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MUCOSAL DELIVERY OF PROGESTATIONAL STEROIDS FROM A CONTROLLED RELEASE DEVICE: IN-VITRO/IN-VIVO RELATIONSHIPS

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ABSTRACT

The mucosal delivery of progestational steroids from a model controlled release device was studied using female

New Zealand White rabbits as the animal model. A controlled release device was developed which was suitable for nasal, rectal and vaginal application. The in-vitro permeation and in-vivo absorption of progesterone from the model controlled release device was investigated through the nasal, rectal and vaginal mucosa. The influence of penetrant hydrophilicity on the in-vitro permeation and in-vivo absorption from the controlled release device was also investigated using the mono-hydroxy, di-hydroxy and tri-hydroxy derivatives of progesterone. The results indicate that the nasal route demonstrates a significantly higher rate of in-vitro permeation and extent of in-vivo absorption than the rectal and vaginal mucosa. The in-vitro permeation rates and steady-state plasma concentrations achieved from the device tend to decrease with increasing penetrant hydrophilicity. A linear in-vitro/in-vivo correlation was obtained for all the mucosa studied. The slope of the relationship between the in-vitro and in-vivo data was similar for the rectal and vaginal mucosa. The results of this investigation agree with the results of a previous investigation with a solution formulation, and suggest that the hydrodynamic and/or membrane barrier properties of the nasal mucosa may differ from that of the rectal and vaginal mucosa.

INTRODUCTION

Administration of drugs via mucosal membranes may bypass the hepato-gastrointestinal "first-pass" metabolism associated with oral administration. This property of mucosal membranes, as well as their dual hydrophilic-lipophilic nature, has resulted in a growing interest in using mucosal membranes as sites for controlled drug delivery. In recent years, there have been many reports of successful delivery of drug entities via the nasal, rectal or vaginal mucosa. Compounds of both a hydrophilic and lipophilic nature have been absorbed through these membranes[1-6]; however, few studies have compared drug absorption from these membranes.

In a previous study, steroid absorption from solution formulations were compared for the nasal, rectal and vaginal mucosa of the rabbit[7]. In the in-vivo study, nasal absorption from progestin solution formulations was significantly greater than rectal or vaginal absorption. The rate and extent of in-vivo solution absorption was found to decrease as progestin hydrophilicity increased for all mucosa. The results indicated that the absorption characteristics of the progestin solutions were influenced by the properties of both the drug molecules and the mucosal membranes. Mucosal membrane factors influencing the absorption of solution formulations may include absorption

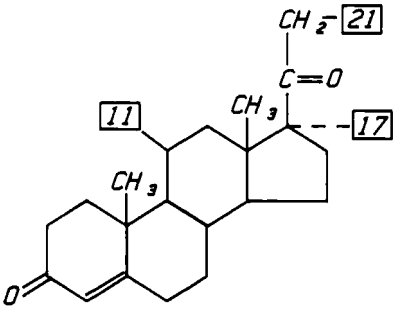
surface area, mucosal layer thickness, and solution clearance rate.

The purpose of this investigation is to expand on the previous solution study by comparing the absorption characteristics of progestins from a model controlled release device. The model controlled release device was designed to be suitable for administration to the rabbit nasal, rectal and vaginal cavities. As in the solution studies, in this investigation, the rate of in-vitro permeation and extent of in-vivo absorption of progesterone from the model controlled release device is investigated through the mucosal membranes. The influence of penetrant hydrophilicity on the in-vitro permeation and in-vivo absorption through these mucosal membranes is also compared using a series of progesterone derivatives. The in-vitro permeation and in-vivo absorption data for the progestins are used to make an in-vitro/in-vivo correlation for the nasal, rectal and vaginal mucosa.

METHODS

Compounds Investigated

A homologous series of progesterone derivatives, possessing the same basic steroid nucleus, but differing in the number of hydroxy groups, was investigated. The structures of progesterone, and its mono-hydroxy (17-alpha



DRUG	HYDROXY GROUP POSITION
PROGESTERONE	NONE
MONO-HYDROXY (17-OH PROGESTERONE)	17
DI-HYDROXY (CORTEXOLONE)	17, 21
TRI-HYDROXY (HYDROCORTISONE)	11, 17, 21

FIGURE 1
Chemical structure of progesterone and hydroxy derivatives

hydroxyprogesterone), di-hydroxy (cortexolone) and tri-hydroxy (hydrocortisone) derivatives are shown in Figure 1. The progesterone derivatives, and all other chemicals and reagents used in this study, were obtained from Sigma Chemical Company.

