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ARTICULO ORIGINAL

Evaluation of the anti-wrinkle efficacy of cosmetic formulations with an anti-aging peptide (Argireline®)

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ABSTRACT

The purpose of this research was to evaluate, by means of in vivo studies, the efficacy of new cosmetic active ingredients which effect of botox, called Argireline®), so that width and depth of wrinkles could be established. For this, it is prepared two formulations: an emulsion with an external aqueous phase for normal to dry skin, and a gel for oily skin. We likewise study the water content of the skin after the application of both formulas, as this must be one of the priority functions of facial treatments in general, as well as the level of satisfaction from the subjective point of view, fundamental for patients and their continuation of the treatment.

After the designed tests, it is possible to verify that there is a remarkable diminution of the wrinkles size tested in each patient during the month of treatment. Besides, it is possible to review how the moisturizing capacity has been increased in all cases.
At the end of the visual test, all the volunteers experienced a reduction in the depth of wrinkles, and from the subjective point of view, the appearance and elasticity of the skin were improved. Finally it is possible to conclude that Argireline® (acetyl hexapeptide-8) shows a great antiaging capacity in all the cases that have been studied and the tried compounds have increased moisturizing power.

KEYWORDS: Botox effect, Argireline®, Acetyl hexapeptide-8, Anti-Wrinkling Efficacy

INTRODUCCIÓN

Independently of the physiological origin of wrinkles, the molecular mechanism involved in the ageing of the skin is directly related to changes in the formation of the triple helix of collagen, the deterioration of the polypeptides of elastin, and disorder in the packing of the skin's lipid matrix. In other words, skin ageing involves a slow impairment of the cellular functions and thus atrophy of the skin in both the epidermis and the dermis[1]. However, in recent years, it has been demonstrated that the contractions that appear in the membranous septa of the connective tissue can be responsible for expression lines[2]. In this regard, favourable results with botulin toxin infiltration led to the development of a new active principle with effects similar to the botox effect, named Argireline®, as

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an alternative to botulinum toxin.

The cosmetic possibilities of botulinum toxin were first realised in 1987, when an improvement in the expression wrinkles of the brow was noted after treating a patient with blepharospasm, though the first publications on the treatment of wrinkles on the glabella and the peri-ocular region did not appear until the start of the nineties[3], and subsequently its usefulness was demonstrated on other areas of the face and neck[4]. However, there are adverse local effects as a result of puncture (pain, erythema, oedema) and diffusion of the toxin (ptosis of the eyelids and eyebrows, asymmetry of the smile, flaccidity, difficulty in speaking)[5].

The search for new compounds to prevent or attenuate skin aging and enhance self-image[6] is a priority of current research on active cosmetics.

However, and despite the great number of substances that promise rejuvenation of the skin produced by the cosmetics industry, very few of them seem to truly have an effect on the skin[7]. Given the social implications surrounding physical appearance, the main aim of this work is to verify the effect of the active cosmetic ingredient, Argireline®, in different types of formulations: gel and cream, which are applied to oily and dry skins, respectively, on women aged between 40 and 57. Likewise, the degree of moisturisation and patient satisfaction of both preparations were studied.

Material and Methods

Material

The products used as components in our formulations were:

Argireline® (acetyl hexapeptide-8), Batch F1460/04, supplied by Lipotec (Barcelona, Spain).

Neo PCL o/w Autoemulsionable® (cera alba, stearyl heptanoate, cetearyl octanoate, cetyl palmitate, stearyl alcohol, steareth-7, steareth-10, stearyl caprylate, isopropyl myristate, myristyl alcohol, dimethicone, paraffinum liquidum, Batch 0512651, supplied by Roig Farma-Fagron (Terrasa, Spain).

Tefose 2561: (PEG-6 stearate, Ceteth-20, Glyceryl stearate, Steareth-20, Batch 0503697, supplied by Roig Farma-Fagron (Terrasa, Spain).

Cyclomethicone pentamer (cyclopentasiloxane), purity 99.26%, Batch 0509565, supplied by Roig Farma-Fagron (Terrasa, Spain).

Sorbitol 70%, Ph. Eur., purity 70.1%, Batch 0405020, supplied by Roig Farma-Fagron (Terrasa, Spain).

Glycerol Ph. Eur., purity 99.8%, Batch 05F0204, supplied by Roig Farma-Fagron (Terrasa, Spain).

