

Evaluation of the *in vitro* Human Skin Percutaneous Absorption of Progesterone in Versabase[®] Using the Franz Skin Finite Dose Model

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Abstract

Women may benefit from hormone replacement therapy to alleviate undesirable menopausal symptoms such as hot flashes, disruption of sleep and fatigue. Transdermal hormone replacement therapy consists in the delivery of hormones through the skin and into the blood stream, avoiding gastrointestinal drug absorption difficulties and the first pass effect. Transdermal compounded medications may be customized to meet the individual needs of women by changing the hormones used, the respective concentrations and the medicine's formulation. Two compounded medications were prepared for progesterone 50 mg/g, as follows: Progesterone in VersaBase[®] Cream and progesterone in VersaBase[®] Cream Gel. The purpose of this study is to determine the *in vitro* human skin percutaneous absorption of progesterone for both test formulations. The Franz Skin Finite Dose Model was the methodology used to evaluate the total absorption, the rate of absorption and the skin content of progesterone applied to the outer surface of *ex vivo* skin. This model has proven to be a valuable tool for the study of percutaneous absorption of topically applied drugs and to accurately predict *in vivo* permeation kinetics. The skin samples were obtained within 24 h to 48 h of death from three adult Caucasian donors and a total of 17 Franz diffusion chambers were prepared for testing. Results are presented as the mean \pm standard error per parameter, for each test formulation. The rate of percutaneous absorption, presented as mean flux, was similar for the two test formulations and characterized by 2 peaks at approximately 6 hours and 28 hours after dose application, followed by a decline in flux over time. The total absorption and the skin content of progesterone were also similar: 21.6% and 21.8% of the applied doses for the test formulations 1 and 2, respectively. Although *in vitro*, these results suggest that progesterone in VersaBase[®] is likely to be delivered through the skin and into the general circulation for systemic effects. Practitioners may then consider transdermal progesterone as a viable route of drug administration for menopausal women.

Keywords: Skin absorption; Progesterone; Hormone replacement therapy; Menopause; Pharmacokinetics

Introduction

Progesterone is a sex hormone naturally produced by a women's body mainly to regulate the menstrual cycle. There are several conditions in which the levels of progesterone are decreased and women may choose to supplement the body's natural production of progesterone by taking hormone replacement therapy (HRT) [1]. Menopausal women, for instance, benefit from HRT to relieve the undesirable menopausal symptoms such as hot flashes, disruption of sleep and fatigue [2,3]. A drug delivery system routinely used for the administration of hormones is the transdermal drug delivery, in which hormones are delivered through the skin and into the general circulation for systemic effects, avoiding gastrointestinal drug absorption difficulties and the first pass effect.

The percutaneous absorption is non-invasive and provides extended therapy with a single topical application. Transdermal HRT may be customized to meet the individual needs of patients by changing the hormones used, the respective concentrations and the medicine's formulation [4]. Customized HRT formulations may be prepared by compounding pharmacists in accordance to the patient's specific hormone needs [1]. Progesterone administered transdermally is

perceived as beneficial over conventional menopausal therapy due to the successful relief of symptoms and individuality of treatment [3,5].

The skin is a major barrier to drug absorption and thus not all drugs are suitable candidates for transdermal delivery. Skin permeation depends on the physical and chemical properties of the drugs, such as: molecular weight, solubility, partition coefficient and dissociation constant [4].

Progesterone is suitable for transdermal delivery provided that it is formulated in an adequate carrier vehicle that facilitates the percutaneous absorption. VersaBase[®] Cream and VersaBase[®] Gel, two compounding proprietary bases by Professional Compounding Centers of America (PCCA; Houston, Texas), were the carrier vehicles selected to evaluate the percutaneous absorption of progesterone. VersaBase[®] Cream is a topical cream base that simulates the natural moisturizing barrier of the skin through its emulsion system whereas VersaBase[®] Gel is a topical gel base that is resilient to low pH and has good compatibility with polar solvents [6].

Progesterone USP micronized 50 mg/g was incorporated in VersaBase[®] Cream and in a mixture of VersaBase[®] Cream and VersaBase[®] Gel (95:5). The human skin percutaneous absorption of the test formulations was evaluated *in vitro* using the Franz Skin Finite Dose Model.

Methodology

The Franz Skin Finite Dose Model was the methodology selected to characterize the percutaneous absorption of progesterone into and through the skin by evaluating the total absorption, the rate of absorption and the skin content of progesterone applied to the outer surface of the skin. This model has proven to be a valuable tool for the study of percutaneous absorption and determination of the pharmacokinetics of topically applied drugs.