Fabrication of Controlled Release Device

The controlled release device was fabricated using a silicone elastomer base (Dow Corning 4-4210). Drug was weighed and dissolved in a small volume of chloroform (1-2ml), and then incorporated into an appropriate amount of silicone elastomer. The mixture was stirred rapidly (Cole Palmer Laboratory Mixer) for 8 minutes to allow for complete recrystallization of drug in the elastomer. After de-aeration under vacuum for 30 minutes, the drug-elastomer mixture was

molded for the in-vitro and in-vivo studies. In order to simplify comparisons of in-vivo absorption from the device, it was desired to develop one device suitable for administration to the nasal, rectal and vaginal cavities. After some preliminary studies, it was determined that a cylindrical device (with a length of 3cm and a diameter of 2mm) would meet this criteria. Following de-aeration, the drug-polymer mixture was loaded into a disposable syringe, and extruded into thermoplastic tubing (C-Flex, Fisher Scientific). After molding, all devices were cured for 3 hours in a 60°C oven. Devices were then removed from the tubing, and cut into 3cm lengths.

In-Vitro Release Studies-

In-vitro release studies were conducted to determine the effect of the loading dose of drug and silicone fluid on the release profiles from the device. Preliminary in-vitro release studies from the cylindrical-shaped devices were conducted by shaking in test-tubes, and were complicated by hydrodynamic boundary layer effects. To overcome the hydrodynamic problems, the devices for in-vitro studies were molded into disks (4cm diameter and 0.5cm width), and cured at 60°C for 3 hours. The in-vitro release profiles of progestins from the controlled release devices were determined by mounting a disk-shaped device on a hydrodynamically well-calibrated Valia-Chien diffusion half-cell. A buffered,

isotonic solution of 10% PEG 400 (pH 7.4) was used as the receptor solution, and samples were taken at specified time intervals.

Drug concentrations for all in-vitro studies were determined by a HPLC method with UV detection at 240nm (Waters Model 590 solvent delivery system, WISP 710B, Kratos Spectroflow 783 detector), using a 15cm uBondapak C-18 column (Waters Associates). The mobile phase consisted of methanol:water (2:1 for progesterone and its mono-hydroxy derivative, 1:1 for its di-hydroxy and tri-hydroxy derivatives) at a flow rate of 1.5ml/min.

In-Vitro Permeation Studies-

To obtain the nasal, rectal and vaginal mucosa for the in-vitro permeation studies, female New Zealand White rabbits (3-4kg) were sacrificed (using T-61 Euthanasia Solution, Hoescht), and the mucosa was excised and rinsed briefly with normal saline. The in-vitro transmucosal permeation studies were conducted by placing three drops of isotonic saline on the surface of a disk-shaped controlled release device (to mimic the aqueous boundary layer under physiologic conditions), and pressing the moistened surface onto the epithelia surface of a mucosal membrane. The device-mucosa combination was then mounted over the opening of a diffusion half-cell, with the serosal side facing the receptor compartment. The receptor was filled with a buffered,

isotonic solution of 10% PEG 400 (pH 7.4), and samples were taken at pre-determined time intervals. Drug concentrations were determined using the HPLC method described in the in-vitro release section.

In-Vivo Absorption and Bioavailability Studies-

The in-vivo studies were performed in ovariectomized female New Zealand White rabbits (3-4kg). Previous studies had shown that ovariectomy resulted in low and stable baseline plasma concentrations of the progestins[8]. Rabbits were anesthetized with Ketamine (35mg/kg) and Xylazine (4mg/kg) (Butler Veterinary Supply Co.) before surgery and during all treatments. Ovariectomies were performed under sterile surgical conditions, and animals were allowed to recover over a two-week period before a study was initiated.

Each of the progestins was investigated in a separate randomized crossover study. There were four rabbits used in the crossover study of each progesterone derivative, and 16 rabbits in total. Crossover treatments consisted of a 60ug/kg IV bolus injection, and nasal, rectal and vaginal administrations by the cylindrical controlled release device. A 10% ethanolic solution of drug in normal saline was used for the IV solution administrations. Rabbits were fasted 18 hours prior to drug administration, and a one-week washout period was allowed between drug treatments.

