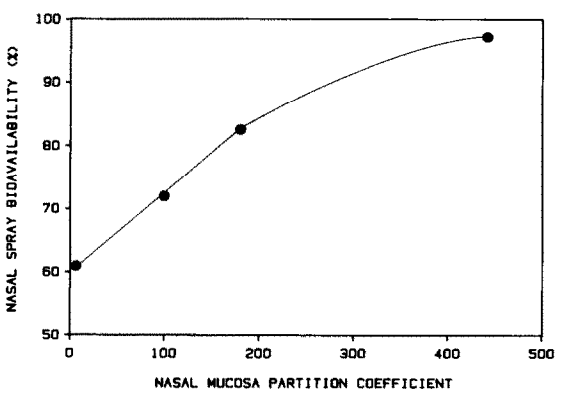


Fig. 6. Relationship between systemic bioavailability following nasal spray administration and nasal mucosa partition coefficients for P progesterone and its hydroxy derivatives.

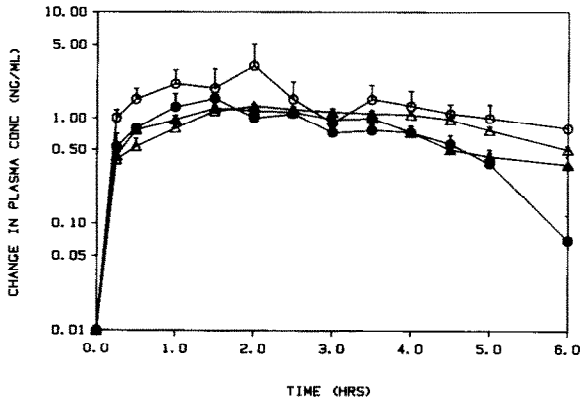


Fig. 7. Time course for the change in the plasma concentrations after intranasal administration (60 μg/kg), using controlled release nasal device, of P (○), HP (●), CT (△), and HC (▲) ($n = 4$).

Systemic bioavailability after nasal administration, by either nasal spray or nasal device, was significantly greater ($P < 0.01$) than oral bioavailability for all 4 progestins. The results demonstrated that nasal delivery can result in a 10–30-fold reduction in the hepatogastrointestinal first-pass metabolism of P and its derivatives.

The systemic bioavailability of HC was significantly greater ($P = 0.02$) after administration by nasal device as compared to nasal spray (77.72% vs 60.90%). The results indicated that the mode of nasal administration may influence systemic bioavailability. As discussed earlier, after nasal administration, a competition may exist between the rates of drug absorption and drug mucociliary clearance. Due to its ability to conform to the nasal mucosa surface, the nasal delivery device may interfere with mucociliary clearance. This would allow a slower absorbed, more hydrophilic drug, like HC, to remain at the absorption site for a longer period of time, resulting in a more complete absorption than that achieved by nasal spray.

TABLE 7

Pharmacokinetic parameters of P and its hydroxy derivatives following intranasal administration by controlled release nasal device in ovariectomized rabbits

Parameter	P	HP	CT	HC
AUC (ng*h/ml)	8.04(±3.71)	4.49(±0.56)	5.22(±0.16)	4.52(±0.09)
AUC/Dose × 10^3 (h/liter)	37.50(±14.80)	16.93(±0.86)	22.42(±0.72)	21.66(±1.69)
F (%)	72.37(±25.71)	87.97(±10.78)	80.87(±5.33)	77.72(±4.35)

Values are mean (±S.E.M.)

The in-vitro permeation rate of P and its hydroxy derivatives across hairless mouse skin was previously investigated and reported to depend on both the the number and position of the hydroxy groups with the rank order of P > CT = HP > HC (Tojo et al., 1987). A similar relationship was found in this investigation for the extent of oral absorption, but not for the extent of nasal absorption. The mechanism of absorption for the nasal mucosa membrane appears to differ from that of the gastrointestinal tract and skin. It may be concluded that the extent of nasal absorption of P and its hydroxy derivatives is influenced by both the mode of nasal administration, and the hydrophilicity of the penetrant, as expressed by the nasal mucosa partition coefficient.

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