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RESEARCH PAPER

Effect of Urea and Pantothenol on the Permeation of Progesterone Through Excised Rat Skin from Polymer Matrix Systems

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ABSTRACT

Standard in vitro permeation experiments, using excised rat skin, were carried out to establish the release profile and permeation behavior of progesterone from polymethacrylate (PMA), polyvinylpyrrolidon (PVP), and polyvinylalcohol (PVA) transdermal systems. Data obtained show significant differences in release characteristics from each polymer systems. The greatest amount of progesterone was released from the PVA system. The influence of urea and pantothenol on progesterone release was also investigated. Release data were compared with the permeation rates of progesterone across excised rat skin. The highest permeation rates were measured from PVA matrices containing 5% urea (860 ± 138 $\mu g/cm^2$; cumulative amount permeated in 24 hr) and from PVP matrices containing 6% pantothenol (660 ± 73 $\mu g/cm^2$; cumulative amount permeated in 24 hr). A good correlation between release and permeation data was found with the polymer matrices; however, this was not the case when additives were included.

INTRODUCTION

Progesterone is the natural progestagen produced by the corpus luteum during the luteal phase of a menstrual cycle. The bioavailability of peroral progesterone is poor owing to extensive first-pass metabolism. This limits the efficacy of peroral administration (1) unless appropriate vehicles and large doses, using micronized progesterone to increase its surface area, are given (2,3). Nonoral delivery of progesterone has been achieved via the nasal, rectal, and vaginal routes. Nasal and rectal applications have proved impractical. Vaginal administration of

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Table 1.

Composition of the Polymer Suspensions: PMA (Eudragit-NE30-D); PVP (Kollidon-30); PVA (Moviol 83)

PMA		PVP		PVA	
PMA	6.1	PVP	1.1	PVA	5.0
Progesterone	0.8	Progesterone	0.8	Progesterone	0.8
HPMC gel 2%	16.0	HPMC gel 2%	16.4	Triethylcitrate	2.6
Triethylcitrate	2.7	Triethylcitrate	3.0	Dem. water	17.2
		Dem. water	4.3		
Total	25.6	Total	25.6	Total	25.6

progesterone is useful, especially for treating luteal phase problems in infertile women (4) and for alleviating the symptoms of premenstrual syndrome (PMS). Recently, a bioadhesive polycarbophil gel preparation of progesterone for vaginal application (Crinone®, Wyeth-Ayerst) was successfully tested (5). The results of other studies suggest progesterone is a suitable symptomatic treatment for PMS (6). PMS may be defined as a combination of physical and behavioral symptoms that occur during the luteal phase of a menstrual cycle but that are absent during the follicular phase. A topically applied progesterone hydrogel (Progestoge®, Nourye Pharma) is suggested for treatment of premenstrual mastodynie. A few studies deal with in vitro percutaneous absorption of progesterone (7–11).

The major problem is bringing sufficient amounts of progesterone through skin. In this study, the influences of polymer-type and additives such as urea and pantothenol on the dosage form release characteristics and permeation rates of progesterone through excised rat skin were investigated.

MATERIAL AND METHODS

Materials

The following materials were used: progesterone (Sigma, St. Louis, MO); polyvinyl pyrrolidon (PVP), Kollidon-30® formula (BASF, Ludwigshafen, Germany); polyethacrylate-methacrylate (PMA), dispersion-Eudragit NE 30D formula, and as an adhesive, polyaminoacrylate (Plastoid E 35 L® formula (Röhm, Darmstadt, Germany); Hydroxypropylmethylcellulose (HPMC), metolose SH-4000 USP/NF formula; and as a plasticiser, triethylcitrate (Merck, Darmstadt, Germany). The backing foil was 3M/1109 Scotchpak polyester film laminate. Supporting foils were Scotchpak polyester film liners, fluoropolymer coated (3 M/9743 or 3M/1022, 3M Medica, Borken, Germany).

Formulations and Preparation

Polymer suspensions based on three different polymer types were prepared using the formulas listed in Table 1. The PMA dosage form was prepared by suspending the drug in the PMA dispersion then adding the HPMC gel. The PVP dosage form was prepared by dissolving the polymer in water, then adding the progesterone, triethylcitrate, and HPMC-gel. The PVA dosage form was prepared by agitating the PVA, water, and triethylcitrate for 20 min at 80°C. The system was then cooled before adding the progesterone. Either urea (5%) or pantothenol (6 or 20%) was then added to each polymer formulation.