At the completion of each mucosal study, the controlled release device was removed, and the amount of drug remaining in the device was determined. The removed device was homogenized, and extracted with 30ml of methanol for 24 hours. The drug content in the methanol extract was then analyzed using the HPLC system previously described. The amount of drug remaining in the device was used to determine the mucosal dose for the bioavailability calculations.

To determine the progestin plasma concentrations for pharmacokinetic analysis, blood samples were withdrawn from the central ear artery of the rabbit at pre-determined time intervals. Samples were immediately centrifuged, and the plasma was separated and stored at -5°C until assay. The plasma concentrations of progesterone, and its mono-hydroxy and tri-hydroxy derivatives were assayed using radioimmunoassay kits obtained from Diagnostic Products Corp. Plasma levels of the di-hydroxy derivative of progesterone were analyzed using a radioimmunoassay kit obtained from ICN Biomedicals, Inc.. Radioimmunoassay samples were counted in a gamma counter (Nuclear Chicago- Searle).

Pharmacokinetic Data Analysis-

Plasma drug concentration-time profiles for individual rabbits were analyzed using a one-compartment pharmacokinetic model. The area under the plasma concentration-time curve was calculated using the linear trapezoidal method.

Bioavailability was calculated by the ratio of the area under the curve for a route of administration (normalized for dose) to the area under the curve for IV bolus administration.

Statistical Analysis-

The values for all experimental data are expressed as the mean (+/-) the standard deviation of 4 determinations. An analysis of variance was performed using the X-Stat computer program (Wiley Professional Software) to make statistical comparisons. Differences were considered to be significant at a level of $p < 0.05$.

RESULTS AND DISCUSSION

In-Vitro Release Studies

The in-vitro release profiles of progesterone derivatives from these matrix-type devices should be described by:

$$Q = [D_p(2A-C_p)C_pt]^{1/2} \qquad \text{(Eq. 1)}$$

where Q is the cumulative amount of drug released, D_p is the effective drug diffusivity in the polymer matrix, A is the drug load in the silicone device, C_p is the drug solubility in the silicone polymer, and t is time[9].

Equation 1 can be rearranged to describe the flux of drug release as:

$$Q/t^{1/2} = [D_p(2A-C_p)C_p]^{1/2} \qquad \text{(Eq. 2)}$$

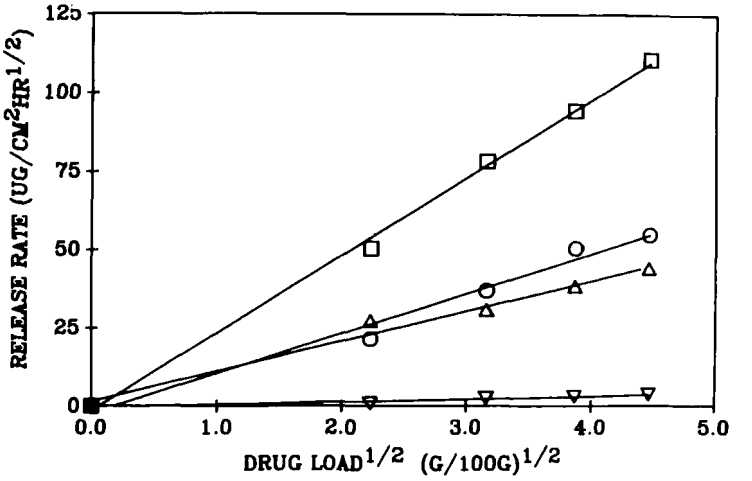


FIGURE 2
Dependence of the in-vitro release rate from the progestin controlled release device on the square root of the drug load of progesterone (□), and its mono-hydroxy (○), di-hydroxy (△) and tri-hydroxy (▽) derivatives.

The effect of increasing the loading dose of drug (A) on the in-vitro release rate for the progestins is shown in Figure 2. As predicted by Equation 2, there is a linear relationship between the release rate ($Q/t^{1/2}$) and the square root of the drug load. The release rate for the progestins at a given drug load decreases as the number of hydroxy groups on progesterone increases. This relationship was previously observed with a different silicone matrix, and was attributed to decreased polymer matrix diffusivity (D_p) and polymer solubility (C_p)[10].

