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Report

Drug Absorption Through Mucosal Membranes: Effect of Mucosal Route and Penetrant Hydrophilicity

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The influence of mucosal route and penetrant hydrophilicity on the *in vivo* absorption of a model lipophilic compound, progesterone, was investigated in ovariectomized rabbits. The absorption rate and systemic bioavailability of progesterone and its monohydroxy, dihydroxy, and trihydroxy derivatives were evaluated and compared following oral, nasal, rectal, and vaginal administrations. Nasal delivery resulted in a significantly higher rate and extent of progesterone absorption than oral, rectal, or vaginal administration. The rate and extent of mucosal absorption decreased as penetrant hydrophilicity increased for the nasal, rectal, and vaginal routes. The results of this investigation indicate that the absorption characteristics of a lipophilic compound, such as progesterone, are influenced by the properties of both the mucosa and the drug.

KEY WORDS: mucosal absorption; nasal; rectal; vaginal; progestins; penetrant hydrophilicity.

INTRODUCTION

In recent years, there has been a renewed interest in using mucosal membranes as sites for noninvasive drug delivery. Drug administration via mucosal membranes, including the nasal, rectal, and vaginal membrane, may bypass the hepatogastrointestinal "first-pass" metabolism which follows oral administration. Although many classes of drug entities have been successfully delivered via mucosal membranes, very few systematic investigations have been designed to compare the extent and mechanism of drug absorption through different mucosal routes.

The objective of this study is to conduct a systematic investigation of the influence of mucosal route and penetrant hydrophilicity on *in vivo* drug absorption in ovariectomized rabbits, using progesterone as a model lipophilic compound. Progesterone has been reported to have a higher absorption and bioavailability following nasal (1–3), rectal (4,5), and vaginal (6–8) administration than oral administration.

In a previous investigation on the nasal absorption of progesterone and its hydroxy derivatives, the extent of absorption of these compounds was found to be influenced by the hydrophilicity of penetrant and the mode of nasal delivery (9). In this investigation, the rate and extent of absorption of the model compound, progesterone, are compared through the nasal, rectal, and vaginal mucosa. In addition, the influence of penetrant hydrophilicity on mucosal absorption and systemic bioavailability is investigated in the ovariectomized rabbit using progesterone and its monohydroxy, dihydroxy, and trihydroxy derivatives.

MATERIALS AND METHODS

Compounds Investigated

A homologous series of progesterone derivatives, possessing the same basic steroid nucleus, but differing in the number and location of hydroxy groups, was investigated. The structures of progesterone and its monohydroxy (17-alpha hydroxyprogesterone), dihydroxy (cortexolone), and trihydroxy (hydrocortisone) derivatives are shown in Fig. 1. Progesterone and its derivatives, as well as all other chemicals and reagents, were used as obtained from Sigma Chemical Company (St. Louis, Mo.).

Experimental Design

The design of this experiment may be viewed as a 5 × 4 factorial design, with route of administration and penetrant hydrophilicity as the factors investigated. The mucosal absorption and pharmacokinetics of each progestin were investigated in a separate crossover study. There was a group of four rabbits used in each progestin crossover study, and 16 rabbits in total. The crossover treatments consisted of an iv bolus injection (60 μg/kg), an oral solution administration (60 μg/kg), and nasal, rectal, and vaginal solution administrations (6 μg/kg), with a 1-week washout period between treatments. The smaller mucosal dose was necessitated by the volume limitations of the rabbit nasal, rectal, and vaginal

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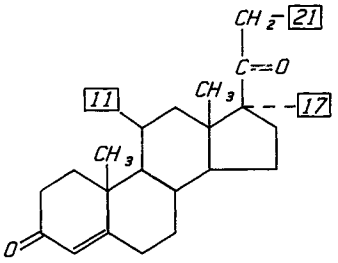

DRUG	HYDROXY GROUP POSITION
PROGESTERONE	NONE
MONO-HYDROXY (17-OH PROGESTERONE)	17
DI-HYDROXY (CORTEXOLONE)	17, 21
TRI-HYDROXY (HYDROCORTISONE)	11, 17, 21

Fig. 1. Chemical structure of progesterone and the hydroxy derivatives investigated in this study.

cavities. The factorial design of this experiment permitted evaluation of the effect of both mucosal route and penetrant hydrophilicity on drug absorption.

