Promoting Production in the Extracellular Matrix Without Compromising Barrier

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Wrinkles are among the first visible signs of aging skin. It has been argued that wrinkles are caused by a lack of collagen, macromolecules found in the dermis and extracellular matrix (ECM). The objective of treating aging skin therefore must be to improve skin appearance through the promotion of ECM while preserving the barrier function of the skin.

TREATMENT OF AGING SKIN

Prior Direct and Indirect Approaches
There are a number of direct and indirect approaches to treat aging skin and promote ECM production. Direct approaches involve the use of vitamins, including vitamin A; vitamin A derivatives such as retinoic acid, retinol, and retinoid esters; vitamin C; and vitamin C derivatives such as ascorbic acid and its esters. Indirect approaches include the use of moisturizers that will stimulate biologic processes in the skin. Barrier repair substances, such as ceramides, help restore the function of the barrier and indirectly promote the production of ECM. Both approaches are documented and active but also have stability and formulation problems, such as the irritation that can be associated with topical vitamin A derivatives.

Peptides
Role—We have developed a novel approach inspired by studies on wound healing processes. This approach showed that lipophilic peptides can be used as messenger signals to trigger ECM activity and production. Peptides can be used because they are natural protein fragments that occur during metabolic processes in the skin, especially during wound healing. They are reused within the tissue to trigger the neosynthesis of tissue. Peptides have well-described roles in skin and other organs. For example, they intervene in wound healing, endocrine function, tanning, fat storage, nerve transmission, and pain moderation.

Peptides in Cell Repair—We can explain the reasons for the use of peptides to promote the production of ECM in the skin by demonstrating the wound healing process. A damaged cell reacts to the insult by releasing a number of peptides and other substances. The first activity of the peptide is to attract cells to the wound. Leukocytes initiate the inflammatory reaction, while fibroblasts act to repair the wound. These cells then release proteases that clean the wound, remove debris, and initiate proteolysis. Proteolysis, which breaks down proteins at the site of the wound, releases other signal peptides that initiate the neosynthesis of tissue. The synthesis of proteins, such as collagen, elastin, fibronectins, proteoglycans, and hyaluronic acid, is stimulated by this process.

A number of peptides with biologic activity that stimulate ECM production have been described in the literature. These include hexapeptides, such as Val-Gly-Val-Ala-Pro-Gly, which are fragments of elastin that contain chemotactic properties. This means that they attract cells to the site of the wound. Arg-Gly-Asp-Ser is a tetrapeptide fragment of fibronectin, which connects cells to each other and to collagen. Tyr-Ile-Gly-Ser-Arg is a tetrapeptide fragment of laminin, which is a structural protein in the skin that is reputed to have ECM activity in animal skin cells. Lys-Thr-Thr-Lys-Ser is a fragment of procollagen I, optimized by nature for ECM synthesis and also optimized by industry for use in topical skin care. We abbreviate this peptide as KTTKS.

A study conducted in Tennessee and funded by the National Institutes of Health discovered that KTTKS stimulates collagens I and III and fibronectin synthesis in fibroblasts in culture. The study concluded that KTTKS is the minimum sequence of collagen fragments that is necessary for the potent stimulation of collagen and fibronectin production in a variety of mesenchymal cells.

FROM CELL REPAIR TO COSMETIC APPLICATIONS
The 4 criteria for a product to successfully repair cells and offer an effective cosmetic application are as follows:

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Promoting Production in the Extracellular Matrix

Figure 1. Comparison of skin biopsies performed and stained for elastin from skin treated with Pal-KTTKS (A, B, C) vs skin treated with vehicle (D, E, F) (orcein nitrate, original magnifications ×63).

- Penetration of the intact stratum corneum
- Retains its bioactivity after penetration
- Visible benefits to the consumer
- Impact on the permeability barrier

Penetration of the Intact Stratum Corneum
This is one of the most difficult criteria, as most peptide fragments are hydrophilic and ion charged and thus do not pass through intact stratum corneum. However, this obstacle can be circumnavigated by attaching a fatty acid, such as palmitic acid, to the peptide. This creates a lipophilic peptide that is able to penetrate into the skin.

