

EVALUATION OF EFFECTIVENESS OF VIPER SERUM FOR TOPICAL USE AS FACIAL ANTI-AGING

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Abstract

In Aesthetic Medicine a revolution to prevent skin aging and fight facial wrinkles has been the introduction into the market (for aesthetic purposes) of botulinum toxin. The action of the toxin is to weaken the muscle through a partial paralysis of peripheral nerves, with a relaxation of the skin. In our study, we tried to assess the anti-aging efficacy of three different oligopeptide preparates to prevent skin aging of the upper third of the face. Within our O.U. Plastic Surgery, we selected, at random, 12 volunteer subjects. We used three preparations will mimic the action of botulinum toxin, through transcutaneous absorption. The two main components are ARGIRELINE® and SYN®-AKE. Short-term treatment showed a general tendency to a reduction in contractile status at the level of the *corrugator* and *orbicularis* muscles. The long-term treatment, instead, showed a valid effect at the level of the *frontalis* muscle.

Keywords: Anti-aging, facial wrinkles, viper serum.

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Introduction

In field of Aesthetic Medicine, cosmetic and pharmaceutical products to prevent and fight the aging of the skin and, in particular, the appearance of facial wrinkles are continually released into the market (1). A real revolution in this area has been the introduction into the market (for aesthetic purposes) of botulinum toxin. The action of the toxin is to weaken the muscle through a partial paralysis of peripheral nerves, which means a relaxation of the district skin (2,3). The three preparations used in our study will mimic the action, but in a "relaxed" way, through transcutaneous absorption (from the outside and not through intramuscular injection as must be done when using diluted toxin).

We tried to assess the effect and the anti-aging efficacy of three different oligopeptide preparates to prevent skin aging and wrinkles formation of the upper third of the face.

Materials and methods

Patients recruitments

Within our U.O. Plastic Surgery, we selected 12 volunteer subjects (after inspection and signing of informed consent) aged between 25-30 years. This population was divided into three groups, each consisting of four individuals. Within each group was given a serum, those below, and therefore all individuals are covered by the first treatment.

Then one individual, chosen at random from each of the three groups, for the subsequent sessions, continued for the full term of 2 months.

The chosen individuals underwent 12 treatments lasting 2 months in total (from October to December 2007).

Drugs

The three gels types used consist of oligopeptides combined in different ways, but the two main components are ARGIRELINE® and SYN®-AKE.

- ARGIRELINE® is an exapeptide identified as the outcome of a study in search for a substance with similar effects to the botulinum toxin, however doesn't share toxic effects. ARGIRELINE® is a ternary complex formed by the proteins VAMP, SINTAXIN and SNAP-25 (synaptosomal associated protein). This complex is involved in the process of anchoring, exocytosis and fusion of vesicles carrying neurotransmitters. ARGIRELINE® is similar to N-terminal protein SNAP-25 and therefore compete with it for a position in the complex Snare, thus modulating its formation (4). When the SNARE complex is slightly destabilized, the vesicles cannot anchor and release neurotransmitters efficiently and therefore the contraction of the muscles are alleviated, providing for the formation of wrinkles (5).

- SYN®-AKE is a synthetic tripeptide that

mimics the effect of Waglerin 1, a peptide that is found in the venom of the Temple Viper. This product act at the post synaptic membrane; it is a reversible antagonist of the muscular nicotinic acetylcholine (ACh) membrane's receptors (6). The tripeptide links to a receptor's subunit, blocking the Ach's attachment; the ionic canal remains closed and there is no uptake of Na⁺ and the muscles remain relaxed (Fig.1A; Fig.1B) (6,7). Muscle contraction is generally reduced and consequently also expression wrinkles (1,7).

In particular, we have to keep in mind that:
 - SNAP-8® is a mimic of the N-terminal end of SNAP-25 which competes with SNAP-25 for a position in the SNARE complex, thereby modulating its formation. If the SNARE complex is slightly destabilized, the vesicle cannot release neurotransmitters efficiently and, therefore, muscle contraction is attenuated, preventing the formation of lines and wrinkles (4,5).

