Dermatologic Therapy, Vol. 20, 2007, 343–349
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DERMATOLOGIC THERAPY
ISSN 1396-0296

Cosmeceutical peptides

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ABSTRACT: Peptide cosmeceuticals are one of the new, popular options to treat aging skin. There are three main categories of cosmeceutical peptides: signal peptides, neurotransmitter-affecting peptides and carrier peptides. Although their benefits currently may not be as rigorously tested as most FDA-regulated drugs, the evidence to support their use is growing. This article attempts to review the various current popular cosmeceutical peptides, the published studies on their theoretical effects, and their practical use in dermatology.

KEYWORDS: carrier peptide, cosmeceutical, neurotransmitter-affecting peptide, peptide, signal peptide

Introduction

Cosmeceuticals are topical creams and lotions designed to improve the appearance of aging skin. These products are not tested and approved as drugs, but rather appear in the consumer arena based on theoretical benefits from in vitro studies of active ingredients. Peptides, which are short chain sequences of amino acids, are a rapidly expanding category of cosmeceuticals. There are three main categories of cosmeceutical peptides: "signal" peptides, neurotransmitter-affecting peptides, and carrier peptides. Of course, for clinical benefit, the active ingredient must be delivered to the target in a stable form and be able to have the desired biologic effect in vivo. The following article is a review of current popular cosmeceutical peptides, the published studies on their theoretical effect, and their practical use in dermatology.

Background

Cosmeceuticals are topical product formulas designed to improve the appearance of the skin. More than cosmetics that adorn or camouflage, but not categorized as drugs that alter cellular functions, cosmeceuticals have grown in popularity in the past two decades.

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One of the most interesting categories is the peptide group. These small sequence amino acid chains are being incorporated in cosmetic formulas to improve the signs of skin aging. Much of the research demonstrating the role of amino acids and peptides in reversing the cutaneous signs of aging has been a secondary benefit of research on wound healing. As far back as the 1930s, yeast extracts have been used in medications for their enhancement of wound healing (1). As technology progressed, this allowed a protein fraction to be recovered from the extract of fermented yeast. Through studies of this protein extract, a beneficial effect on wound healing and improved collagen synthesis has been demonstrated (2,3). These actions have been attributed to low molecular weight peptides that are enzymatically manufactured by the growing yeast. The peptides are theorized to up-regulate cellular growth factors leading to skin healing as a result of the stimulation of angiogenesis and granulation tissue and new collagen synthesis (4). To date, more than 500 assorted proteins have been identified from the yeast *Saccharomyces cerevisiae* (5). From this early breakthrough, the category of peptide cosmeceuticals was born.

Signal peptides

Peptides with the ability to increase fibroblast production of collagen or decrease collagenase breakdown of existing collagen should potentially

improve the clinical appearance of the fine and coarse wrinkles visible in both chronologically and photo-aged skin. As a result of wound healing research and genomic studies on the growth and stimulation of human skin fibroblasts, certain bioactive amino acid chains have been discovered, which stimulate human skin dermal fibroblast growth in vitro and decrease the depth and length of wrinkles in vivo. A few of these novel signal peptides will be discussed.

A valine-glycine-valine-alanine-proline-glycine peptide was discovered in one study of elastin-derived peptides, which significantly stimulated human dermal skin fibroblast production, whereas simultaneously down-regulating elastin expression (6,7). Additionally, this amino acid sequence was shown to be chemotactic for fibroblasts (8). The peptide was combined with palmitic acid to aid in peptide penetration of the epidermis and is marketed currently in many cosmeceutical products under the name of palmitoyl oligopeptide (9). Another study examined the tyrosine-tyrosine-arginine-alanine-aspartame-aspartame-alanine peptide sequence. It was found that the peptide inhibited procollagen-C proteinase, which cleaves C-propeptide from type I procollagen, thus leading to decreased collagen breakdown (10).

