ARGIRELINE®

A New Anti-Wrinkle Peptide (Acetyl Hexapeptide-3)

Argireline: Inhibits Wrinkle Formation

Argireline from Lipotec S.A. is a unique new peptide that both reduces the degree of existing facial wrinkles and has been demonstrated effective against their development. It's been shown to inhibit the formation of the SNARE complex as well as catecholamine release. These inhibitions confer anti-aging activity on Argireline; they closely relate to the basic biochemical mechanisms of wrinkle formation.

Controlled studies have also demonstrated that facial wrinkle depth can be reduced, especially in the forehead and around the eyes, and that Argireline can prevent apparent facial skin aging. Skin topographic analysis performed on healthy female volunteers confirmed the validation of the proposed biochemical mechanism of action.

Causes of Facial Wrinkling

Facial wrinkling associated with aging is caused – and exacerbated – by many factors. Beyond the physiological pathways, molecular mechanisms involved in facial aging include changes in collagen conformation, elastin polypeptide degradation, and problems of the skin's lipid matrix. Recent studies clearly establish that these changes can be significantly reduced by inhibiting SNARE complex formation, a core of membrane proteins that mediate neuronal exocytosis. Their inhibition by short synthetic peptides can decrease facial wrinkle formation, and thereby the appearance of aging. The overproduction and release of catecholamines also encourages the formation of wrinkles and fine lines.

The Technology

Argireline's specific sequence was discovered within Lipotec's combinatorial library of hexapeptides and shown to inhibit the SNARE complex formation and catecholamine release. Once identified, it was synthesized by solid phase peptide synthesis and then purified and characterized.

Argireline solution was found to inhibit vesicle docking by preventing formation of the essential ternary SNARE Complex. Inhibition of noradrenaline and adrenaline release was also demonstrated in a second *in vitro* study.

In vivo tests further demonstrated the benefits of Argireline solution. Facial topography analysis (for measuring the effectiveness of an O/W emulsion containing 10% Argireline solution) was performed using silicon replicas from around the eyes at 0, 15 and 30 days of a twice-a-day treatment regimen. Analyses of the imprints were performed by confocal laser scanning microscopy. It was observed that the severity of wrinkles around the eyes decreased up to 17% after 15 days of treatment and up to 27% after 30 days of treatment, substantiating the proposed biochemical mechanism hypothesis.

Argireline Efficacy Testing

The anti-wrinkle effect of Argireline solution was ascertained in two different *in vitro* tests directly related to the formation of wrinkles in the epidermis as well as a separate *in vivo* test performed on healthy human volunteers.

IN VITRO TESTING

SNARE complex modulation in chromaffin cells

This test evaluates inhibition of the SNARE complex formed by peptides from the N-terminus of SNAP-25 (synaptosome-associated protein of 25kDa). Argireline solution modulates SNARE complex formation at concentrations in the mM range (see Fig. 1).

Chromaffin cells were prepared and further separated from erythrocytes and other impurities by centrifugation gradient. Cells were maintained in monolayer cultures. The ternary SNARE complex was precipitated and incubated with Argireline and other related peptides, or without them (as a control). Immunocomplexes were analyzed under non-reducing conditions and immunoblotted with an anti-syntaxin mAb.

Argireline proved to modulate vesicle docking by attenuating the formation of the essential ternary SNARE complex.

Modulation of catecholamine release in chromaffin cells

Inhibition of the release of catecholamines was determined by monitoring the neurotransmitters adrenaline and noradrenaline. Chromaffin cells were incubated with tritiated noradrenaline/adrenaline and Argireline. The release of catecholamines, as well as the total cell content, was determined by liquid scintillation counting. The significant modulation of both neurotransmitters at nM concentrations of Argireline solution is a clear indicator of the potent anti-wrinkle activity of this hexapeptide (see Fig. 2).

