

Article

The Efficiency and Safety of Leuphasyl—A Botox-Like Peptide

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Abstract: Peptides of synthesis are a very new strategy in cosmetic science and technology for at least two reasons: (1) they are small molecules, easily penetrable in the skin and (2) they are able to induce a very specific action, because all skin cells (keratinocytes, fibroblasts, nervous cells) have membrane receptors for peptides. This group of cosmeceutics includes the botox-like peptides, represented by acetyl hexapeptide 3 (Argireline) and pentapeptid-3 (Leuphasyl). The latter is less known and has been less studied. This substance inhibits the neuromuscular synapses in the mimic muscles, acting as enkephalins. It links the enkephalin receptor to nervous cells, thereby modulating the release of acetylcholine in synaptic space. This cellular activity will be translated *in vivo* in a relaxation of the muscle and a reduction of expression wrinkles. The aim of our study is to evaluate the optimal concentration of Leuphasyl for skin application at the mimic muscle level, the efficiency and the safety of this peptide. We formulated three emulsions of different concentrations (0.5%, 1%, 2%) which were applied to the skin, at the level of mimic muscles (1) at the eyebrows zone (above the *corrugator supercilii* muscle) and (2) at the periorbital zone (above the *orbicularis oculi* muscle). We evaluated the regression of the wrinkles between the eyebrows using an imagistic method: pro-derm Analyser. The study is of interest to discussions concerning how to apply these kinds of cosmetic products at the mimic muscle skin level and not at the level of the wrinkles.

Keywords: wrinkles; aging skin; botox-like peptides

1. Introduction

Among efforts focused to increase life quality are those in the field of dermo-cosmetic anti-aging innovation, mainly to decrease (and to prevent) the extension of facial wrinkles. To a large extent, wrinkles were ignored as a serious subject by most scientists until about 35 years ago when scientific articles began to appear on the nature and origin of wrinkles.

To find pathogenic treatments against wrinkles (or to prevent them) it is necessary to understand how wrinkles develop. According to their development, there are four types of wrinkles [1]: (1) Atrophic Crinkling Rhytids—fine lines on the face that are almost parallel to each other. They show up on different areas of the face and body but tend to disappear when skin is stretched transversally (this just means they shift when your body posture changes). These wrinkles are associated with loss of elasticity (loss of collagen); (2) Permanent Elastic Creases—crease lines in the skin that become increasingly permanent over time, especially with sun exposure. They show up most frequently on the cheeks, the upper lip and the base of the neck. Sun exposure and also smoking are involved in extension of those wrinkles; (3) Dynamic Expression Lines—wrinkles that are caused by facial muscle movement. Most of the facial muscles have involuntary movements. In this case the wrinkles' form is perpendicular to the long axis of the muscle. The relaxation of the muscles over which the skin is pulled can improve those wrinkles; (4) Gravitational Folds—as the name implies, these lines are due to the effects of gravity and become increasingly obvious as skin begins to fold and sag. The location of these wrinkles is related to the thickness of skin.

While elastic creases could be prevented by reducing sun exposure and others types of wrinkles could be reduced by using collagen stimulators, dynamic expression lines are still a problem. Their most efficient treatment is represented by botox (botulinum toxin) injections (chemodenervation), but this imposes a lot of side effects (facial asymmetry, additional dynamic wrinkles in botox-free facial zones) besides the fact that they are expensive for the patient. These are sufficient arguments for cosmetic industry to search for innovating cosmetic botox-like molecules.

In the group of botox-like cosmetic active substances there are two main peptides: acetyl hexapeptide-3 (Argireline) and pentapeptid-3 (Leuphasyl). Argireline is well known and already used [2]. The latter is less known and has been less studied. This substance inhibits the neuromuscular synapses in the mimic muscles, acting like enkephalins. It links the enkephalin receptor to nervous cells, and thereby modulates the release of acetylcholine in synaptic space. This cellular activity will be translated *in vivo* in a relaxation of the muscle and a reduction of expression wrinkles [3].