The most widely published studies and popular signal peptide is the sequence lysine-threonine-threonine-lysine-serine (KTTKS) found on type I procollagen. This pentapeptide has been demonstrated to stimulate feedback regulation of new collagen synthesis and to result in an increased production of extracellular matrix proteins such as types I and II collagen and fibronectin (11). KTTKS is linked to palmitic acid in order to enhance delivery through the epidermis for action in the dermis. It is currently marketed under the name of palmitoyl pentapeptide-3 or Matrixyl® (Sederma) (12). In a 12-week double-blind, placebo-controlled, split-face, left-right randomized clinical study of 93 Caucasian women aged 35–55, pal-KTTKS was well tolerated by the skin and provided significant improvement versus placebo control for reduction in wrinkles/fine lines by both qualitative technical and expert grader image analysis (13).

The tripeptide glycyl-L-histadyl-L-lysine (GHK) is primarily known as carrier peptides, but it has also been shown to have some signal peptide effects. GHK without copper has been shown to enhance collagen production by stimulating fibroblasts (14). GHK has also been linked with palmitic acid and marketed as Biopeptide-CL. In vitro and in vivo studies have been performed by the company (Sederma), substantiating stimulation of collagen and gylcosaminoglycans (GAG) synthesis and reduction in length and depth of wrinkles and decreased roughness (15). Lipospondin, a tripeptide linked to elaidic acid (elaidyl-KFK or elaidyl-lys-phe-lys), was designed to simultaneously activate latent transforming growth factor-beta (through its peptide domain) and inhibit matrix metalloproteinase mRNA: messenger ribonucleic acids (MMP) (through its lipophylic moiety, elaidic acid). Studies have demonstrated that it is able to up-regulate collagen and tissue inhibitor of metalloproteinase (TIMP)-1 production and down-regulate MMP-1 in fibroblast cultures (16). Another tripeptide, lysyl-valine-lysine, has been shown to have similar effects on the up-regulation of transforming growth factor-beta when combined with palmitic acid and bistrifluoroacetic acid. This peptide is currently marketed under the name of palmitoyl-tripeptide-3/5 or Syn®-Coll (17). The same company is marketing an elastin derived peptide, Biopeptide-EL (Pal-val-gly val-val-ala-proyl-glycine), which is purported to have similar effects (18). Peptamide-6, the commercial name of a hexapeptide containing phe-val-ala-pro-phe-pro (FVAPFP), which was isolated from yeast extracts, has a similar sequence as yeast heat shock proteins. It has an unknown mechanism of action; however, company-sponsored in vitro studies of human dermal fibroblasts demonstrate up-regulation of human skin genes related to stress and extracellular matrix function, and in vivo studies show increase in skin firmness (19).

Neurotransmitter-affecting peptides

The neurotransmitter-affecting peptides currently incorporated into cosmeceutical products were developed as topical mimics of the botulinum neurotoxins. Currently, only Botulinum neurotoxin type A (BTX-A) has been U.S. Food and Drug Administration (FDA) approved for subcutaneous, intradermal and intramuscular injection for facial wrinkles (20). All botulinum neurotoxin serotypes (A-G) are single-chain polypeptides which inhibit acetylcholine release at the neuromuscular junction (NMJ) in a three-step process. The single-chain polypeptides are activated by proteases and cleaved into a double chain consisting of heavy and light chain moieties. Upon cleavage, the heavy chain binds to a high-affinity receptor on the presynaptic nerve terminal, which enables internalization of the bound toxin into the cell. The light

chain moiety is a zinc-dependent endopeptidase that cleaves membrane proteins that are responsible for docking acetylcholine vesicles on the inner side of the nerve terminal membrane. The cleavage of these proteins inhibits the fusion of the vesicles with nerve membrane, thereby preventing release of acetylcholine into the NMJ. The intracellular target of BTX-A is synaptosome-associated protein of molecular weight 25 kDa (SNAP-25), which is a protein essential for the successful docking and release of acetylcholine vesicles with the presynaptic vesicle. In contrast, botulinum neurotoxin type B (BTX-B) cleaves vesicle-associated membrane protein (VAMP), which is also known as synaptobrevin. Like SNAP-25, VAMP is essential for the docking and fusion of the synaptic vesicle to the presynaptic membrane for the release of acetylcholine. Use of these polypeptides inhibits the repetitive contraction of the intrinsic muscles of facial expression and thereby reduces hyperkinetic facial lines (21). The topical neurotransmitter-affecting peptides that are currently marketed in cosmeceuticals reportedly function to decrease facial muscle contraction and thus reduce lines and wrinkles by raising the threshold for minimal muscle activity, requiring more signal to achieve movement and reducing subconscious muscle movement over time. Most of these peptides act on the soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) complex, whereas others target different parts of the NMJ or certain neurotransmitters. Although a promising idea, it has yet to be proved whether these topical neurotransmitter-affecting proteins can penetrate to the level of the NMJ.