IN VIVO TESTING

Determination of efficacy against facial wrinkling

An independent study of the effect of Argireline solution on the elasticity of the skin around the eyes was performed. Using silicone facial replicas and confocal microscopic analysis, the researchers measured changes in the depth of skin wrinkling.

The skin replicas below (see Figs. 3, 4) show the improvement in facial smoothness at 15 and 30 days post-Argireline treatment, compared with the results obtained without the incorporation of Argireline solution into the test cream.

The researchers concluded that Argireline solution reduced the depth of wrinkles up to 17% after 15 days and 27% following 30 days of treatment.

CYTOTOXICITY

Human dermal fibroblasts

The test was conducted on human dermal fibroblasts at concentrations between 10 µg/ml and 1 mg/ml with a cell density of 21,000 cell/cm^2. No signs of cytotoxicity were observed.

Human epidermal keratinocytes

The test was carried out on human epidermal keratinocytes at concentrations between 10 µg/ml and 1 mg/ml with a keratinocyte density of 15,000 cell/cm^2. The results showed no signs of cytotoxicity at the concentrations assayed.

Cosmetic Application

Argireline can be incorporated in cosmetic formulations such as emulsions, gels, sera, etc., where the reduction in deep lines or wrinkles in the forehead or around the eyes area is desired. The recommended dosage of Argireline is 5%.

Product Specifications

Argireline solution

Code:	PD010	*INCI name:*	Water, Acetyl Hexapeptide-3
Appearance:	Transparent solution	*% Argireline powder:*	0.05%

Please contact us for complete study results, further technical information, and samples.

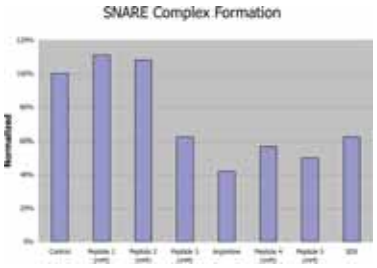

Fig. 1. Modulation of SNARE complex formation by analogues of the N-terminus of SNAP-25.

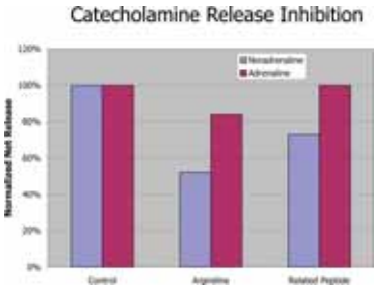

Fig. 2. Modulation of catecholamine release

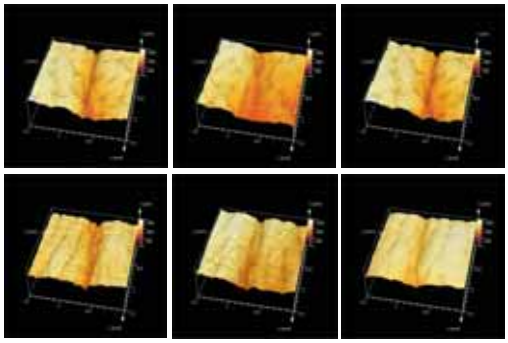

Fig. 3. Silicone replicas of skin that was untreated (top row) and treated with an Argireline-containing cream formulation.

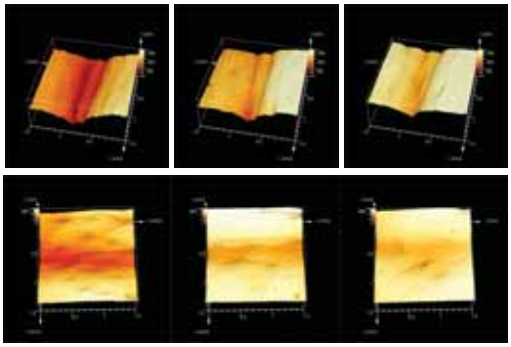

Fig. 4. Additional replicas of skin that was treated with an Argireline-containing cream formulation at 0, 15, and 30 days.

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