As shown in Table 1, each patch consisted of a 25.6 g drug-polymer suspension, which was spread evenly onto foil supported on a glass plate, then dried at 40°C for up to 24 hr. The average area was 320 cm^2, which corresponded to approximately 80 mg of drug-polymer suspension per square centimeter. The amount of progesterone per square centimeter was approximately 2.5 mg. After preliminary drying, an adhesive layer was applied using a template, then dried at 60°C for 30 min. Backing foil was then rolled over the polymer-adhesive layer. Finally, the TTSs were cut to size for the diffusion cells (area = 0.95 cm^2). For all experiments, the thickness of the polymer drug matrices as well as for the adhesive layers was 100 μm, which corresponded to 200 μm of total thickness.

Analytical Procedure

Samples were assayed for progesterone content using a previously reported modified HPLC method (7) at a flow rate of 1 ml/min with a UV detector (series 200 LC, Perkin Elmer) at a detection wave length of 240 nm. The stationary phase was a Nucleosil 100 5C-18 column (24 × 4.6 mm). Any polymer residues were held back on a Nucleosil 100-5C-18 precolumn (40 × 4.0 mm). The

mobile phase was methanol : water (90:10). Samples (20 μl) were injected by autosampler (ISS-100, Perkin Elmer). The retention time for progesterone was ~4.4 min. Calibration curves were calculated on the basis of peak area measurements. The linearity interval established in the diffusion receptor phase of propylene glycol : water (40:60 w/w) was 0.01 to 54.5 μg/ml (r = 0.999971).

Release/Permeation Studies from TTS

The release of progesterone was investigated using Franz-type (LG-1083-PC; ERWEKA) diffusion cells. The effective area available to diffusion was 0.95 cm^2. The receptor compartment was filled with propylene glycol : water (40:60 w/w), themostated to 32°C, and continuously stirred with a magnetic bar. Drug-polymer matrices came into direct contact with the receptor phase.

Rat skin permeation profiles were determined using the same diffusion model. The excised skin was mounted on the cell, stratum corneum uppermost, with the dermal side facing the receptor compartment. The TTS was placed on top of the stratum corneum. At regular intervals, aliquots were taken from the receptor phase for assay. All samples were analyzed using HPLC for progesterone content. All experiments were repeated at least three times.

Skin Membrane Preparation

After anesthetizing with diethyl-ether, the rats were sacrificed by cervical dislocation. Their abdominal skin was surgically removed and any adherent subcutaneous fat carefully removed. Abdominal hair from female rats weighing 250 to 300 g was shaved off using hand razors. The processed skin was stored for 12 hr in phosphate buffer (pH, 7.2; 0.137 M).

Solubility of Progesterone

Excess progesterone was suspended in phosphate buffer (7.2; 0.01 M) or water : propylene glycol (60:40) and stirred overnight at 32°C. After centrifugation (13,500*g*; 30 min), the supernatant was analysed using HPLC.

Statistical Data Analysis

Results are expressed as the mean of at least four experiments ±SD. Statistical analysis was performed using the *t* test with $p < 0.05$ as the minimum level of significance.

Table 2.

Solubility of Progesterone at 32°C

Medium	Solubility (μg/ml)
Phosphate buffer pH 7.2; 0.01 M	8.2 (±1.0)
Water : propylene glycol (60:40, w/w)	335 (±0.001)

Values are the mean ± SD of four HPLC Injections.

RESULTS AND DISCUSSION

As seen in Table 2, the solubility of progesterone in phosphate buffer is <10 μg/ml. To have enough driving force, water : propylene glycol (60:40; w/w) was used as the receptor medium for the diffusion and release experiments. As seen in Table 2, the progesterone solubility is more than 40 times higher than in the tested phosphate buffer. The evaluated parameters were polymer type and type of pentration modifier. The TTS formulas are summarized in Table 1.

The lowest release rates for progesterone occurred with the PMA matrix systems, whereas the PVA and PVP systems improved the cumulative amount of progesterone released over a 24-hr period.