It consists in using *ex vivo* human trunk skin mounted on specially designed Franz diffusion chambers which provide an intimate contact between the dermal layers of the skin and the receptor solutions, parallel to the *in vivo* contact between the underlying tissues of the skin and the blood stream. Furthermore, these chambers allow the skin to be maintained at a temperature and humidity that match normal *in vivo* conditions. Because of these characteristics, the Franz Skin Finite Dose Model has proven to accurately predict *in vivo* percutaneous absorption kinetics [7].

Preparation of skin samples and Franz diffusion chambers

The percutaneous absorption of progesterone was measured using *ex vivo* human trunk skin samples, without obvious signs of disease, obtained within 24 h to 48 h of death from three adult Caucasian donors. The skin samples were dermatomed, prepared for cryopreservation, sealed in a water-impermeable plastic bag and stored at approximately -70°C until the day of the experiment.

Prior to use, the skin samples were thawed in water at approximately 37°C and rinsed in tap water to remove any adherent blood or other material from the surface. The skin samples from each donor were then cut into small sections to fit on the nominal 1.0 cm² chamber compartment.

The receptor solution compartment was filled to capacity with a reservoir solution of phosphate-buffered isotonic saline (PBS), pH 7.4 ± 0.1, and the chamber chimney left open to ambient laboratory conditions. All Franz diffusion chambers were mounted on a diffusion apparatus in which the bathing solution was stirred magnetically at approximately 600 RPM and the skin surface temperature was maintained at 32.0°C ± 1.0°C.

The integrity of each skin section was evaluated by testing its permeability to titrated water prior to the experiment. Following a brief equilibrium period of 30 minutes to 1 hour, 3H₂O (NEN, Boston, MA, sp. Act. ~0.5 µCi/mL) was layered across the top of the skin with a dropper so that the entire exposed surface was covered (approximately 200 µL to 500 µL).

After 5 minutes, the 3H₂O aqueous layer was removed and after 30 minutes, the receptor solution was collected and analyzed for radioactive content by liquid scintillation counting. The skin samples were considered acceptable when absorption of 3H₂O was less than 1.56 µL-equ/cm².

Preparation of test formulations

Two test formulations were prepared, in duplicate, containing the active pharmaceutical ingredient (API) Progesterone USP, PCCA Special Micronized (particle size average <10 microns) 50 mg/g and the wetting agent Pentylene Glycol 10%. The test formulation 1 was incorporated in VersaBase® Cream and the test formulation 2 was incorporated in a mixture of VersaBase® Cream and VersaBase® Gel

(95:5), named below as VersaBase® Cream-Gel. The test formulations were prepared at PCCA and shipped to PRACS Institute, Ltd. (Fargo, North Dakota, USA) for *in vitro* testing.

Dosing application and diffusion

The receptor solution was replaced with a solution of phosphate-buffered isotonic saline (PBS) with 0.1% Volpo and placed bathing the inner surface of the skin sections in order to measure the rate of appearance of progesterone. The chamber chimney was removed from the diffusion cell to allow full access to the epidermal surface of the skin. Each test formulation was applied to the skin sections of the three donors, in triplicate, using a positive displacement pipette set to deliver a finite dose of 5 µL formulation/cm²/skin section, and it was spread across the surface with the Teflon tip of the pipette.

Due to the limited number of skin sections by one donor that passed the integrity test, the test formulation 1 was replicate twice instead. As a result, there were a total of 8 Franz diffusion chambers for the test formulation 1 and 9 Franz diffusion chambers for the test formulation 2. One non-dosed chamber was included in the study to serve as blank control.

The percutaneous absorption of progesterone was evaluated over a period of 48 hours. At pre-selected times after dose application (4, 8, 12, 24, 32 and 48 hours), the receptor solution was removed entirely and replaced with new solution; an aliquot of the receptor solution was saved for posterior analytical assay testing.

After the collection of the last aliquot at 48 hours, the skin surface was washed twice with equal parts of methanol and water in order to collect unabsorbed test formulation from the surface of the skin. Subsequently, the skin section was removed from the chamber, tape stripped to remove the stratum corneum and separated into epidermis and dermis for evaluation of the skin content of progesterone.

Analytical assay testing

The quantification of progesterone from the aliquots was performed by the analytical method High Performance Liquid Chromatography with Ultraviolet Detection (HPLC/UV). A volume of 10 µL of each aliquot was injected in the HPLC/UV, which was conducted on a Hewlett-Packard 1100 Series system with an Agilent 1100 Series LC and a diode array detector at a wavelength of 245 nm (5 nm) to 480 nm (50 nm). The solvent system consisted of 85% methanol (solvent A) and 15% water (solvent B), which was run through a Phenomenex Luna C18 column (100 mm × 4.6 mm; 3 µ) at a flow rate of 0.5 µL/min, the column temperature was maintained at 40°C.