Acetyl hexapeptide-3 (AC-gly glu-met-gln-arg-arg-NH2) is a synthetic peptide patterned from the N-terminal end of the protein SNAP-25 that inhibits SNARE complex formation and catecholamine release. This peptide is currently marketed as Argireline® (McEit [Tianjin] International Trade Co., Ltd.) (22). Acetyl hexapeptide-3 was found to inhibit vesicle docking by preventing formation of the ternary SNARE complex, which is involved in synaptic vesicle exocytosis (23,24). One open label-trial of 5% acetyl-hexapeptide-3 cream applied twice daily on 10 women demonstrated a 27% improvement in periorbital rhytids after 30 days as measured by silicone replica analysis (25). Theoretically, this peptide may mimic the effects of botulinum toxin injections.

Pentapeptide-3, a peptide of unpublished amino acid sequence currently marketed as Vialox® (Cellular Skin, Rx) is purported to act similarly to tubocurarine, the main active ingredient of curare. The peptide is a competitive antagonist at the acetylcholine postsynaptic membrane receptor. As the acetylcholine receptors are blocked, the sodium ion channel remains closed. Therefore, there is no sodium ion influx to depolarize the cell and lead to muscle contraction, and smooth muscles stay relaxed. Company-performed in vitro and in vivo studies have been performed to confirm efficacy at reducing muscle contraction and decreasing wrinkle size and skin roughness (26). Leuphasyl® (Lipotec S.A.), another pentapeptide of unpublished amino acid sequence, is proposed to modulate calcium channels by mimicking enkephalins. Enkephalins are endogenous opioids that inhibit neuronal activity. Their receptors are on the outside of neurons, coupled to inhibitory G-proteins (Gi). The docking of enkephalins on these receptors results in the release of G-protein subunits (alpha, beta or gamma) in the cell. These subunits close calcium ion channels and open potassium ion channels. Preventing the entry of calcium ions into the neuron avoids vesicle fusion and consequently inhibits acetylcholine release across the synapse to the muscle (27,28). This enkephalin-like peptide couples to the enkephalin receptor on the outside of nerve cells and a conformational change initiates a cascade inside the neuron that results in a decrease of excitability and modulates the release of acetylcholine, thus diminishing muscle contraction. In vivo and in vitro placebo-controlled cosmeceutical studies performed by the company (Lipotec) reportedly confirm efficacy at reducing neurotransmitter release and decreasing wrinkle depth as assessed by skin topography analysis of silicon imprints. The studies also showed a synergistic effect when both Leuphasyl® and Argireline® were applied together (29). Tripeptide-3 (beta-Ala-Pro-Dab-NH-benzyl $\times$ 2 AcOH), currently marketed as Syn®-Ake (Lipotec S.A.), is proposed to act similarly to Walglerin-1. Walglerin-1 is a neurotoxin found in the venom of the temple viper, which causes reversible antagonism of muscular nicotinic acetylcholine receptors (mnAChR) at the postsynaptic membrane. It is proposed to bind to the epsilon subunit of the mnAChR which prevents binding of acetylcholine to the receptor, preventing it from opening. In the closed state, there is no uptake of sodium ions so that no depolarization takes place and the muscles remain relaxed (30). The company (Pentapharm) has performed their own in vitro and in vivo tests confirming its efficacy in decreasing muscle contraction and reducing wrinkle depth (31).

