Evaluation of the *in vitro* Human Skin Percutaneous Absorption of Progesterone in PCCA VersaBase® Anhydrous HRT

SUMMARY: The human skin percutaneous absorption of PCCA Progesterone Special Micronized in PCCA VersaBase Anhydrous HRT and PCCA VersaBase® Cream was evaluated *in vitro* and it was shown that VersaBase Anhydrous HRT performs comparably to our industry-leading VersaBase Cream, and with a steadier absorption rate. Since VersaBase Anhydrous HRT has a water activity below 0.6 (Aw <0.6), it may provide an excellent option for compounding pharmacists who rely on anhydrous topical formulations for extended default beyond-use dates (BUDs) without compromising on permeation performance.

Introduction:

The steroid hormone progesterone is an important component in hormone replacement therapy (HRT) for postmenopausal women. Progesterone is commonly prescribed to women with an intact uterus when they are under estrogen replacement, to prevent estrogen-induced endometrial hyperplasia and endometrial cancer [1]. It can also be used to control vasomotor symptoms caused by menopause [2]. Micronized progesterone is the preferred form due to its enhanced bioavailability and less peaking effect [3]. Progesterone can be delivered via oral, vaginal or topical route. VersaBase Cream is one of the suggested bases among compounding pharmacists to deliver hormones topically, and it has been shown to facilitate penetration of progesterone through full-thickness skin following topical application in an *in vitro* model [4].

PCCA VersaBase Anhydrous HRT is a new proprietary base for topical delivery of female hormones into and through the skin with extended stability. Compared with VersaBase Cream that contains water, VersaBase Anhydrous HRT has a water activity below 0.6 (Aw<0.6), classifying it as an anhydrous base. This allows extended default beyond-use dates (BUDs) for preparations that do not have stability studies.

The purpose of this study is to compare the percutaneous absorption of PCCA Progesterone Special Micronized incorporated in PCCA VersaBase Anhydrous HRT and in VersaBase Cream (Table 1.) in an *in vitro* dermatomed skin model.

Progesterone 100mg/Gm Topical Gel (VersaBase Anhydrous HRT)	
Progesterone USP, PCCA Special Micronized	10%
Propylene Glycol USP	10%
Base, VersaBase Anhydrous HRT	80%

Progesterone 100mg/Gm Topical Cream (VersaBase Cream)	
Progesterone USP, PCCA Special Micronized	10%
Propylene Glycol USP	10%
Base, VersaBase Cream	80%

Table 1. *Compounded formulas (PCCA formulas 13466 and 12114[a]) were used in the percutaneous absorption study.*

[a] *Modification was made to F12114 in order to compare with F13466. Propylene glycol 10% was used instead of propylene glycol 8% and ethoxy 2% blend.*

Methodology:

Skin Preparation

The percutaneous absorption of progesterone was measured using human cadaver abdomen skin tissue from three Caucasian female donors. Dermatomed skin samples were purchased from BioIVT (Westbury, NY) and were cryopreserved and stored at -20°C in tightly sealed plastic bags. Prior to use, the skin samples were defrosted and then soaked in diffusion medium for at least 30 min at room temperature. The samples were visually checked for any significant damages, such as cuts, or holes. Skin tissues from 3 donors and 3 replicates were used for each compounded formula.

Franz Cell Diffusion

The Franz diffusion system (surface area of 1.77 cm^2) was used in this study. The diffusion cells were mounted in the diffusion apparatus and the physiological diffusion medium was added to the receptor compartment. A skin integrity test was performed using a Precision LCR meter. Intact skin has transcutaneous electrical resistance at least 2 times greater than the diffusion medium. The finite dose, approximately 5 mg/cm^2 of the compounded formula was applied on each skin sample using a positive displacement pipette and a pellet pestle to spread the product across the skin surface. The receptor solution (HBSS #14175-079, 25 mM HEPES, #15630-080 and 50 µg/mL Gentamicin, #15750-060, Gibco) was stirred magnetically at 600 rpm with the water jacket temperature maintained at 32±0.5°C. During the exposure period, samples of the receptor solutions (1 mL) were removed at predetermined time points: 2, 4, 6, 8, 12 and 24 hours after applying the compounded formula.

Progesterone Quantification

The quantification of progesterone in receptor solution was performed by ELISA (Cayman, Ann Arbor, MI) following manufacturer's instruction.

Results and Discussion:

Progesterone that was applied on the skin passed through the stratum corneum, epidermis and dermis layers of the skin model, and finally reached receptor solution. This process mimics progesterone penetrating through the skin and into systemic circulation *in vivo*. Percutaneous absorption of progesterone in this study refers to the amount of progesterone detected in the receptor solution and is shown in Figure 1. Progesterone was detected as early as 2 hours after skin application, and the amount continued to increase, but not significantly different between the two compounded formulas until the 24-hour time point. By the end of 24 hours, percutaneous absorption of progesterone facilitated by VersaBase

Evaluation of the *in vitro* Human Skin Percutaneous Absorption of Progesterone in PCCA VersaBase® Anhydrous HRT

Anhydrous HRT was 2851±66 ng/cm^2 of skin surface, which is significantly higher than 1867±125 ng/cm^2 by VersaBase Cream.