The aim of our study is to evaluate the optimal concentration of Leuphasyl for skin application at the mimic muscle level, the efficiency of those different concentrations, the behavior of a cosmetic formulation containing Leuphasyl for two different facial skin zones (frontal region and periorbital region) and the safety of this peptide.

2. Materials and Methods

2.1. Leuphasyl[®]

Leuphasyl[®] is a Lipotec product, (Lipotec SAU, Barcelona, Spain), an active botox-like molecule [4]. Using this substance, we formulated 3 emulsions of different concentrations (0.5%, 1% and

2%). The composition of the formulation was the following: Polysorbate 60, 2.0%; Cetostearyl alcohol, 10%; Vaseline, 18%; Glycerol, 10%; Dimethiconol Blend 20, 5%; Liquid paraffin, 2%; Distilled water, add up to 100%.

Distilled water is decreased with the amount of active agent.

2.2. Methodology for the Cosmetic Product Rheological Evaluation

In order to apply the product on volunteers' skin we made a rheological evaluation of the cosmetic product. The rheological profile of the samples was studied by PaarPhysica MCR101 rheometer (Anton Paar GmbH, Graz, Austria). A cone-plate measuring device was used in which the cone angle was 1° and the thickness of the sample was 0.046 mm in the middle of the cone. The measurements were carried out at 25 °C. Flow curves of the different samples were determined.

2.3. Volunteers

Our three formulas (concentration) of this active molecule were applied on 20 volunteers, who accepted to perform this study, for 2 months. Experimental protocol had the following admission and exclusion criteria:

Admission criteria for volunteers:

- The written accept of enrolling in the study;
- Minimum age of volunteers: 30 years old, meaning that it is necessary to have evident chronoaging skin signs (dynamic wrinkles);
- No signs of skin pathology on face;
- Ceasing to use other anti-aging products, mainly at the level of dynamic wrinkles, which are evaluated during those 60 days.

Exclusion criteria for volunteers:

- Acne or sebaceous syndrome, pathologies which represent contraindication for anti-ageing emulsions;
- Volunteers without botulinicum toxin injections at the level of face muscles having minimum 1 year history (can generate false positive effect).

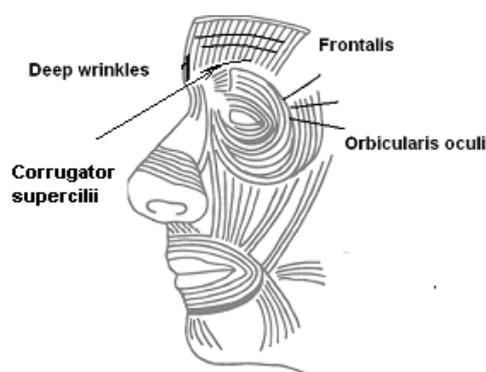
2.4. Instrumental Skin Evaluation

The wrinkles' involution in the period of the experiment was performed with Proderm Analyzer (NU SKIN, Provo, UT, USA). This device registers the wrinkles size in two dimensions, in a 1/1 cm of skin surface and the software of this instrument transforms the wrinkle into a blue line, in order to be measured.

Our emulsion was applied by all volunteers daily, at the mimic muscles' level. We were interested in two main anatomical regions of facial muscles mimics (Figure 1): frontal region and periorbital region.

The instrumental evaluation was registered once a week during the period of the test.

Figure 1. Facial anatomic regions which were explored in the study.