- LEUPHASYL® is an enkephalin modified for enhanced stability that provides a modulation of acetylcholine release from neuron cell cultures. This may reduce muscle contraction, preventing the formation of lines and wrinkles.

Particularly, the three serums were so composed:

Serum 1: homogeneous gel of average consistence enriched with Argireline® 4%,

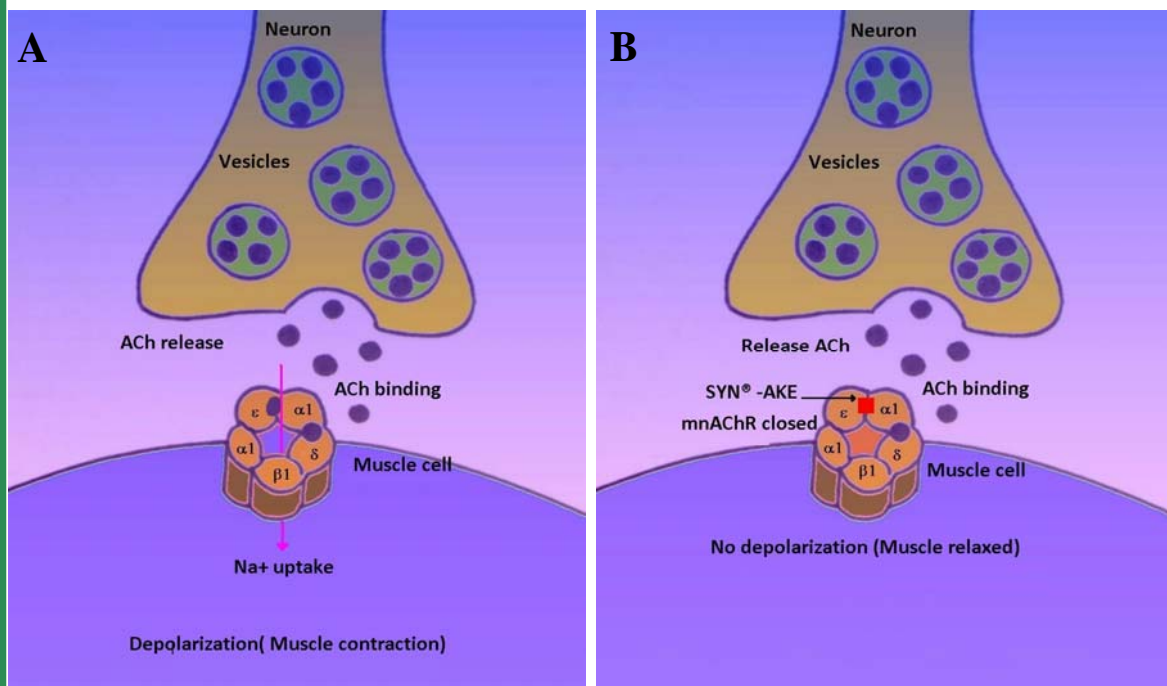


Fig. 1: A) An open ionic canal: there is uptake of Na⁺ and muscle contraction; B) SYN®-AKE links to the receptor's subunit blocking uptake of Na⁺.

Syn®-Ake 4%, oligopeptides (Carnosine, Silimarine, Vitis vinifera, Avena sativa, low molecular weight hyaluronic acid, Vitamin C, Vitamin E in liposomal form and with high biodisponibility, Beta-glucan and Oryzanol).

Serum 2: homogeneous gel of average consistence enriched with Argireline® 5%, Leuphasyl® 5% (LIPOTEC®) and oligopeptides C. These are two powerful anti expression wrinkles with complementary mechanisms of action both on acetylcholine receptor; upstream and downstream of the smooth muscle.