Retains Its Bioactivity After Penetration
In Vitro—Palmitoyl-KTTKS (Pal-KTTKS) has been shown in vitro to stimulate collagen IV synthesis. This is a special collagen that is located at the epidermal-dermal junction. Stimulation of glucosaminoglycans also occurs. The mechanism was investigated through a DNA array study. We wanted to see what genes were activated to explain the effects that are witnessed in in vitro culture. The genes that are activated by Pal-KTTKS are among those that are identified in wound healing processes (C. Mas-Chamberlin et al, unpublished data, 2000).

Ex Vivo—we conducted an ex vivo study (skin biopsies) where we could measure the stimulation of collagen I synthesis in full-thickness human skin. First, we confirmed the penetration of the peptide into the skin but not through the skin; the peptide remains in the dermis (C. Mas-Chamberlin et al, unpublished data, 1999).

In Vivo—we measured biologic activity in vivo in human skin when we performed biopsies on the facial skin of volunteers. Figure 1 shows histologic slides taken from biopsies after 2 and 4 months. They compare the Pal-KTTKS peptide versus the placebo or vehicle, which contains the same cream but without the peptide. We noted, for the first time in this type of study, the stimulation of elastin synthesis in the skin.

Visible Benefits to the Consumer
Next, we conducted 4 in vivo studies: 2 versus vehicle sample, 1 versus vitamin C, and 1 versus retinol. Only retinol and Pal-KTTKS showed in vivo activity that relates to reduced wrinkles.

In our most recent study, we divided 60 volunteers into 2 groups. One group of 30 people used 5 ppm of Pal-KTTKS in vehicle and the other group used the placebo (vehicle alone). We carried out image analysis of skin replicas, which
Figure 2. Results from an in vivo study of Pal-KTTKS vs placebo show changes from baseline in roughness (A), wrinkle volume (B), and wrinkle depth (C).

Figure 3. Before (A) and after (B) treatment with Pal-KTTKS.
showed a decrease in roughness, wrinkle volume, and wrinkle depth after 2 and 4 months (Figure 2). We also observed visible effects on a significant number of panelists (Figure 3). The vehicle had no significant effect.

The previous study compared the peptide in the same vehicle with retinol. Cream containing 3 ppm of lipopeptide was compared with 700 ppm of retinol for 2 to 4 months on 16 volunteers. We measured skin replicas with digital image analysis, applied visual scoring by a dermatologist, and conducted echography studies to measure skin thickness. The volume of the wrinkle depth and length decreased similarly in the 2 actives. The scoring of the dermatologist also showed progressive improvement of the skin appearance with Pal-KTTKS and retinol. Results of echography studies show an approximately 9% increase in the skin thickness for both actives after 4 months, despite the fact that the effects of the peptide are more apparent after 2 months.

**Impact on the Permeability Barrier**

It was important to determine whether this success had been achieved at the expense of damage to the barrier. We discovered that the barrier was not damaged. A study was conducted on 92 subjects comparing the Pal-KTTKS peptide versus the placebo. The levels of transepidermal water loss, the most significant parameters of the barrier, were not affected. There was no difference between the vehicle and placebo (Figure 4).

**Other Toxicology Studies**

Other toxicological aspects also have been undertaken on lipophilic peptides. The peptide and its various solutions have been tested for oral and ocular irritation, skin irritation, mutagenicity, and sensitization. These tests, using concentrations of at least 100 ppm (ie, 30 times greater than the concentration recommended for use), have demonstrated the safety of the product.

**CONCLUSION**

We conclude that lipophilic oligo-peptides, optimized for ECM production in damaged cells, show potential biologic activity that can be used for topical cosmetic treatment of wrinkles and aged skin. Although these lipopeptides do not penetrate through the skin, they demonstrate in vitro, ex vivo, and in vivo biologic activity to stimulate the production of ECM. Visible improvement in human subjects has been achieved at very low concentrations in a number of studies. A negative impact on any parameter on the skin barrier has not been shown. No other toxicity has been noted.

**REFERENCES**