In an effort to increase the release flux further, the effect of adding modifiers which might change the

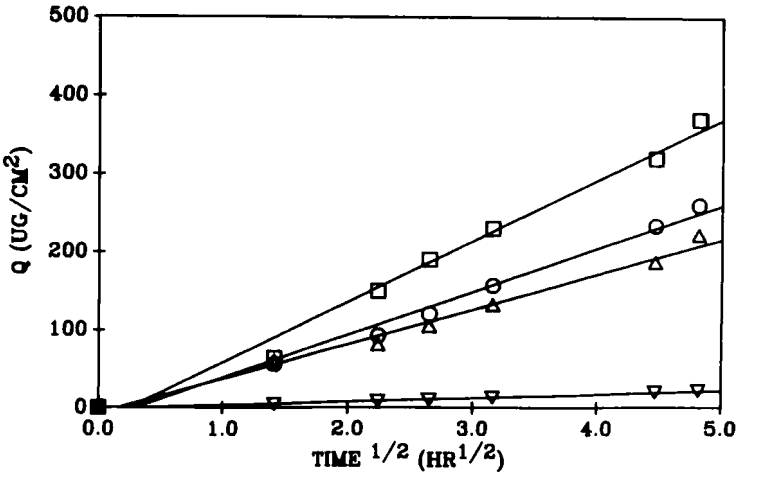


FIGURE 3
In-vitro release profiles from a controlled release device containing 20% drug load and 20% silicone fluid load for progesterone (□), and its mono-hydroxy (◯), di-hydroxy (△) and tri-hydroxy (▽) derivatives.

cross-linking density of the polymer matrix and alter drug release, was investigated. The addition of 5-20% of glycerin or polyethylene glycol 400 to this polymer matrix was found to inhibit curing. The addition of 5-20% silicone fluid to the polymer matrix did not inhibit curing, but had only a moderate effect on progestin release. Since for the in-vivo studies, it was desired to have a device with the highest possible release rate, 20% silicone fluid (which increased release flux 25%) was incorporated into the final device.

The release profiles for the progestins from the device containing a 20% drug and 20% silicone fluid load are shown in Figure 3. As predicted by Equation 1, the cumulative amount

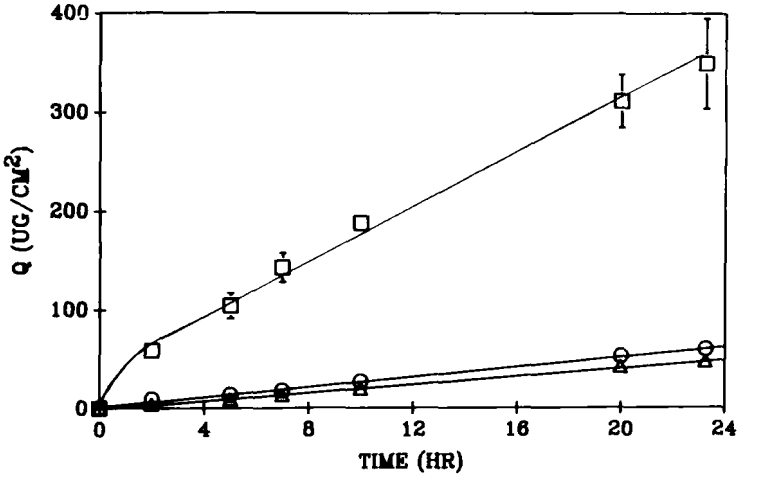


FIGURE 4
In-vitro permeation profiles of progesterone from the controlled release device through the nasal (□), rectal (○) and vaginal (△) mucosa.

of progestin released (Q) is proportional to the square root of time. The release rate fluxes for progesterone, and its mono-hydroxy, di-hydroxy and tri-hydroxy derivatives are 124.7, 62.6, 50.7 and 4.5 ug/cm^{2}hr$^{1/2}$, respectively.