Serum 3: homogeneous gel enriched with Argireline® 4%, Snap-8® 4% and oligopeptides (Carnosine, Silimarine, Vitis vinifera, Avena sativa, low molecular weight hyaluronic acid, Vitamin C, Vitamin E in liposomal form and with high biodisponibility, Beta-glucan and Oryzanol). These are two powerful anti expression wrinkles, with complementary mechanisms of action, always related to the modulation of acetylcholine release in smooth muscle (one acting upstream on the acetylcholine receptor and the other on post-synaptic channel receptor, modulating the superficial muscle contraction). Industries are different, LIPOTEC® and PENTAFARMA®, but, in America, the combination has already been tested successfully.

The effect of these three different preparations are reversible and side effects are absent, unless the conditions described in the following contraindications are observed:

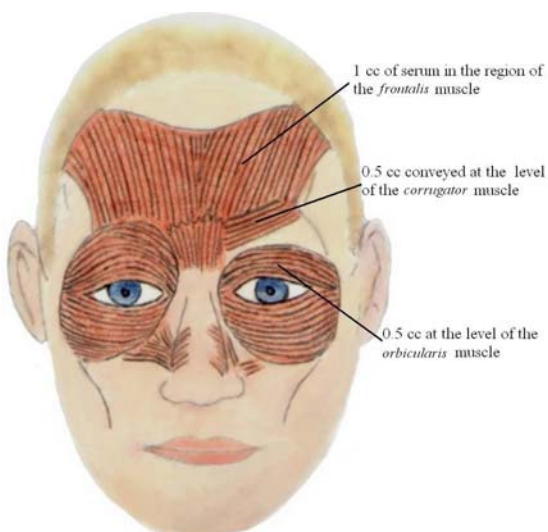


Fig.2: Application areas in the superior one-third of the face.

A) ABSOLUTE

- Hypersensitivity to each component of the formulation (Syn®-Ake, Argireline®, Leuphasyl®, Snap-8®)

- Generalized disorder of muscle (eg. Myasthenia gravis, etc.)

- Presence of infection or inflammation in the region of inoculation

- Patients already undergone Aesthetics Surgery or Aesthetics Medicine in the upper third of the face

- Pregnancy and/or breastfeeding

B) RELATIVE

- Low patient compliance

Treatments

These treatments consisted in transcutaneous application of different types of serum conveyed by radio waves generated by Thermotone Plus. The selected preparations were applied to different locations in the upper third of the face (Fig.2).

The applications time for each region was of 5 minutes (total treatment duration equal to 20 minutes) and post-treatment surveys were always carried out by the same operator.

For the first month, 8 treatments were applied with a frequency of 2 times per week (Monday and Friday), while the "maintenance regime" for the second month was one treatment per week (Friday), for a total of 4 treatments.

Evaluation of the results

In order to obtain quantitative values on the effectiveness or otherwise of different preparations, we used an electromyography (Fig. 3), connected to the electrodes, Infiniti ProComp™ (Thought Technology.Ltd) that, through a computer software Infiniti BioGraph®, has given us real-time



Fig.3: Electrodes positioned in frontal region.