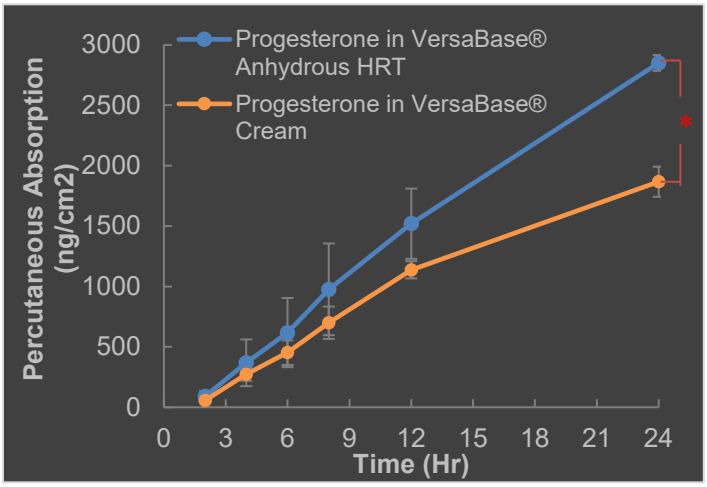

Figure 1. *Across donor summary: mean skin percutaneous absorption of progesterone (ng/cm^2 of skin) in two compounded formulas during 24 hours diffusion. Percutaneous absorption was represented by the total amount of progesterone recovered from receptor solution at each time for each cm^2 of skin surface area (* p<0.05).*

In order to understand what led to the significant difference in percutaneous absorption of progesterone in two formulas at 24-hours, the rate of absorption, or flux rate, was determined and is shown in Figure 2. The rate of percutaneous absorption shows a rapid penetration upon application and the maximum flux was achieved at approximately 7 hours post-application in both VersaBase Anhydrous HRT and VersaBase Cream formulas, followed by a slow decline. The mean flux profiles of progesterone were similar in both compounded formulas, except for the absorption rate, which in VersaBase Anhydrous HRT declined slower than in VersaBase Cream after 12 hours. In VersaBase Cream, flux rate during 12 to 24 hours was significantly lower than the rate between 8 to 12 hours. In contrast, flux facilitated by VersaBase Anhydrous HRT maintained a steady rate, without significant change compared with the 8 to 12 hours period.

In summary, profiles of progesterone in both VersaBase Anhydrous HRT and VersaBase Cream are very similar during the first 12 hours. However, VersaBase Anhydrous HRT maintains progesterone absorption in a steadier rate compared with VersaBase Cream from 12 to 24 hours, resulting in a higher total percutaneous absorption.

Conclusions:

This *in vitro* study performed in the dermatomed human skin model has demonstrated that the proprietary topical base VersaBase Anhydrous HRT facilitates the percutaneous absorption of progesterone across human cadaver skin. The profile of absorption is comparable to VersaBase Cream, but with a steadier absorption rate throughout the 24 hours. One of the desired characteristics for an ideal female hormone delivery base is to produce steady delivery without quick peaking or declining. VersaBase Cream, as our industry-leading base, has already received satisfactory responses from patients. With a similar delivery capability and even superior ability to maintain a steady absorption rate, VersaBase Anhydrous HRT could provide a reliable option to compounding pharmacists to extend default BUDs with the assurance of an excellent permeation performance.

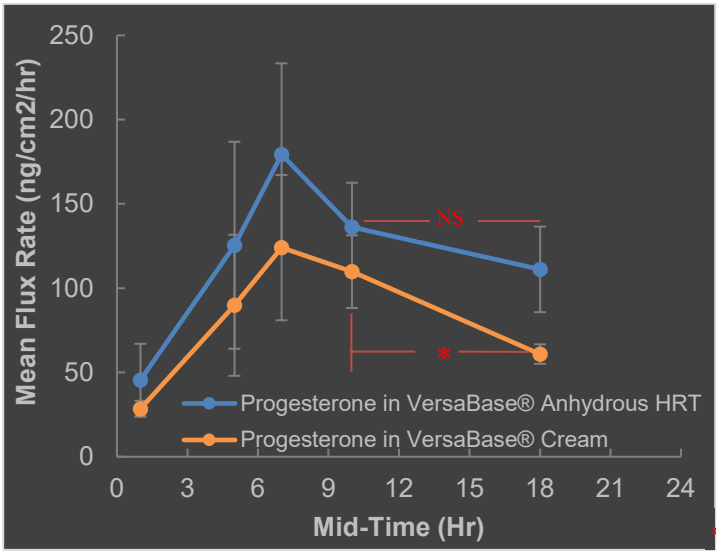

Figure 2. *Across donor summary: mean flux rate of progesterone in (ng/cm^2/hr) in two compounded formulas (* p<0.05. NS, p>0.05, not significant). Absorption values were reported at midpoint of sample collection.*

References:

1. Stanczyk FZ. Treatment of postmenopausal women with topical progesterone creams and gels: are they effective?. Climacteric. 2014;17 Suppl 2:8-11.
2. Hitchcock CL, Prior JC. Oral micronized progesterone for vasomotor symptoms--a placebo-controlled randomized trial in healthy postmenopausal women. Menopause. 2012;19(8):886-93.
3. Fitzpatrick LA, Good A. Micronized progesterone: clinical indications and comparison with current treatments. Fertil Steril. 1999;72(3):389-97.
4. Bassani AS, Banov D, Carvalho M. Evaluation of the in vitro Human Skin Percutaneous Absorption of Progesterone in Versabase® Using the Franz Skin Finite Dose Model. J Women's Health Care. 2017; 6(4). DOI: 10.4172/2167-0420.1000384