Evaluation of the *In Vitro* Percutaneous Absorption and Stability of Progesterone Topical Compounded Medications

ICSC 4 RRM
20th International Clinical & Scientific Congress for Restorative Reproductive Medicine

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Background & Purpose

Progesterone plays an important role in early pregnancy by thickening the uterine lining and creating an appropriate environment for implantation of the fertilized egg. Progesterone supplementation is commonly prescribed to women as part of infertility treatments. The exogenous hormone may be delivered through various routes of administration, most commonly oral, topical/transdermal, vaginal and intramuscular. Transdermal compounded medications, using permeation-enhancing topical bases, represent a promising dosage form which is non-invasive, easy to administer and customizable to meet the individual patient's needs.

In this *in vitro* study, progesterone USP (special micronized) was incorporated in a proprietary anhydrous permeation-enhancing gel base (APEB), and also in a proprietary water-containing cream base (VBC) for comparison purposes. The skin percutaneous absorption of the hormone was evaluated using the Franz skin finite dose model and mass spectrometry. Additionally, the stability of the hormone was determined using a validated, stability-indicating Ultra-Performance Liquid Chromatographic (UPLC) method.

Methods

The Franz skin finite dose model used donated human cadaver abdomen skin tissues from one donor and the skin samples were fitted into the Franz diffusion system. All Franz diffusion chambers were connected to a circulating water bath and the skin surface temperature was maintained at 32.0°C ± 1.0°C. The receptor medium was mixed at approximately 600 RPM using a magnetic stirring device to ensure appropriate homogenization of the release drug in the receptor phase throughout the experiment. A positive displacement pipette was used to apply ~10 mg/cm^2 of the compounded medications (progesterone in either APEB or VBC) on each skin sample. The amount of progesterone in the skin sample was determined after 4 hours by mass spectrometry. Images were collected on a Bruker timsTOF fleX QTOF mass spectrometer in positive ion mode at 40 µm spatial resolution over the m/z range 100-1000 (Figure 1).

The stability of progesterone was determined using UPLC to separate progesterone from its degradation products. The mobile phase consisted of water with 0.1% trifluoroacetic acid (A), acetonitrile with 0.1% trifluoroacetic acid (B), acetonitrile (C), and 50% methanol in water (D). A Waters UPLC Cortecs column maintained at 25ºC was used to separate molecules with a flow rate of 0.35 mL/min. Molecules were detected at a 243 nm wavelength. Samples (1 µL) were injected into the Aquity UPLC separation module equipped with a photodiode array detector. Data were acquired and analyzed using Empower version 3 (Figure 2). The UPLC method was validated in accordance with current guidelines for system suitability, linearity, accuracy, precision, robustness, solution stability, and specificity.

Results & Discussion

The chemical composition of the compounding base is critical for the skin permeation of drugs. The methods used to quantitatively analyze the drugs are equally important. The percutaneous absorption of progesterone in APEB and VBC formulations were compared using mass spectrometry imaging, which is an effective method for the quantitative analysis of progesterone that permeated through the skin. Figures 1A-1C show the H&E-stained tissues, progesterone signals and superimposed images. The bright signals in Figure 1B represent progesterone which were measured quantitatively as shown in Figure 1D. Progesterone in APEB showed an average optical density of 1699 compared with progesterone in VBC which had an average optical density of 550. Statistical analysis shows a significant difference (p<0.029) in the skin permeation of progesterone compounded in APEB or VBC. These results suggest that anhydrous APEB is potentially a better base to compound progesterone compared with water containing VBC. The better efficacy of APEB may be attributed to its chemical composition. APEB contains phosphatidylcholine and jojoba esters, which may have contributed to its permeation-enhancing property. The anhydrous property of APEB also provides for better solubility of the nonpolar progesterone and unfavorable conditions for growth of microorganisms, without compromising the efficacy of the skin permeation.

The stability of progesterone in APEB was tested at room temperature for 180 days and it was concluded that the concentration of progesterone ranged between 96% – 106% relative to its initial concentration, as shown in Figure 2. As such, progesterone in APEB has an extended beyond-use-date of 6 months, which reinforces the benefits and convenience of using this anhydrous formulation. Results from this pre-clinical *in vitro* evaluation can be used as basis for a warranted clinical trial in using APEB as a base for compounded topical progesterone in hormone replacement therapy.