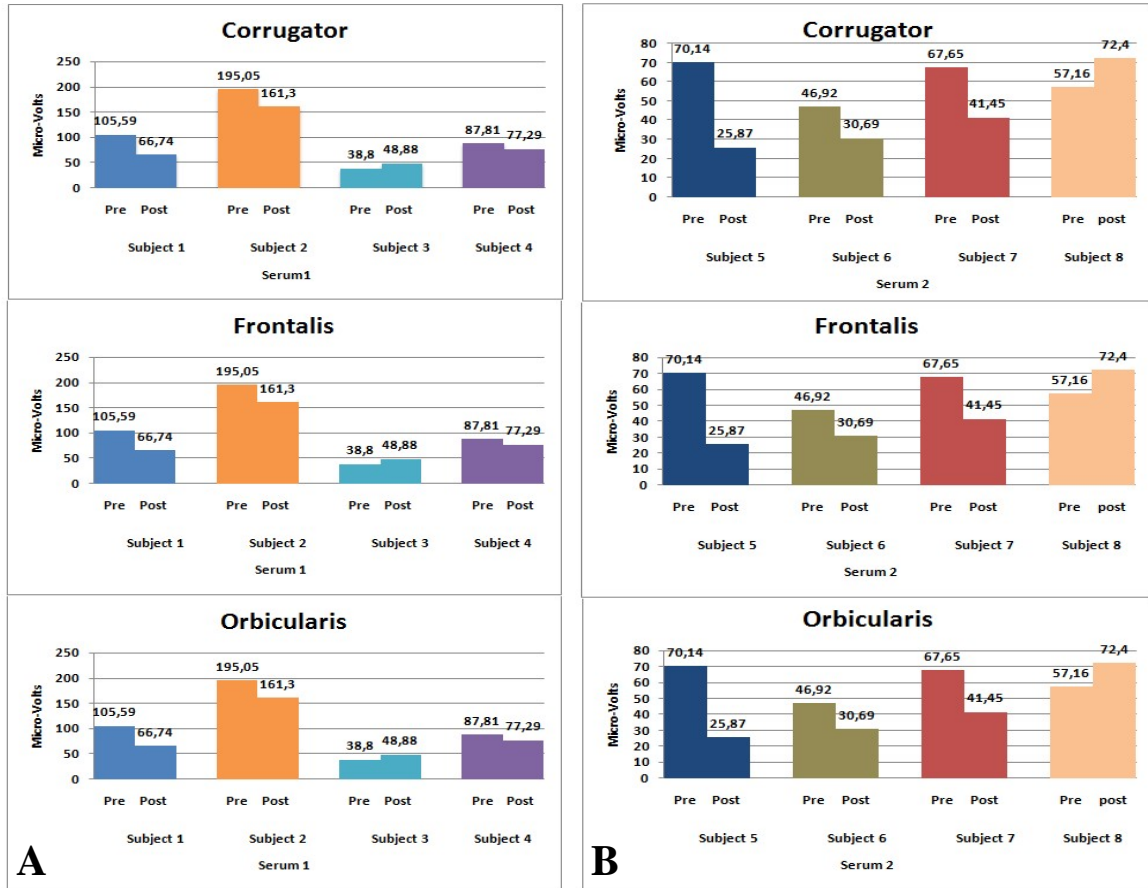
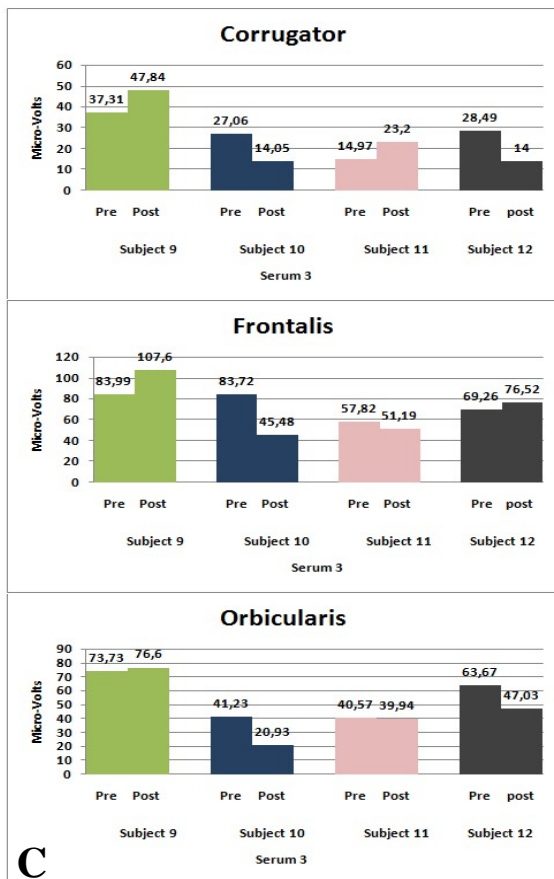


Fig.4: Results after first treatment with serum 1 (A), 2 (B), and 3 (C).



values of the contractile status of different muscles at rest or in contraction according to the directions of the operator. These are "contract" or "release" the muscle.