There was no significant influence on the release rates of progesterone from the PMA polymer systems when urea was included in the formulation (Fig. 1); however, inclusion of 6% pantothenol in the formulation caused a slight increase of 1.1-fold and incorporation of 20% pantothenol a 1.1-fold increase in release rates.

After 24 hr, the PVP systems increased progesterone release 1.6-fold compared with the PMA systems. Progesterone release was further increased by the addition of pantothenol to the PVP formulations: 1.3-fold with

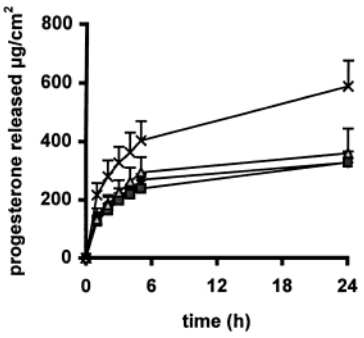

Figure 1. Release of progesterone from PMA-matrix-based systems. ♦ Indicates without additive; ■, with 5% urea; △, with 6% pantothenol; ×, with 20% pantothenol (mean ± SD; $n = 4$).

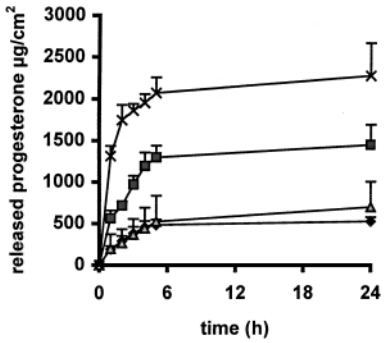

Figure 2. Release of progesterone from PVP-matrix-based systems. ♦ Indicates without additive; ■, with 5% urea; △, with 6% pantothenol; ×, with 20% pantothenol (mean ± SD; $n = 4$).

6% pantothenol and 4.3-fold with 20% pantothenol. The addition of 5% urea to the PVP formulations increased progesterone release 2.7-fold compared with the PVP patch (Fig. 2).

The highest progesterone release per square centimeter occurred with the PVA systems; however, the addition of urea or pantothenol to the PVA formulations was not significant. Inclusion of 20% pantothenol had no influence on progesterone release (Fig. 3). Similar results were obtained with rat skin over the same time period. However, as expected, the cumulative amount of progesterone permeated was lower.

PMA systems had the lowest permeation rate over 24 hr, corresponding to 146 μg/cm^2 of progesterone. In the PMA systems, no significant influence on progesterone permeation could be observed by the inclusion of urea or pantothenol in the formulations (Fig. 4).

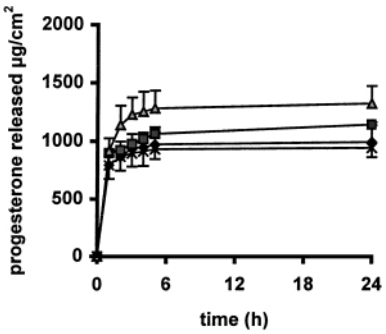

Figure 3. Release of progesterone from PVA-matrix-based systems. ♦ Indicates without additive; ■, with 5% urea; △, with 6% pantothenol; ×, with 20% pantothenol (mean ± SD; $n = 4$).

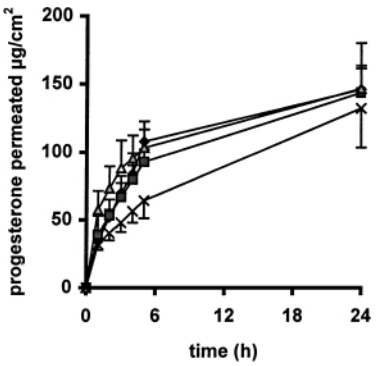

Figure 4. Permeation of progesterone from PMA-matrix-based systems through excised rat skin. ♦ Indicates without additive; ■, with 5% urea; △, with 6% pantothenol; ×, with 20% pantothenol (mean ± SD; $n = 4$).

In the PVP systems, progesterone permeation was 157 μg/cm^2, only marginal higher than seen with the PMA systems. However, inclusion of pantothenol in the PVP formulations had a positive influence on progesterone permeation: 6% pantothenol increased the progesterone amount 4.5-fold, whereas 20% pantothenol increased it only 2.5-fold compared with the PMA system. Urea had no influence (Fig. 5).

The highest permeation of progesterone, 444 μg/cm^2, occurred with the PVA systems. The inclusion of 5% urea in the PVA formulations increased progesterone permeation 2.1-fold (Fig. 6).