In-Vitro Permeation Studies

The results of the in-vitro mucosal permeation studies for progesterone from the controlled release device are shown in Figure 4. The permeation rate is significantly higher through nasal mucosa than through rectal and vaginal mucosa. A summary of the permeation rates from the progestin devices is shown in Table 1, in conjunction with the estimated device release rates. For progesterone, and its mono-hydroxy and

TABLE 1

Summary of Release and Permeation Rates From The Controlled Release Device (ug/cm^2hr)

	Progesterone	Mono-Hydroxy	Di-Hydroxy	Tri-Hydroxy
Release	14.6 (±2.4)	8.3 (±2.1)	6.7 (±1.6)	0.7 (±0.2)
Nasal	12.6 (±2.6)	3.2 (±1.2)	3.4 (±0.9)	0.7 (±0.3)
Rectal	2.6 (±0.6)	1.3 (±0.5)	0.9 (±0.3)	0.6 (±0.1)
Vaginal	2.2 (±0.5)	1.1 (±0.3)	0.9 (±0.2)	0.5 (±0.1)

di-hydroxy derivatives, permeation rates are similar through the rectal and vaginal mucosa, while nasal permeation is significantly higher (Table 1).

The permeation profiles and rates through the nasal mucosa appear to be at least partially controlled by the release of the device for the progestin derivatives. For the tri-hydroxy derivative, which has the lowest release rate, there is no significant difference between the release rate and permeation rates through the nasal, rectal and vaginal mucosa. The results indicate that, at high device release rates, the permeation through the rectal and vaginal mucosa may be controlled by the mucosal barrier. However, at low device release rates, permeation through all of the mucosa may be controlled by the release rate of the device.

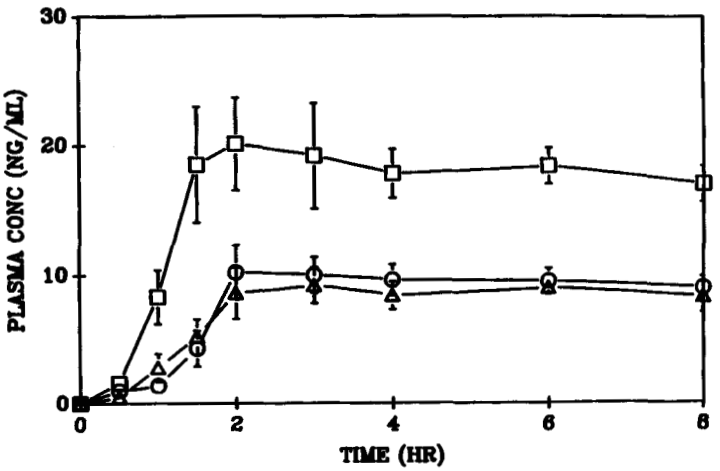


FIGURE 5
Time course for the change in plasma concentration following mucosal administration of the progesterone controlled release device by the nasal (□), rectal (○) and vaginal (△) routes.

In-Vivo Absorption and Bioavailability Studies

After IV bolus administration, progesterone and its hydroxy derivatives are rapidly eliminated. The elimination rate profiles can be adequately described by a one-compartment pharmacokinetic model. In a previous investigation in rabbits[8], it was found that the plasma profiles of the progestins follow linear pharmacokinetics. The presence of linear pharmacokinetics allows bioavailabilities to be compared for different treatment dosages.

The in-vivo mucosal absorption of progesterone from the controlled release device is shown in Figure 5. As predicted

from the in-vitro permeation studies, nasal administration results in higher plasma concentrations than rectal and vaginal administration. Based on the plasma concentration-time profile, it appears that absorption may be more rapid after nasal administration than rectal or vaginal administration.

The rate and extent of in-vivo absorption should be influenced by the properties of the hydrodynamic and mucosal barriers. Previous in-vitro studies on the barrier properties of these mucosal membranes suggested that the apparent higher absorption of the nasal mucosa may be related to differences in the properties of its mucosal aqueous and lipoidal barriers.[11] A more rapid and complete absorption of progesterone after nasal administration, as compared to rectal or vaginal administration, was also observed in the previous in-vivo solution studies.[7]

The plasma concentration-time profiles for the progestin derivatives after nasal, rectal and vaginal administration are shown in Figures 6, 7 and 8, respectively. It can be seen that steady-state plasma concentrations are achieved between 2 and 4 hours after application of the device, and are maintained throughout the study period. The steady-state plasma concentrations achieved after mucosal administration of the controlled release device are summarized in Table 2. For all the progestins investigated, nasal administration results