Since after the first treatment carried out on all subjects of the population, we have performed measurements to evaluate the response after the first application. Each subject was subjected to electromyographic study to evaluate the contraction activities of the three muscles under study (*Corrugator*, *Frontalis*, *Orbicularis*), obtaining the following results.

Results

The values reported in the histograms represent averages of measurements (Max value and Min value) performed after each treatment session (Fig. 4).

The results obtained after the first treatment, based on the measurements taken with an electromyograph, allowed to identify a reduction of the contractile status, and thus a higher degree of relaxation of the examined muscles, applied to 21 out of 36 measurements (58,3%). The measurements indicated that the best results, after

the first treatment, were obtained with serum 2, applied to 8 out of 12 measurements (66,7%). These results were evident at the level of the *corrugator* and *orbicularis* muscles. After these initial results, the three individuals, randomly selected for the study, underwent comprehensive treatment: for the first month, a total of 8 treatments with a frequency of 2 times per week (Monday and Friday); for the second month, a "maintenance regime" consisting of one treatment per week (Friday), finally a number of 4 treatments. The measurement was taken using electromyograph after each application and absorption of the serum. The results of the long-term treatment, instead, showed an effect at the level of the *frontalis* muscle. The effect consisted in a reduction of the contractile status values for all the subjects. These results of the long-term treatment have led to the following three graphs with logarithmic curves showing the relative trend (Fig. 5).

Discussion

The short-term treatment showed a general tendency to a reduction in contractile status. These results were evident at the level of the *corrugator* and *orbicularis* muscles. This reduction applied to 21 out of 36 measurements (58,3%) after the first application of the three serums. Specifically, the best results were obtained with the application of serum 2, which proved more effective as far as the