Experimental Protocol

The *in vivo* studies were performed in ovariectomized female New Zealand White rabbits (3–4 kg). Previous studies had shown that ovariectomy resulted in low and stable baseline plasma concentrations of the progestins (9). Rabbits were anesthetized with ketamine (35 mg/kg) and xylazine (4 mg/kg) (Butler Veterinary Supply Co.) for both surgery and all treatment dosing and blood sampling. The ovariectomy procedure was performed under sterile surgical conditions and was followed by a 2-week recovery period.

Due to their low water solubility, solutions of the progestins were prepared in normal saline containing 10% (v/v) ethanol and used for the iv, oral, and mucosal administrations. Animals were fasted 18 hr prior to drug administration, and the oral solution was administered via a gastric tube. Some investigators have reported that the surface area of application is an important factor in determining the extent of mucosal absorption (10). In this study, an attempt was made to minimize the influence of application surface area by administering the drug solution to the mucosal site by two actuations of a 100-μl-metered dose spray (BLM Packaging Co.).

To determine plasma progestin concentrations for pharmacokinetic analysis, blood samples were withdrawn from the central ear artery of the rabbit at predetermined 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 monohydroxy (17-hydroxyprogesterone) and trihydroxy (hydrocortisone) derivatives were assayed using radioimmunoassay kits obtained from Diagnostic Products Corp., while those of the dihydroxy derivative (cortexolone) were assayed using a radioimmunoassay kit obtained from ICN Biomedicals, Inc. Samples were analyzed using a gamma counter (Nuclear Chicago-Searle).

Pharmacokinetic Data Analysis

A one-compartment pharmacokinetic model was used to analyze the plasma drug concentration–time profiles for individual rabbits. The value of the elimination rate constant (k_e) was determined from linear regressional analysis of the terminal log-linear segment of the concentration–time profile. The value of the absorption rate constant (k_a) was determined using the method of residuals. The area under the plasma concentration–time curve was calculated using the linear trapezoidal method with extrapolation to infinite time. Bioavailability was calculated by the ratio of the area under the plasma–concentration time curve (AUC) for a route of administration (normalized for dose) to the AUC for iv bolus administration.

The values for all experimental data are expressed as the mean (±SE) of four 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

Effect of Route of Administration on Progesterone Absorption

The nasal, rectal, and vaginal absorption of progesterone is compared to that after oral administration (normalized for dose) in Fig. 2. The elimination rate constants obtained for these routes of administration are not significantly different from that after iv bolus administration [k_e: iv, 6.4 (±1.7) hr^{-1}; nasal, 5.4 (±1.3) hr^{-1}; rectal, 5.3 (±1.0) hr^{-1}; vaginal, 5.0 (±0.9) hr^{-1}]. The pharmacokinetic absorption parameters and systemic bioavailability following administration by the different mucosal routes are summarized in Table I. The absorption rate constant (k_a), maximum plasma concentration (C_{max}), and systemic bioavailability (F) are significantly greater following nasal administration than rectal, vaginal, or oral administration. The greater rate constant of absorption after oral administration than after rectal and vaginal administration may reflect the absorptive function of the gastrointestinal mucosa. The systemic bioavailability after mucosal

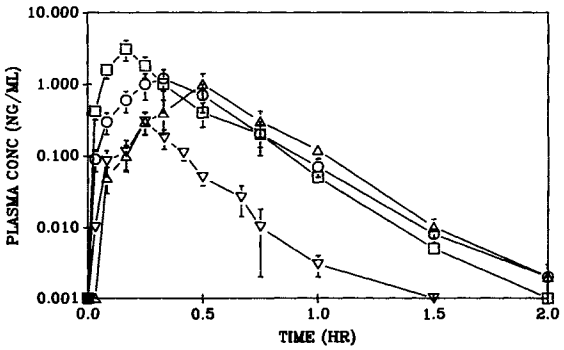

Fig. 2. Time course for the change in plasma concentration following the nasal (□), rectal (○), vaginal (△), and oral (▽) administration of progesterone (oral plasma concentrations normalized for dose).