Hispagel 200®, Batch 0409242, supplied by Roig Farma-Fagron (Terrasa, Spain).

Propylene glycol Ph. Eur., water content <0.1%, Batch 04K23FP, supplied by Roig Farma-Fagron (Terrasa, Spain).

Phenonip® (phenoxyethanol, methylparaben, butylparaben, ethylparaben, propylparaben), Batch 0510592, supplied by Roig Farma-Fagron (Terrasa, Spain).

Kathon CG® (methylchloroisothiazolinone 1.5%, methylisothiazolinone 0.37%), Purity 75.2%, Batch 0504885, pH 2.6.

Deionized distilled water, supplied by Interapothek (Murcia, Spain).

Methods

1.Preparation of formulations

Was prepared an oil/water emulsion for normal-to-dry skin and a cream for oily skin. The used technique was the same one that the published study[8].

Both formulations were stored at room temperature (25 ºC).

2. In Vivo Trial: Hydrating and Anti-Wrinkling Efficacy

The measurement apparatus was Clinipro Antiaging SD, using the IMAGE DB system, which makes it possible to determine both the width and depth of the wrinkles. The first day of the trial and prior to the application of the formulations, the depth and width of a given wrinkle were measured, which would be the same throughout the study period. In addition, it has a flat probe with surface area of 1.5cm^2, which is applied at constant pressure on the skin. This probe determines the amount of water present in the surface layers of the skin.

From the values obtained, the percentage of reduction during the study period was calculated.

Photographs were also taken of these wrinkles on all the volunteers before starting the test and after 30 days of application of the product. The images were taken of the wrinkles under study with facial scanner that processes the images taken of the wrinkles under study, which were subsequently processed by software.

A fixed amount of the studied formulation was deposited, by means of syringe, onto the entire surface of the faces of 20 volunteers aged between 40 and 57 years. The face was then gently and uniformly massaged for approximately 30 seconds, until complete absorption of the formulation.

The volunteers were divided into two groups, those with dry skin and those with oily skin, 50% of the volunteers in the study had oily skin and the other 50% dry skin.

The study was always carried out by the same person in order to reduce possible experimental errors, and throughout the trial period the volunteers undertook not to use any other type of preparation except that under study, twice a day.

The environmental conditions were between 23-25ºC and relative humidity of 60%.

Two types of trials were carried out[9]: It was studied whether the two formulations could affect the aqueous content of the skin and the evolution of the wrinkle, in the short-term and long-term.

Short-term trial: the formulas were applied every 12 hours and measurements were taken 24 hours after application.

Long-term trial: To do this, the volunteers were treated with the cream or gel for one month, twice a day. The evolution of the aqueous content of the skin was determined after 7, 15, 21 and 30 days.

3. Sensorial Evaluation

The subjectivity of the evaluator may condition the evaluation of a cosmetic[10], and may even lead to interruption in the use of it. Therefore, in order to obtain their personal perceptions, the volunteers were asked about both the product during its application and its action at the end of the study[11].

The tests carried out were:

- Acceptability of the products based on the absence or presence of sensorial properties. The organoleptic characteristics were evaluated in accordance with the following descriptive terminology[12]: thick, hard, creamy, smooth, soft,dry, thin, spreadable, cool or warm.
- Adequacy[13]: this was assessed by the patients themselves, who classified them within the following categories: Satisfactory, Passable, Non-satisfactory.
- Improvement in the texture and elasticity of the skin: evaluated in terms of the appearance and feel of the skin, assigning one of the following categories: Very Good, Good, Passable, Bad.

The parameters were visually assessed by the researchers.

The researchers assigned points to the reduction in the wrinkles, comparing each time point with the photograph taken at the start of the study: 1. No reduction, 2. Slight reduction, 3. Medium reduction, 4. Considerable reduction

