

In Vitro Skin Structure Benefits with a New Anti-aging Peptide, Pal-KT

Rosemarie Osborne, Ph.D.¹, Lisa A. Mullins, B.S.¹, Bradley B. Jarrold, M.S.¹, Rebecca A. Taylor, B.S.¹, Robert Ha, Ph.D.¹, Larry R. Robinson, Ph.D.¹, Karl Lintner, Ph.D.²

¹P&G Beauty, Cincinnati, Ohio, USA, ²Sederma, France

INTRODUCTION

Peptides applied topically in moisturizer-type cosmetic products are known to improve the appearance of signs of facial skin aging (1). For example, palmitoyl-KTTKS pentapeptide has been shown to improve the appearance of fine lines and wrinkles in aging facial skin when applied twice daily in a facial moisturizer (2). A screening program was undertaken to identify additional peptides that may improve the appearance of aging facial skin. A peptide identified via the screening program was Pal-KT (palmitoyl-lysine-threonine), which proved to have activity superior to other peptides tested in human derived fibroblasts and human skin equivalents on skin structure- related biomarkers.

OBJECTIVE

The goal of the current work was to evaluate *in vitro* the activity of Pal-KT peptide on skin structural biomarkers linked to anti-aging benefits.

METHODS

• Human Dermal Fibroblasts

Human dermal fibroblasts were treated with Pal-KT for 72 hours. Collagen I, collagen IV and fibronectin were measured in cell extracts by ELISA, using commercial kits.

• Human Skin Equivalents

As a second-tier screen, Pal-KT at 1, 2, and 4 ppm was evaluated in human skin equivalent cultures (MatTek Human Skin EpiDermFT Skin Model, MatTek Corp., Ashland, MA USA). These skin cultures reproduce key structural aspects of natural skin, including a differentiated epidermis, a basement membrane zone and a dermal fibroblast-containing dermal matrix. Pal-KT (5.5 ppm, 100 ul/culture) in simple vehicle was applied topically to the stratum corneum surface of the skin equivalent cultures for 24 hrs at 37°C. Levels of expression of skin biomarkers were measured using RT-PCR for mRNA (Qiagen Inc, Valencia CA USA), and ELISA (commercial kits) for Hyaluronic Acid (HA), and for Type-1 Procollagen C and Fibronectin proteins, as normalized to total protein (Coomassie Blue).

References:

- (1) Int. J. Cosmetic Science 2000; 22: 207-218.
- (2) Ann. Dermatol. Venereol. 2002; 129: 1S456.

RESULTS

Figure 1. Human Dermal Fibroblasts

Human dermal fibroblasts treated for 72 hours with 1, 2 and 4 ppm Pal-KT demonstrated a dose-responsive increase in Collagen I, and maximal stimulation of Collagen IV and Fibronectin over this range of doses.

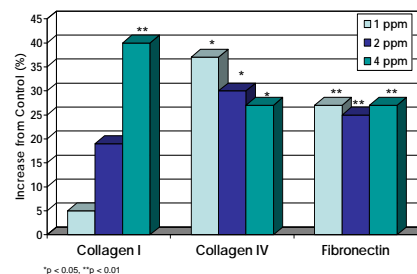


Figure 2. In Vitro Human Skin Equivalents

Pal-KT was evaluated further in human skin equivalent cultures. Histologic examination reveals that these cultures contain a stratified, cornified epidermis, a basement membrane zone (BMZ), and a dermal matrix containing dermal fibroblasts.

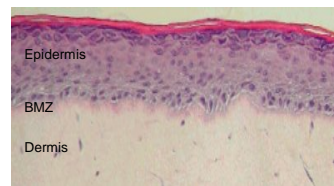


Figure 3. Human Skin Equivalents - mRNA

Topical treatment of human skin equivalents for 24 hrs with 5.5 ppm Pal-KT produced significant (RQ > 0.2, line) increases in mRNA expression of basement membrane, dermal fibroblast and dermal matrix components, including dermal remodeling.

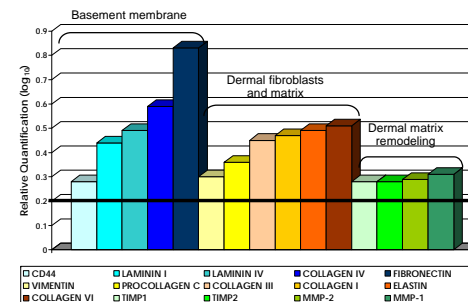
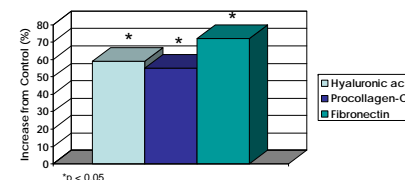


Figure 4. Human Skin Equivalents – HA & Proteins

Topical treatment of human skin equivalents with 5.5 ppm Pal-KT significantly increased Hyaluronic acid, Procollagen and Fibronectin expression.



CONCLUSIONS

Pal-KT, a palmitoylated dipeptide, increases *in vitro* expression of skin biomarkers indicative of effects on dermal fibroblasts (vimentin), dermal matrix (collagens, elastin) including dermal matrix remodeling (MMPs and TIMPs), basement membrane (laminins, collagen IV) and wound healing (fibronectin). Thus, Pal-KT produces promising *in vitro* skin responses that are widely believed to be consistent with skin anti-aging benefits.