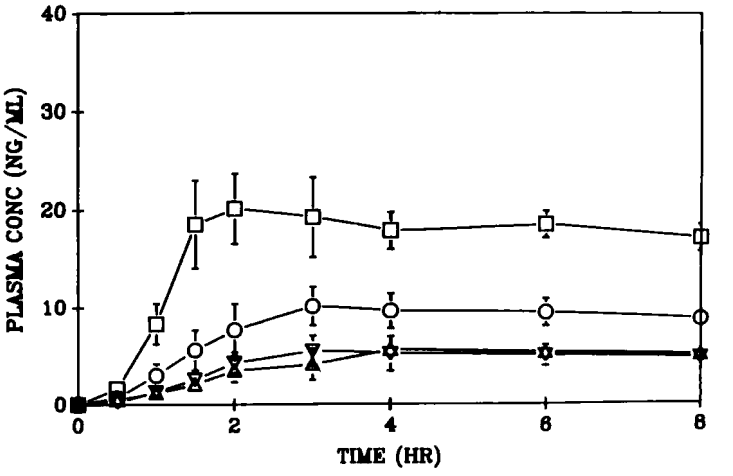


FIGURE 6
Time course for the change in plasma concentration following nasal administration of the controlled release device containing progesterone (□), and its mono-hydroxy (○), di-hydroxy (△) and tri-hydroxy (▽) derivatives.

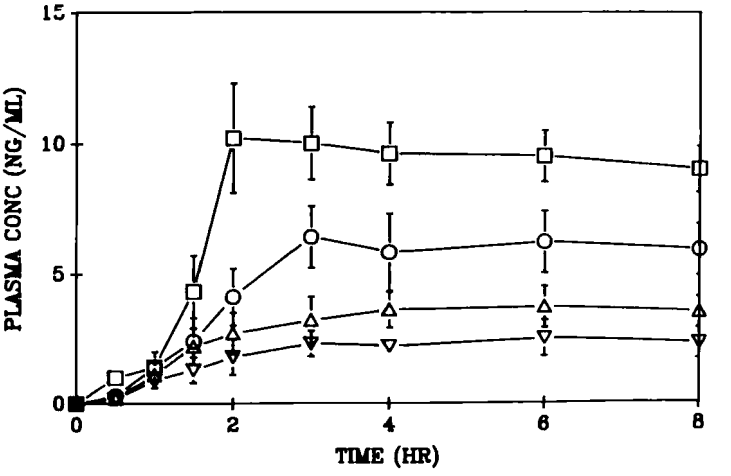


FIGURE 7
Time course for the change in plasma concentration following rectal administration of the controlled release device containing progesterone (□), and its mono-hydroxy (○), di-hydroxy (△) and tri-hydroxy (▽) derivatives.

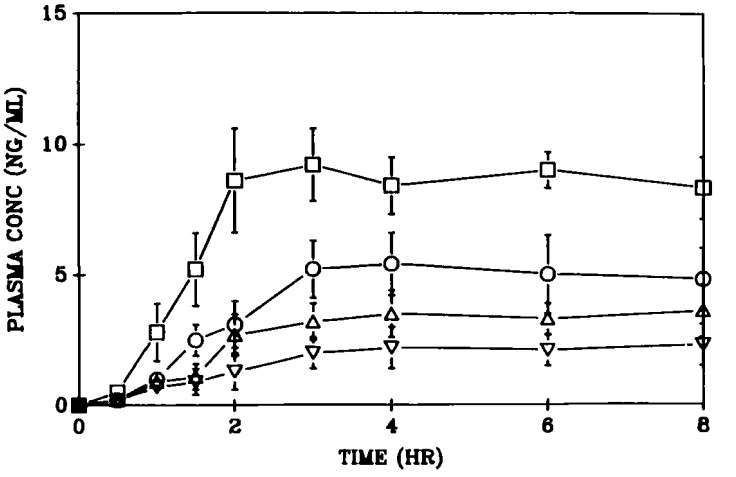


FIGURE 8
Time course for the change in plasma concentration following vaginal administration of the controlled release device containing progesterone (□), and its mono-hydroxy (◯), di-hydroxy (△) and tri-hydroxy (▽) derivatives.