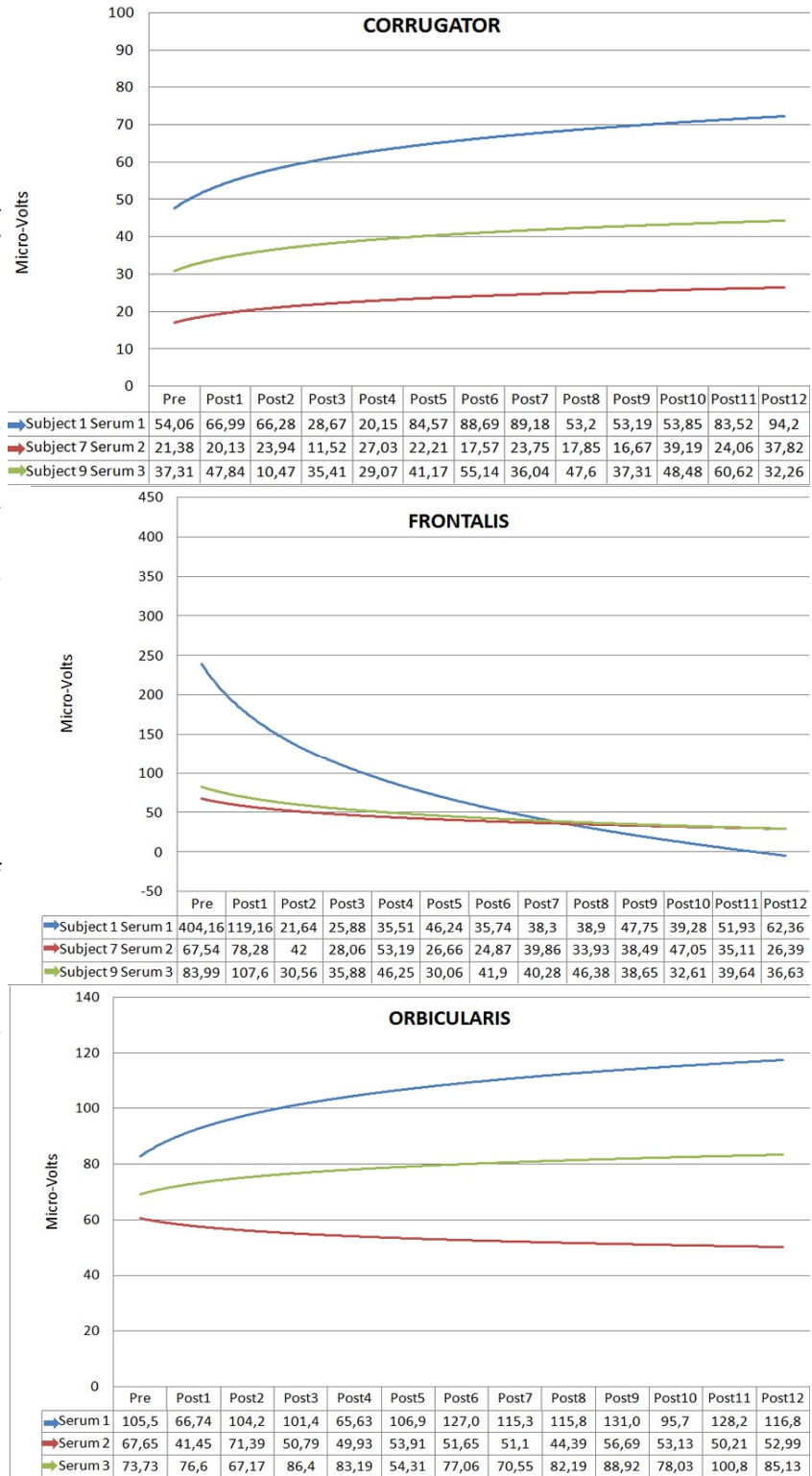


Fig. 5: Logarithmic curves showing the relative trend in long-term treatments.

corrugator and *orbicularis* muscles. This application allowed for the comparative assessment of the effectiveness of the

three serums.

The results of the long-term treatment, instead, showed an effect at the level of the *frontalis* muscle. The effect consisted in a reduction of the contractile status values for all the subjects. In the long-term treatment, all serums showed a clear effect on the *frontalis* muscle, possibly due to anatomical factors. The muscle, in fact, displays a wider surface suitable for the absorption of the serums through the skin. The application of the long-term treatment allowed for the comparative assessment of the responses for the examined muscles. The study has shown an approach to the assessment of the effectiveness of the different preparations containing the above mentioned. The validity of the method for the application and subsequent measurements has been demonstrated. Surely, in order to thoroughly assess the effectiveness of the products, it will be necessary to significantly expand the sample of the population to have a larger number of measurements.

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VALUTAZIONE DELL'EFFICACIA DEL SIERO DI VIPERA QUALE ANTI-ETA' TOPICO FACCIALE

Una rivoluzione in Medicina Estetica, nella prevenzione dell'invecchiamento cutaneo e nel combattere le rughe facciali, è stata l'introduzione nel mercato della tossina botulinica (ad uso estetico). L'azione della tossina consiste nel ridurre la contrattilità muscolare attraverso una parziale paralisi dei nervi periferici, con conseguente rilassamento cutaneo. Nel nostro studio, abbiamo cercato di verificare l'effetto anti-aging di tre differenti preparati oligopeptidici per prevenire l'invecchiamento cutaneo nel terzo superiore del distretto facciale. All'interno della nostra U.O. di Chirurgia Plastica, sono stati selezionati casualmente 12 soggetti volontari. Abbiamo usato tre preparati che mimano l'azione della tossina botulinica attraverso assorbimento transcutaneo. I due componenti principali sono ARGIRELINE® e SYN-AKE®. Il trattamento a breve termine ha dimostrato una generale tendenza a ridurre lo stato contrattile a livello dei muscoli corrugatore e orbicolare. Il trattamento a lungo termine ha mostrato una valida risposta a livello del muscolo frontale.

Parole chiave: Invecchiamento cutaneo, rughe facciali, siero di vipera.

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