The type of polymer used in the matrix systems has a significant influence on progesterone permeation through

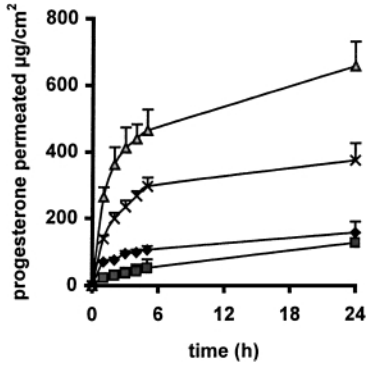

Figure 5. Permeation of progesterone from PVP-matrix-based systems through excised rat skin. ♦ Indicates without additive; ■, with 5% urea; △, with 6% pantothenol; ×, with 20% pantothenol (mean ± SD; $n = 4$).

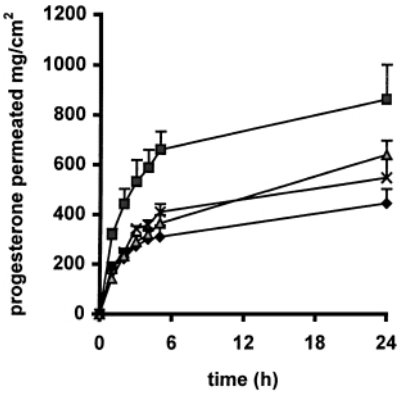

Figure 6. Permeation of progesterone from PVA-matrix-based systems through excised rat skin. ♦ Indicates without additive; ■, with 5% urea; △, with 6% pantothenol; ×, with 20% pantothenol (mean ± SD; $n = 4$).

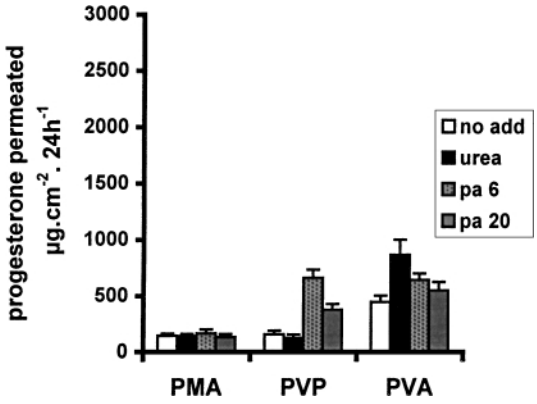

Figure 8. Comparison of the cumulative amount of progesterone permeated from the different matrix formulations through rat skin (mean ± SD; $n = 4$). pa 6 = 6% pantothenol; pa 20 = 20% pantothenol.

rat skin. PVP and PVA have higher progesterone release and permeation rates than does PMA. A comparison of 24-hr release data to permeation data through rat skin shows a good correlation when no additives are included in the matrix systems (Fig. 7). In the permeation experiments, only urea has a positive effect on the permeation of progesterone in PVA systems, whereas pantothenol can enhance progesterone permeation in PVA as well as in PVP systems (Fig. 8).

In conclusion, it is possible to achieve high progesterone permeation through rat skin with PVA or PVP matrix systems and suitable additives. The greatest progesterone permeation, 860 ± 138 μg/cm^2 after 24 hr, was measured from PVA with 5% urea. The second highest permeation, 660 ± 73 μg/cm^2 at 24 hr, was seen from PVP patches with 6% pantothenol.

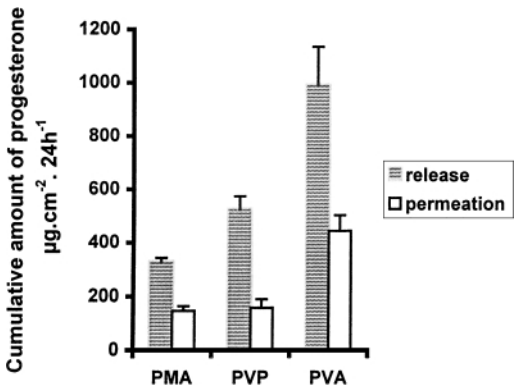

Figure 7. Comparison between release and permeation through excised rat skin of progesterone within 24 hr. Formulations are without additives (mean ± SD; $n = 4$).

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