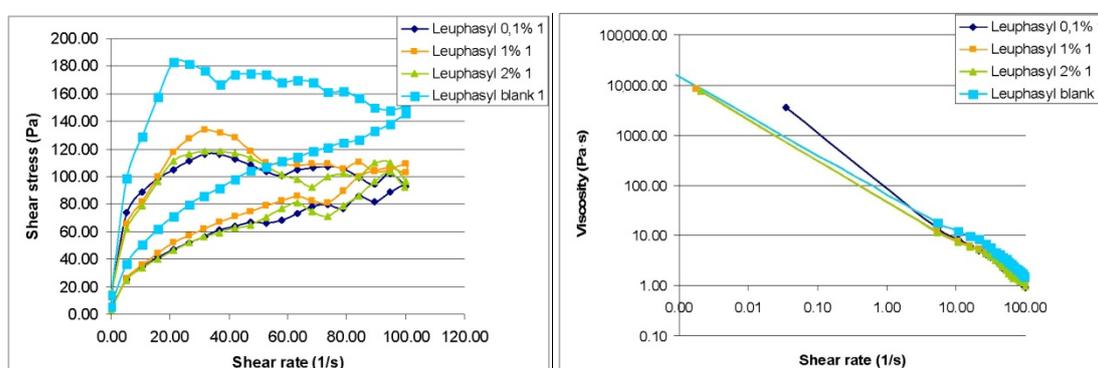


3. Results

3.1. Results for Rheological Determinations

Using the described rheometer we have demonstrated that the shear rate was increased from 0.1 to 100 1/s (up curve), and then decreased from 100 to 0.1 1/s (down curve). The shearing time was 300 s in case of both segments (Figure 2). The cosmetic product containing Leuphasyl can be easily spread on skin, with a good adhesion to the skin surface.

Figure 2. Rheological properties of Leuphasyl cosmetic formulation.



3.2. Results of Instrumental Skin Evaluation with Proderm Analyzer

As we already specified, Proderm Analyser is a device for imagistic skin exploration, which shows the wrinkles in 2 D (in blue colors). All images taken during the period of the test were printed in a standardized size (5 cm/3 cm), in order to be compared (Figure 3). For all those, the sizes of wrinkles were measured (mm). We have compared the dynamic wrinkles' involution (in time), for both anatomic regions (frontal and periorbital).

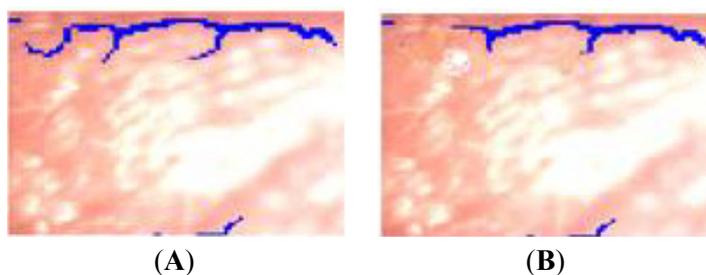
Our results emphasize that only for Leuphasyl 2% there are important ameliorations. The size of the wrinkles trajectory was smaller with the following average:

- 34.7% for frontal region (inter eye-brows zone);
- 28.4% for periorbital zone.

The best results were obtained for the frontal region (inter eye-brows zone) compared to periorbital region. A possible explanation is that for the periorbital region, a big group of muscle fibers is

involved (*orbicularis oculi*), but at the inter eyebrows zone level there is only one important mimic muscle: *corrugator supercilii*.

Figure 3. Samples of images (5 cm/3cm) of a periorbital wrinkle (in blue), in evolution. (A) left latero- orbital wrinkle of a female volunteer aged 47 years before Leuphasyl treatment; (B) left latero- orbital wrinkle of a female volunteer aged 47 years after Leuphasyl treatment.



In most of the cases, wrinkles decrease starting from the external part, mainly for the periorbital wrinkles. Our methodology in 2D does not permit us to evaluate the wrinkles depth. This parameter remains a finding of the subjective evaluation, obvious for the inter eyebrows zone.

We must admit also that at the end of the experiment there was not a total disappearance of the wrinkles for any of the subjects. This result may be present after the butulinum toxin injections, but this fact happens with the price of losing the facial expressivity [5].

3.3. Subjective Evolution

Without knowing the results of the instrumental exploration, volunteers were asked to estimate the beneficial effect (using scores from 1 to 10), at the end of the test, for each facial region. Table 1 shows the scores average for both facial regions.