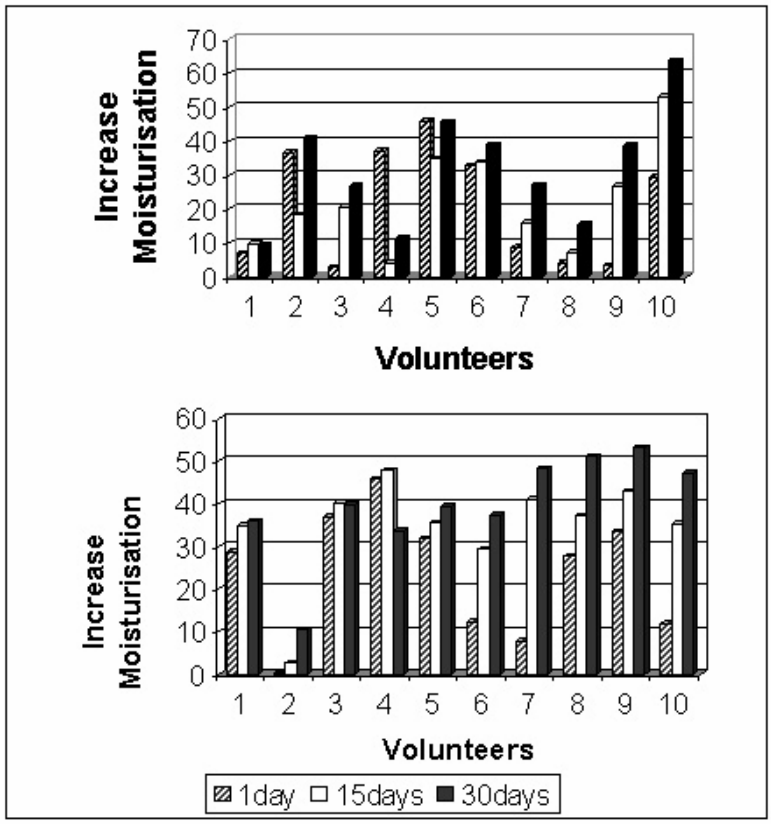
Results and Discussion

Three determinations were carried out on each volunteer. The average values and the standard deviation were calculated for each one of the trials. All the results underwent an Anova statistical treatment to give a confidence level of 95%, in order to check whether there were any significant differences between the averages compared.

1. In Vivo Trial: Hydrating and Anti-Wrinkle Efficacy

Moisturisation Test:

Figure 1. Evolution of the wrinkle depth on dry skin (a) and oily skin (b).



The increase in the water content was immediate, appearing in all cases after 24 hours. The skin

moisturisation values increased considerably in both the short- and long-term studies, varying, at the end of these, between 11.43%-64.28% and 35.71-81.43%, for dry and oily skins, respectively. It was noted that in some patients the aqueous content on the first days of the treatment was greater than that obtained on successive days.

In the course of the study, there was a progressive increase in the moisturisation level of the face. The differences noted between zero hours and 24 hours, 15 and 30 days after the application of the cream or gel, are shown in figure 1 as a % of moisturisation gained over the course of the two trials. The greatest increase over the course of the study was after 30 days, with the exception of one patient for each skin type. The results show how, at the end of the study, the aqueous content of the skin had increased in all the patients, compared to day 0, with values of between 9.65 - 63.54 and 10.33 -53.02, for dry and oily skins, respectively.

Anti-Wrinkle Test

Figure 2 show the evolution in the depth and width of the wrinkles for both formulations. Particularly notable is the reduction in depth compared to the reduction in width of the wrinkles. However, the results show a reduction in both parameters after 30 days of treatment. The values of depth of the wrinkle reduced significantly in both the short-term and long-term studies. The same is not true for the width, as in the majority of patients with dry skin, the differences in width over time are not very significant. In patients with oily skin, these differences were only relevant after 30 days of treatment. Despite this, reductions were achieved in the width, which varied between 8.36 - 75.17% and 11.85 - 88.88 %, for dry and oily skin, respectively.

On the other hand, there was a clear reduction in the depth 24 hours after the application, compared to the start. After one week, this difference had increased. The same was true after 15, 21 and 30 days. The greatest difference in the reduction of the depth of the wrinkle was after 30 days, with reductions of between 41.83 - 77.79% and 64.16 - 78.25 %, for dry and oily skins, respectively.

In general, and in accordance with the results obtained, the values of cutaneous moisturisation, depth and width of wrinkle were slightly more favourable for the volunteers with oily skin.