TABLE 2

Steady-State Plasma Concentration (ng/ml) After Mucosal Administration of Controlled Release Device

	Progesterone	Mono-Hydroxy	Di-Hydroxy	Tri-Hydroxy
Nasal	19.0	9.6	5.3	5.0
Rectal	10.0	6.2	3.7	2.3
Vaginal	8.8	5.4	3.5	2.1

TABLE 3

Systemic Bioavailability (%) of Progestins After Mucosal Administration of Controlled Release Device

	Progesterone	Mono-Hydroxy	Di-Hydroxy	Tri-Hydroxy
Nasal	89.6 (±10.1)	85.2 (±11.6)	82.1 (±10.6)	69.9 (±8.2)
Rectal	55.1 (± 9.7)	51.3 (± 8.1)	55.9 (± 6.4)	41.2 (±5.0)
Vaginal	53.5 (± 7.8)	45.4 (± 6.3)	48.5 (± 6.7)	40.0 (±4.8)

in higher plasma concentrations than rectal and vaginal administration. In addition, as predicted from the in-vitro studies, the steady-state plasma concentrations of the progestins tend to decrease as the number of hydroxy groups increase.

The systemic bioavailability of the progestins after mucosal administration of the controlled release device is summarized in Table 3. It can be seen that nasal administration results in a significantly higher bioavailability than rectal and vaginal administration. Although the mucosal bioavailability tends to decrease as the number of hydroxy groups increase, the differences are not significant. The systemic bioavailability after administration of the controlled release device by any of the mucosal routes is significantly greater than the oral bioavailabilities previously reported[8]. This indicates that mucosal administration of progestins may bypass some of the

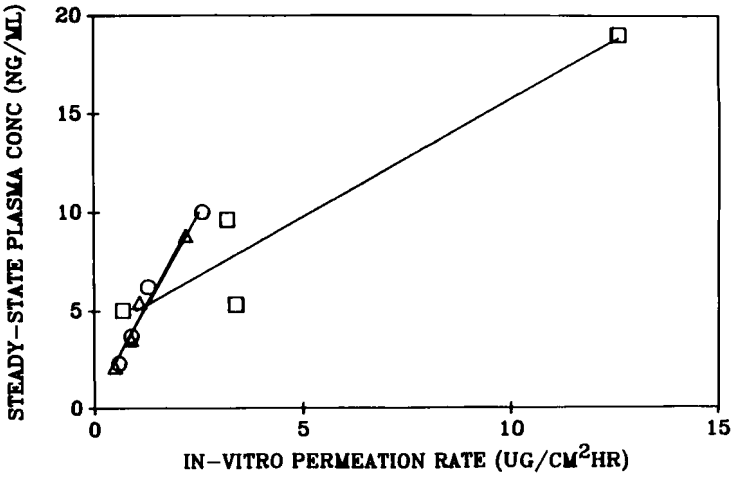


FIGURE 9
Relationship between the in-vitro permeation rate and the in-vivo steady-state plasma concentration of the progestins from the controlled release device for the nasal (□), rectal (○) and vaginal (△) mucosa.

hepatic "first-pass" metabolism associated with oral administration.

In-Vitro/In-Vivo Relationship

The relationship between the in-vitro permeation rate from the progestin devices and the steady-state plasma concentration achieved after administration of the devices is shown in Figure 9. It can be seen that there is a fairly linear correlation for all mucosal routes, indicating that the in-vitro permeation results may be useful in projecting in-vivo absorption. The linear relationships for the rectal and vaginal mucosa have nearly identical slopes, which may suggest similarities in their in-vivo hydrodynamic and/or

mucosal barrier properties. The differences between the slope of this relationship for the nasal mucosa and the rectal and vaginal mucosa may reflect the previously observed differences in the properties of the hydrodynamic and membrane barrier of the nasal mucosa.[11]

CONCLUSION

To summarize the results of this investigation, a good correlation was found between the in-vitro mucosal permeation and the in-vivo mucosal absorption from a model controlled release device. As in the previous solution studies , the results indicate that the nasal route demonstrates a significantly higher rate of in-vitro permeation and extent of in-vivo absorption than the rectal and vaginal route. The similar trends in the solution and controlled release device studies suggest that at sufficiently high device release rates, the observed permeation and absorption may be controlled by the hydrodynamic and mucosal barrier properties of the membranes.

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