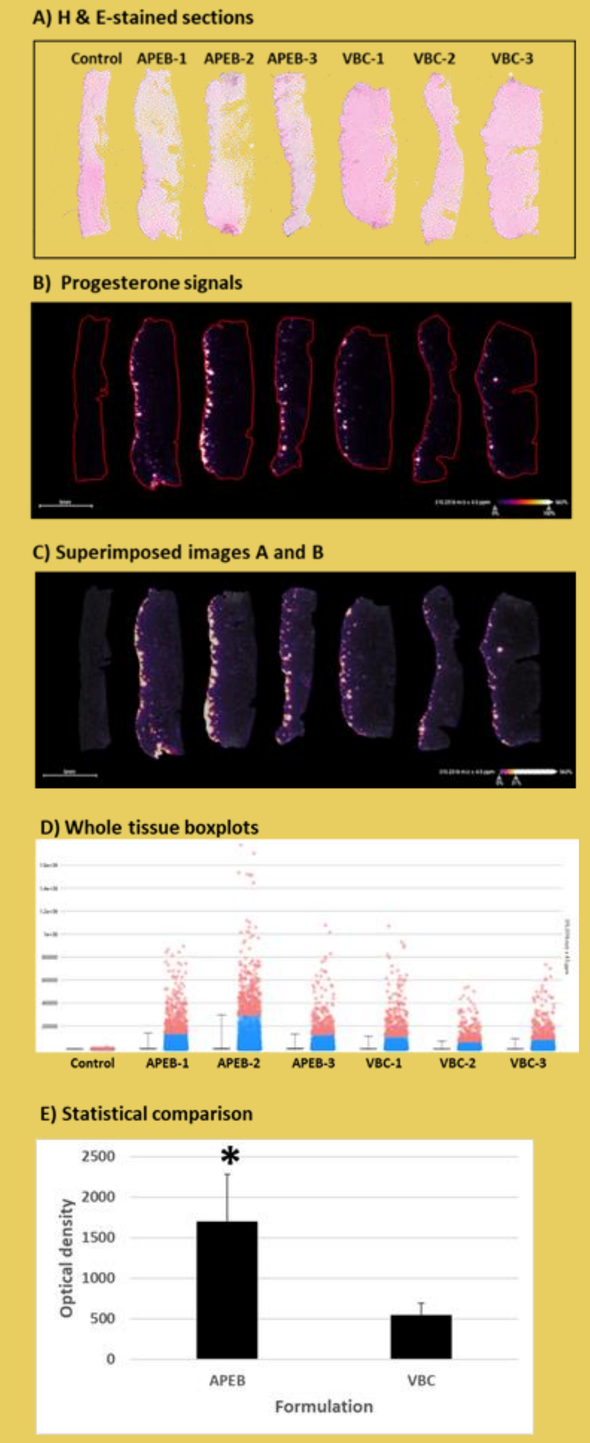

Figure 1 (A-E).
Mass spectrometric analysis of the skin permeated progesterone. Skin sections were stained (A), and mass spectrometry images were collected (B). Panel C shows the superimposed images in A and B. All spectrum signals were quantitated and presented as bar graphs in D. The average optical densities of the progesterone signals were compared using the two compounding bases (E).

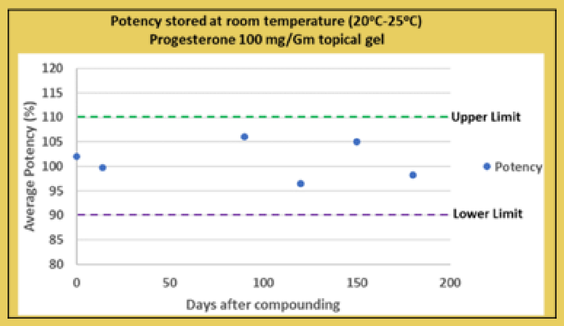

Figure 2.
Stability of progesterone in APEB over 180 days. The dashed lines represent the specific range of 90.0% to 110.0% acceptable limits.

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Acknowledgements: Mass spectrometry imaging was performed in the University of Texas Mass Spectrometry Imaging Facility supported by Cancer Prevention and Research Institute of Texas award RP190617.

Conflicts of Interest Disclosure: The authors are employees of PCCA, the manufacturer of the proprietary compounding bases APEB and VBC discussed in this study.

References: Banov, D., Song, G., Ip, K., Seeley, E. H., Linehan, S. T., Bassani, I., Ferron, G., Bassani, A. S., & Valdez, B. C. (2024). In vitro evaluation of the percutaneous absorption of progesterone in anhydrous permeation-enhancing base using the Franz skin finite dose model and mass spectrometry. Archives of Dermatological Research, 316(6), 291. https://doi.org/10.1007/s00403-024-03040-x.

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