Table I. Pharmacokinetic Parameters Following Progesterone Administration (Normalized for Dose)

Route	K_a (hr^{-1})	C_{max} (ng/ml)	F (%)
Oral	7.3 (±0.9)	0.3 (±0.1)	9.5 (± 6.2)
Nasal	11.4 (±1.1)	3.1 (±0.8)	88.4 (±10.1)
Rectal	4.5 (±0.6)	1.2 (±0.4)	58.8 (± 6.7)
Vaginal	4.8 (±0.5)	1.0 (±0.4)	46.6 (± 5.4)

administration via the nasal, rectal, or vaginal route is significantly greater than oral administration.

Effect of Penetrant Hydrophilicity on Rate and Extent of Absorption

As shown in Fig. 3, after iv bolus administration, the elimination of progesterone and its hydroxy derivatives can be adequately described by a one-compartment pharmacokinetic model. In a previous investigation in rabbits (9), it was found that the plasma profiles of the progestins follow linear pharmacokinetics. The presence of linear pharmacokinetics allows the bioavailabilities to be compared for different treatment dosages.

The plasma profiles after nasal, rectal, and vaginal delivery of progestins are shown, respectively, in Figs. 4A, B, and C. Progestins are rapidly absorbed through these mucosa, with elimination rate constants similar to those after iv bolus administration. The effect of penetrant hydrophilicity on the rate of mucosal absorption is shown in Table II. For nasal delivery, as the number of hydroxy groups on the progesterone molecule is increased, there is a significant decrease in the absorption rate constant. This trend also exists for the rectal and vaginal routes, although the rate constants for the rectal and vaginal absorption of progesterone and its monohydroxy derivative are not statistically different. As observed for progesterone, the rate constants for nasal absorption of all the hydroxy derivatives are consistently higher than those for rectal and vaginal absorption.

The effect of penetrant hydrophilicity on the extent of mucosal absorption, or systemic bioavailability, is shown in Table III. It can be seen that the systemic bioavailabilities of progesterone and its monohydroxy derivative are similar.

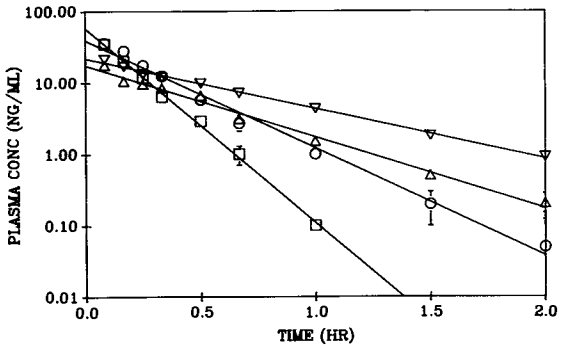

Fig. 3. Time course for the change in plasma concentration following the intravenous injection (60 μg/kg) of progesterone (□) and its monohydroxy (○), dihydroxy (△), and trihydroxy (▽) derivatives.