Carrier peptides

Carrier peptides function to stabilize and deliver important trace elements necessary for wound healing and enzymatic processes. The most commonly encountered carrier peptide is used to stabilize and deliver copper into cells. Copper is an elemental metal that enhances wound healing, enzymatic processes, and angiogenesis. There are several mechanisms whereby copper may have beneficial effects in the skin. Lysyl oxidase is an important enzyme in collagen and elastin production. It is dependant upon the action of copper. Tyrosinase and cytochrome-c oxidase require copper as well. The detrimental effects of free radicals on the skin have been elucidated in basic science research into skin photoaging. Superoxide dismutase acts as an important antioxidant and requires copper as a cofactor. Copper is an essential cofactor for collagen and elastin formation, down regulates MMPs and reduces the activity of collagenase. It is also a required cofactor for the enzyme lysyl oxidase, an important enzyme in collagen and elastin production. The tripeptide complex, glycyl-L-histidyl-L-lysine (GHK) spontaneously complexes with copper and facilitates the uptake of copper by cells (32). This peptide sequence is found in proteins of the extracellular matrix such as the alpha chain of collagen, and it is believed to be released during wounding and inflammation. As a cosmeceutical, copper peptide is thought to improve skin firmness and texture, fine lines, and hyperpigmentation. GHK-copper complex increases levels of MMP/TIMPs and aids in dermal tissue remodeling (33). It also causes stimulation of collagen I, glycosoaminoglycans, cytochrome-c oxidase, and tyrosinase (34,35). As mentioned earlier in the carrier peptide section, a feedback stimulation of collagen repair has also been proposed for this peptide (14), but the main benefit to photoaged skin is believed to be its ability to enhance delivery of copper. Both the tripeptide alone and the copper-tripeptide complex have been found to have beneficial effects on collagen stimulation. This carrier peptide-copper combination has also been found to increase levels of MMP-2 and MMP-2 mRNA, as well as TIMP-1 and -2. As such, it could function in collagen remodeling (33). Experiments using GHK-Cu have demonstrated stimulation of both type I collagen and the GAG's dermatan sulfate and chondroitin sulfate in rat wounds as well as cultured rat fibroblasts (34). Human fibroblast cultures showed increased synthesis of dermatan sulfate and heparin sulfate after addition of the tripeptide-copper complex (36). Limited clinical trials with patients using a facial cosmeceutical product containing the complex as the active ingredient did demonstrate an improvement in the appearance of fine lines as well as an increase in skin density and thickness (37).

FIG. 1. Right side before cosmeceutical peptide treatment.

How to incorporate peptides in a facial antiskin-aging protocol

Practicing dermatologists, as well as their patients, may be confused as to the real benefit of peptide cosmeceuticals and if and when to utilize them. The clinical benefit of improved skin hydration and resulting clinical improvement of the skin's appearance can be very important. Remember the original benefit of cosmeceutical proteins such as hydrolyzed collagen, was improved skin hydration. In the present authors' practice, they

FIG. 2. Right side after 4 weeks of daily use of cosmeceutical peptide.