Results and Discussion

To characterize the percutaneous absorption of progesterone, the following parameters were determined for each Franz diffusion chamber: rate of absorption, total absorption, skin content (epidermis and dermis) and surface wash. The absorption of progesterone refers to the percutaneous diffusion of the drug through the skin whereas the skin content refers to the distribution of the drug into the skin. Results are presented as the mean ± standard error (SE) per parameter, for each test formulation. Statistical significance was determined using p-values obtained from a student's t-test. A p-value of <0.05 is considered statistically significant.

The rate of percutaneous absorption of progesterone was determined by dividing the amount of progesterone collected in the

receptor solutions during a specified time interval, over the 48-hour study period, for all 17 Franz diffusion chambers. This is a time-averaged parameter presented as flux ($\mu\text{g}/\text{cm}^2/\text{h}$) and reported at midpoint of sample collection. The mean flux profile of percutaneous absorption for progesterone in both test formulations is shown in Table 1 and Figure 1.

Mid-Time (hr)	Progesterone VersaBase® Cream	in Progesterone in VersaBase® Cream-Gel
2	0.094 ± 0.024	0.099 ± 0.015
6	0.156 ± 0.021	0.192 ± 0.020
10	0.150 ± 0.012	0.158 ± 0.017
18	0.104 ± 0.010	0.101 ± 0.017
28	0.12 ± 0.011	0.229 ± 0.127
40	0.092 ± 0.018	0.101 ± 0.032

Table 1: Mean flux ($\mu\text{g}/\text{cm}^2/\text{hr} \pm \text{SE}$) of percutaneous absorption of progesterone in VersaBase® Cream and in VersaBase® Cream-Gel over 48 hours (across donor summary). Time is shown as midpoint of sample collection.

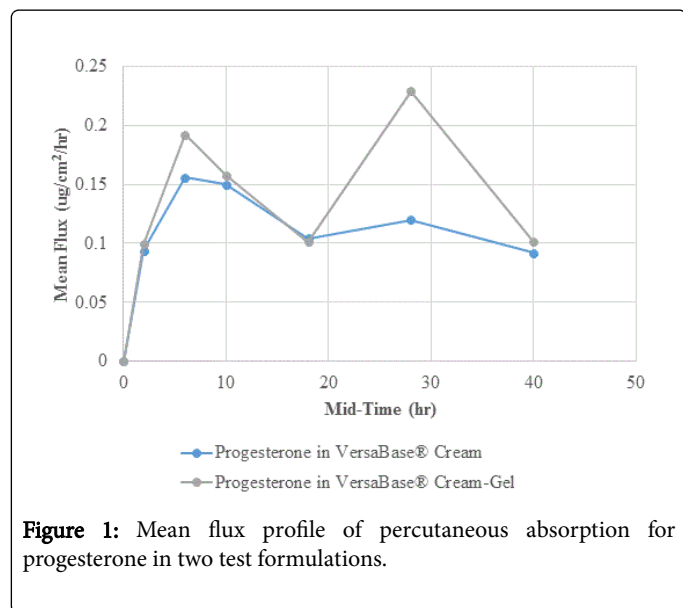


Figure 1: Mean flux profile of percutaneous absorption for progesterone in two test formulations.

The two test formulations exhibit similar penetration profiles, characterized by a rise in absorption to a peak at approximately 6 hours after dose application, followed by a slow decline in flux over time. A secondary peak is present for both formulations at approximately 28 hours after dose application, though progesterone in VersaBase® Cream-Gel exhibits a much higher peak (Figure 1), possibly due to a depot of some of the applied dose in the epidermis.

The total absorption of progesterone corresponds to the amount of progesterone (μg) collected in the receptor solutions over the 48-hour study period, for all 17 Franz diffusion chambers. The mean total absorption of progesterone in VersaBase® Cream was $5.285 \mu\text{g} \pm 0.682$, which corresponds to 2.1% of the applied dose; whereas the mean total absorption of progesterone in VersaBase® Cream-Gel was $6.470 \mu\text{g} \pm$

1.814 , which corresponds to 2.6% of the applied dose, as displayed in Table 2.

Parameter	Progesterone VersaBase® Cream (%)	in Progesterone in VersaBase® Cream-Gel (%)
Total absorption	2.114 ± 0.273	2.588 ± 0.726
Epidermis	18.376 ± 10.098	18.696 ± 3.500
Dermis	1.088 ± 0.284	0.520 ± 0.095
Surface wash	67.145 ± 13.663	68.436 ± 4.036

Table 2: Total absorption, skin content (epidermis and dermis) and surface wash (mean \pm SE) of progesterone in VersaBase® Cream and in VersaBase® Cream-Gel as percent of applied dose over 48 hours (across donor summary).