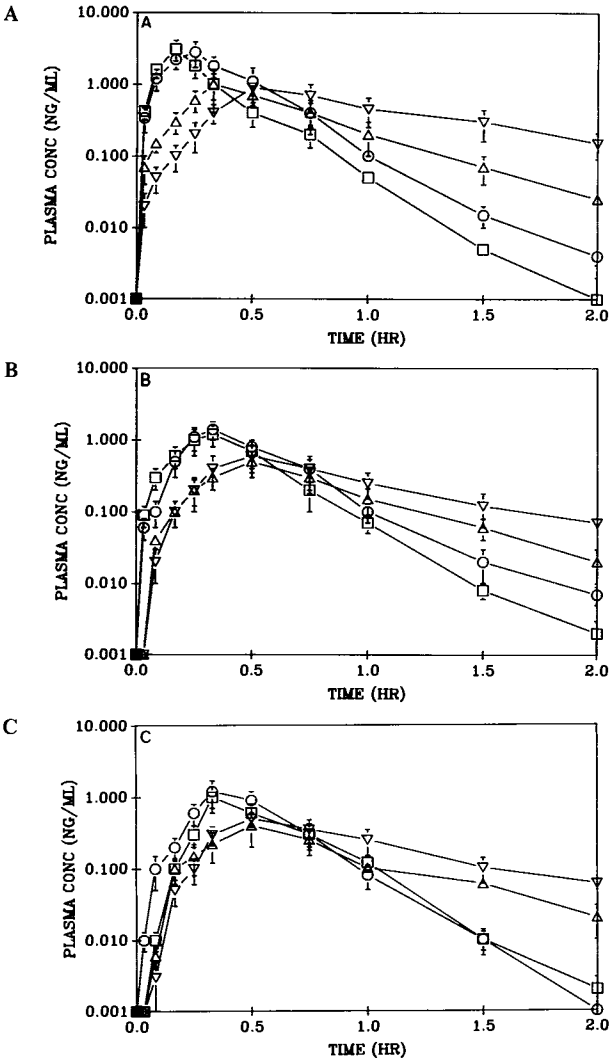

Fig. 4. Time course for the change in plasma concentration following the nasal (A), rectal (B), and vaginal (C) delivery of progesterone (□) and it monohydroxy (○), dihydroxy (△) and trihydroxy (▽) derivatives (dose = 6 μg/kg).

However, as the number of hydroxy groups on the progesterone molecule increases from one to three, there is a significant decrease in the systemic bioavailability of the penetrant for all routes of mucosal administration. The effect of penetrant hydrophilicity is more dramatic for nasal absorption than for rectal and vaginal absorption.

DISCUSSION

The low systemic bioavailability of many compounds

Table II. Rate Constant of Absorption (hr^{-1}) After Mucosal Delivery of Progestins

Route	Progesterone	Monohydroxy	Dihydroxy	Trihydroxy
Nasal	11.4 (±1.1)	9.0 (±0.7)	6.0 (±0.6)	3.6 (±0.2)
Rectal	4.5 (±0.6)	4.9 (±0.7)	3.2 (±0.4)	1.4 (±0.3)
Vaginal	4.8 (±0.5)	5.4 (±0.7)	2.0 (±0.3)	1.8 (±0.3)

Table III. Systemic Bioavailability (%) of Progestins After Mucosal Administration

Route	Progesterone	Monohydroxy	Dihydroxy	Trihydroxy
Nasal	88.4 (±10.1)	90.7 (±9.4)	74.1 (±7.6)	55.3 (±6.2)
Rectal	58.8 (± 6.7)	52.8 (±6.2)	43.6 (±5.1)	37.9 (±4.2)
Vaginal	46.6 (± 5.4)	43.8 (±6.7)	34.0 (±4.3)	32.1 (±3.9)

following oral administration has been attributed to extensive hepatogastrointestinal first-pass metabolism. Mucosal routes have been investigated for their potential to, at least partially, bypass this first-pass metabolism. The results of this investigation confirm that administration of progesterone by the nasal, rectal, and vaginal routes in rabbits achieves a significant improvement in systemic bioavailability over oral administration. Based on histologic similarities between rabbit and human mucosal membranes, the rabbit appears to be a good model for studying mucosal absorption.

In this study, nasal delivery was characterized by a faster absorption and greater bioavailability than rectal or vaginal administration. These results may be explained by differences in drug absorption and/or metabolism in these mucosa. *In vitro* permeation studies are currently underway to compare the absorption barrier properties of the nasal, rectal, and vaginal mucosa. The degree of mucosal and hepatic "first-pass" metabolism after absorption through these mucosa has not yet been fully characterized.