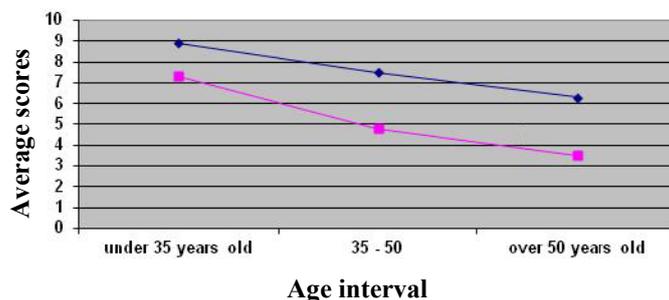
Table 1. Scores average for inter eyebrows zone and periorbital region, in the volunteers perception.

Type of wrinkle	Under 35 years old	Between 35 and 50 years old	Over 50 years old
Inter eyebrows zone	8.96	7.56	6.33
Periorbital region	7.32	4.84	3.56

Figure 4 represents a comparison between the two volunteer perceptions, for frontal region (in blue) and for periorbital region (in pink). This comparison emphasizes two important things:

- Aged skin responds later and more slowly to a specific cosmetic emulsion than young skin;
- Like in instrumental measures, the benefit for the frontal region (inter eye-brows zone) is superior to the periorbital zone.

Figure 4. Comparison between the two volunteer perceptions, for frontal region (in blue, the upper line) and for periorbital region (in pink).



3.4. Comparison between Instrumental and Subjective Results

It is quite impossible to compare an imagistic exploration (in mm) with a subjective result (scores given by volunteers, according to their own perception). In spite of this difficulty, there are two main evidences, which transpired in those two explorations:

- Aged persons respond with more difficulty to a specific cosmetic emulsion than young persons;
- The benefit for the frontal region (inter eye-brows zone) is superior to the periorbital zone.

4. Discussion

Our study raises some interesting discussions.

The frontal wrinkles can be better reduced compared to periorbital wrinkles, and that evidence was observed in both tests: instrumental and client test. We hypothesized that in periorbital muscles there are more muscular fibers, situated in a deep anatomic layer, difficult to be penetrated by the cream.

The client test shows another important thing: in young people the amelioration is more evident and more rapid than in mature people and a possible explanation is that young skin having more trophic factors, is able to regenerate easier.

Even if results obtained using Leuplasyl in this present study are beneficial, in literature results given by Argireline are quite superior. In our opinion, it is ideal to use a mix of Leuplasyl and Argireline for a synergic effect. Each one of those substances have another pathway of action in mimical muscles, so a mix of those could induce a longer and a more lasting effect. When using Leuphasyl in antiaging products it is absolutely necessary to respect the minimal concentration. Respecting indicated concentration remains an ethical problem of each production laboratory.

Above all those observations, the most important thing to be underlined by us is the importance of the anatomic skin region where the cosmetic botox-like product must be applied [6]. Some production laboratories show the application of the cosmetic product on the wrinkles' lines (Figure 5). In our opinion this is the biggest mistake in the advice process. The wrinkles are the result of involuntary muscular contractions and it is necessary to block (or to relax) those muscle fibers, so the cosmetic product must be applied on the skin region above the muscle.

Figure 5. The wrong way of applying a botox-like cosmetic product. The example was included in the prospectus of a pharmaceutical product.



5. Conclusions

Leuphasyl is an active synthesis peptide, having botox-like action, and a proven efficacy for minimum 2% concentration. The efficiency of this molecule is evidently inferior to botulinum toxin injections, but it is absolutely free from side effects and it confers an aspect of wrinkles' attenuation, also preserving facial expressivity.

In our opinion, there are two conditions of efficiency for cosmetic botox-like molecules: (1) to respect the minimal active concentration (in this case 2%) and (2) to apply the botox-like cosmetic product on the mimic muscular skin zones and not on the trajectory of the dynamic wrinkles.

It would be possible to increase the efficiency of Leuphasyl by its association with Argireline. Even if their cellular pathway of action is different, the benefit would be more visible.

Conflicts of Interest

The authors declare no conflict of interest.

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