The two formulations were found to be statistically similar with regards to the total absorption of progesterone since the p-value obtained was 0.574 (>0.05). These results show that the bioavailability of transdermal progesterone may not be high but it is important to consider that the skin samples used referred to the torso, which is thicker and less irrigated than other body parts commonly used in transdermal therapy, such as the inner wrists. Furthermore, the test formulations were applied to 1.0 cm^2 skin samples, which is a very small area in comparison to the *in vivo* potential areas of exposure.

The skin content of progesterone corresponds to the amount of progesterone (μg) recovered from the epidermis and the dermis layers of the skin sections following the 48 hours post-dose application, for all 17 Franz diffusion chambers. For the progesterone in VersaBase® Cream, the mean distribution content of progesterone in the epidermis was $45.939 \mu\text{g} \pm 25.244$ (18.4%) and in the dermis was $2.721 \mu\text{g} \pm 0.710$ (1.1%).

For the progesterone in VersaBase® Cream-Gel, the mean distribution content of progesterone in the epidermis was $46.739 \mu\text{g} \pm 8.750$ (18.7%) and in the dermis was $1.301 \mu\text{g} \pm 0.237$ (0.5%) (Table 2). When combining the total absorption and the skin content of progesterone, it is concluded that 21.6% (test formulation 1) and 21.8% (test formulation 2) of the applied doses permeated the skin.

The surface wash of progesterone corresponds to the amount of progesterone (μg) recovered from the surface of the skin sections following the 48 hours post-dose application, for all 17 Franz diffusion chambers. The mean surface wash of progesterone in VersaBase® Cream was $167.861 \mu\text{g} \pm 34.158$, which corresponds to 67.1% of the applied dose; whereas the mean surface wash of progesterone in VersaBase® Cream-Gel was $171.090 \mu\text{g} \pm 10.090$, which corresponds to 90.2% of the applied dose (Table 2).

Despite the vast majority of the applied dose for both test formulations was found to be either on the epidermal layer of the skin or remaining on the surface of the skin, as demonstrated above, progesterone was able to penetrate through and into the skin. These *in vitro* results may be used in practice to predict the *in vivo* rate and extent of percutaneous absorption for progesterone. Future clinical studies are needed though to establish a correlation between the *in vitro* results and the *in vivo* conditions. However, there is already clinical evidence regarding the efficacy of multiple hormones, including progesterone 100 mg/mL, in VersaBase® applied to postmenopausal women. According to the pilot study by Glaser et al., both saliva and blood testing proved the systemic absorption of

hormones following topical application of a combination cream to the mucous membranes of the labia and vagina [8]. These *in vitro* and *in vivo* studies are important evidence to support the use of transdermal progesterone in HRT.

The total recovery of progesterone, calculated as a sum of all parameters (total absorption, skin content and surface wash), was 88.7% for the test formulation 1 and 90.2% for the test formulation 2. Although the mass accountability of the applied dose was high, a total recovery of progesterone was not achieved potentially due to the limitations of the study. For instance, due to adsorption of progesterone to the Franz diffusion chamber, binding of progesterone to skin tissue proteins, concentration of progesterone in the test formulations lower than labeled, instability of progesterone or incomplete extraction of progesterone for analytical assay testing.

Albeit the widespread use of the Franz Skin Finite Dose Model, there are additional limitations that should be considered when studying the percutaneous absorption of drugs. Inadequate study protocols generate inadequate data; the experimental conditions must be carefully designed taking into account the formulation tested and the physicochemical characteristics of the drug [9]. Preferably, the study protocols should be validated to reduce data variability and to enhance reproducibility [10].

Animal skin is often employed in these studies due to the limited availability of human skin excised from cadavers or obtained from plastic surgeries; these samples do not always correlate to the human skin absorption as a result of differences in thickness, follicular structure and vascular anatomy, among others [9]. When human skin is used, variables such as the anatomical site, the skin hydration and the age of the person also affect the skin absorption [11]. Another limitation to consider is the number of the samples used to obtain significant results; it is not always possible to employ as many samples as required for statistical power. Furthermore, *in vitro* evaluations cannot fully reproduce the complexity of biological systems; when extrapolated, the study results should be considered only a prediction of the *in vivo* skin absorption [9].

Conclusions

Women may benefit from transdermal progesterone to alleviate undesirable menopausal symptoms. The Franz Skin Finite Dose Model has demonstrated that progesterone does penetrate through and into *ex vivo* skin, following topical application of both test formulations. Although *in vitro*, these results suggest that progesterone in VersaBase® is likely to be delivered through the skin and into the general

circulation for systemic effects. Practitioners may then consider transdermal progesterone as a viable route of drug administration for menopausal women.

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