Several investigators have previously shown that the nasal absorption of compounds is influenced by many factors, including the hydrophilicity (9,11,12), as well as the molecular weight and structure (13,14) of the penetrant. Progesterone was chosen as a model lipophilic compound in this study since the addition of hydroxy groups to its large steroid nucleus produces significant changes in its hydrophilicity, while molecular structure and molecular weight remain relatively unchanged. This permits a comparison of the effect of penetrant hydrophilicity on the rate and extent of absorption through different mucosa, with minimal complications from molecular weight and/or structure variations.

The effect of penetrant hydrophilicity, as indicated by the reported log (octanol/water) partition coefficient of the progestins (15), on the absorption rate constant from the nasal, rectal, and vaginal routes is shown in Fig. 5. It can be seen that increases in penetrant hydrophilicity result in linear decreases in the rate constant of mucosal absorption. This result suggests that penetrant hydrophilicity is an important factor in determining the rate of mucosal absorption. Similar results have been reported for the *in situ* nasal absorption of steroids (16). The slope of the linear relationship in Fig. 5 is similar for the rectal and vaginal mucosa, while the nasal mucosa has a steeper slope. This may indicate that the nature of the nasal mucosa and its interactions with the penetrant may differ from that of the rectal and vaginal mucosa.

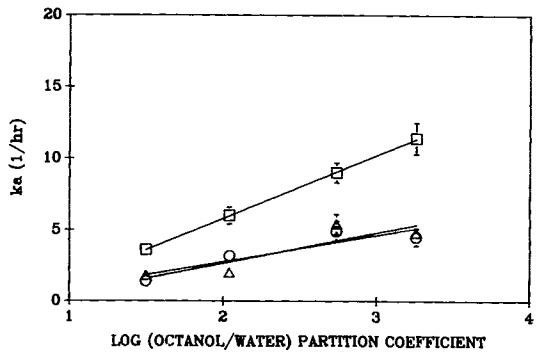

Fig. 5. Relationship between the rate constants of mucosal absorption following nasal (□), rectal (○), and vaginal (△) delivery and the log (octanol/water) partition coefficient of the progestins.

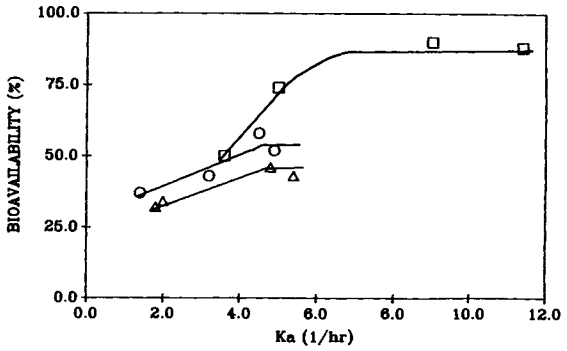

Fig. 6. Relationship between systemic bioavailability after nasal (□), rectal (○), and vaginal (△) administration and the rate constant of mucosal absorption for the progestins.

The results of this study also indicate that the extent of absorption, as indicated by the systemic bioavailability, also decreases with increasing penetrant hydrophilicity. This trend may be related to the influence of the (octanol/water) partition coefficient on the rate constant of mucosal absorption (Fig. 5). The effect of the absorption rate constant on the systemic bioavailability of progestins is shown in Fig. 6. It can be seen that bioavailability initially increases as absorption rate increases and appears to plateau at k_a values above 5 hr^{-1}. Assuming that there is a time-dependent drainage or diffusion of drug from the site of absorption, one would expect that compounds with a faster rate constant of absorption would have a higher bioavailability. Therefore, the decrease in mucosal bioavailability with the increase in penetrant hydrophilicity may be a function of the relative absorption rates of these compounds. In addition, mucosal bioavailability may be influenced by metabolism in the mucosal membranes. *In vitro* metabolism of progesterone in nasal mucosal homogenates has been reported (17); however, the *in vivo* significance of mucosal metabolism has not been established.

In summary, the absorption characteristics of a lipophilic compound, such as progesterone, may be influenced by both the physicochemical properties of the penetrant and the biophysicochemical nature of the mucosal membrane. The characteristics of and interactions in the nasal mucosa which result in a more rapid and complete absorption of these lipophilic compounds remain to be investigated.

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