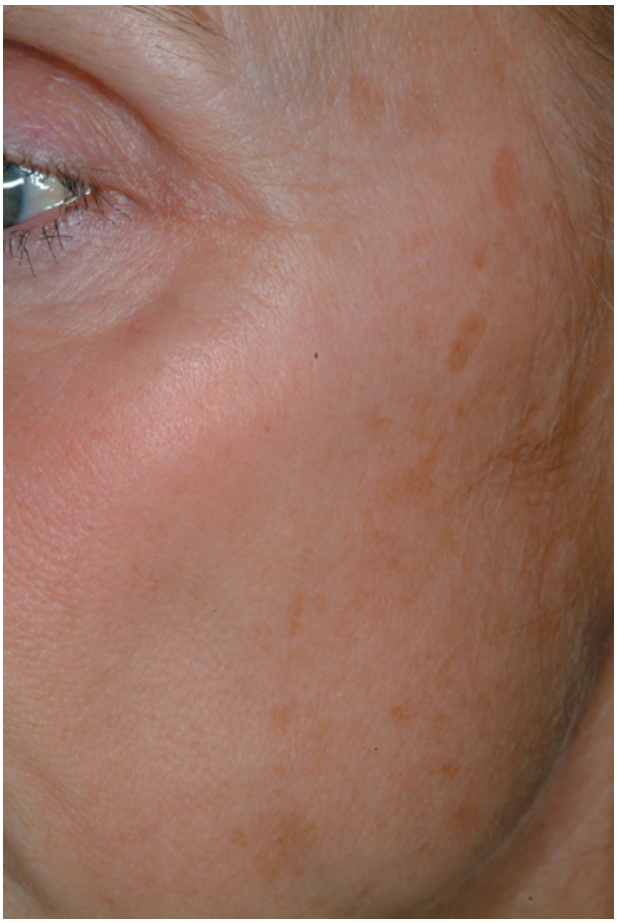

FIG. 3. Left side before cosmeceutical peptide treatment.

use these products in a complementary manner to retinoids, which, although approved by the FDA to improve the appearance of photoaged skin, often cause dryness and irritation. When compliance with retinoids is improved, clinical results improve. In a comprehensive skin care program for photoaged skin, the daily use of sunscreens in the daytime and retinoids at night is the gold standard. The addition of cosmeceuticals such as peptides may speed visible results either by enhancing collagen production, relaxing mimetic wrinkling, improving hydration and barrier function, or by a combination of these benefits. Unless intolerance to retinoids exists, cosmeceuticals should never be used instead of retinoids, but rather in addition to them to improve skin hydration and perhaps have a complementary benefit on their own. Peptides, however, are just one option. Antioxidants, alpha hydroxy acids, and growth factors are other possible options. These cosmeceuticals can be used during the daytime in addition to sunscreens, alternated with retinoids at night, or applied in addition to the retinoids in the evening.

Often the only way to assess individual response is by trial and error, as the only real goal of any cosmeceutical is to improve the appearance of aging skin in an individual. FIGS. 1 and 3 demonstrate the baseline picture of a patient prior to treatment with a proprietary cosmeceutical signal peptide. FIGS. 2 and 4 are the clinical results after 4 weeks of daily use. It must always be remembered that cosmeceutical trials yield results that could be classified by scientists as merely anecdotal. This does not mean that the product is not worthy of use, but rather it should be used with a level of appropriate skepticism. Certainly no one would argue that the clinical effects of new cosmeceuticals have improved through advances in genomic studies and improved formulations that enhance penetration. Practicing dermatologists must accept

FIG. 4. Left side after 4 weeks of daily use of cosmeceutical peptide.

that cosmeceutical companies have no incentive to make drug claims that will result in scrutiny by the FDA.

Conclusion

Peptides in cosmeceuticals are one of the new, popular options to treat aging skin. Most studies used to justify the incorporation of these ingredients into skin care products are in vitro. As dermatologists well know, these results do not always translate into in vivo actions. For any active ingredient to work, it must be absorbed in a stable form into the viable dermis. It is not an easy task to penetrate the barrier of the skin. Double-blinded, placebo-controlled drug study data is lacking, as it is with all cosmeceuticals as a result of regulatory concerns by industry. There is, however, soft clinical data and anecdotal evidence to suggest that they are beneficial and may have a place in a comprehensive skin care protocol for aging skin.

Appendix

Cosmeceutical: Topical products designed to improve the appearance of the skin by various mechanisms of action. They are not considered drugs and are therefore not regulated by the U.S. Food and Drug Administration.
Peptide: Sequence of amino acids.
Signal peptide: Peptide with the ability to increase dermal remodeling through directly stimulating human dermal skin fibroblasts, inhibiting collagenases and increasing ground substance production.
Neurotransmitter-affecting peptide: Peptide that decreases muscle contraction through its interactions at the neuromuscular junction.
Carrier peptide: Peptide that aids in the delivery into the dermis of a cofactor required for wound healing and various other enzymatic processes necessary for maintaining the dermis.

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