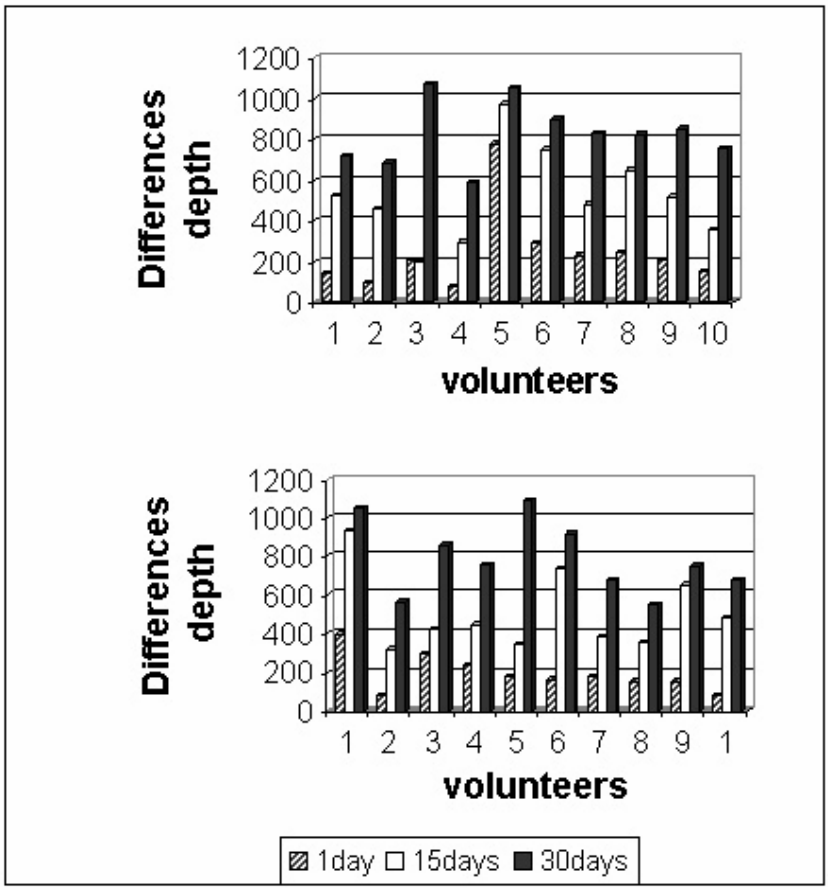
The appearance of the skin depends on its general condition, and in particular its level of moisturisation, as this regulates the elasticity, flexibility and smoothness of the skin[14-16].

The integrity of the extracellular matrix, especially the collagen fibres, is essential to ensure that the epidermis is firmly anchored to the dermis. It has been suggested that the deterioration of the collagen fibres, at the dermis-epidermis union, weakens it, which finally leads to the appearance of wrinkles[17-18].

In expression wrinkles, the contraction of the muscle leads to contraction of the fibroblasts and with this the contraction of the collagen fibres and even deterioration of the extracellular matrix in the affected area. Consequently, the hydrating properties of all the components of the matrix will be affected.

According to the results obtained in the moisturisation and anti-wrinkle tests and based on the bibliography consulted regarding the action mechanism of Argireline[19], it could be said that it is capable of relaxing the fibroblasts, which will decontract the collagen and elastin matrix, which could be decisive in preventing deterioration of the functions of collagen, such as water retention. Thus, it could be deduced that by reducing the wrinkle, greater moisturisation is achieved.

Figure 2. Differences in reduction of depth compared to day 0 for dry skins (a) and oily skins (b).



2. Sensorial Evaluation

Favourable results were obtained for both formulas:

With regard to the organoleptic characteristics of the preparations, all the patients classified them as satisfactory. All of them described the cream as smooth, creamy and spreadable, and the gel as smooth, soft and cool.

From the subjective point of view, the appearance and elasticity of the skin were improved.

Figure 3 are photographs of a wrinkle of volunteers with dry and oily skin, respectively, taken at the start and after 30 days of treatment. In the photographs, is possible to observe that the formulations studied improve the appearance of the wrinkles.

Table I shows both the points and the statistical analysis of the visual evaluation of the reduction in depth and width. The average variation in the depth of the wrinkle is given in figure 4. The Friedman test was used.

The qualitative reduction of the wrinkles was determined, obtaining improvements in 90 and 100% of the volunteers, with dry and oily skin, respectively. According to Friedman test, the data obtained after 15 and 30 days are significant (Table II). At the end of the visual test, all the volunteers experienced a reduction in the depth of wrinkles.

Figure 3. Photograph of the wrinkle of a volunteer with dry skin (0 day-A); (30 day-B). Oily skin (0 day-C); oily skin (30 day-D).

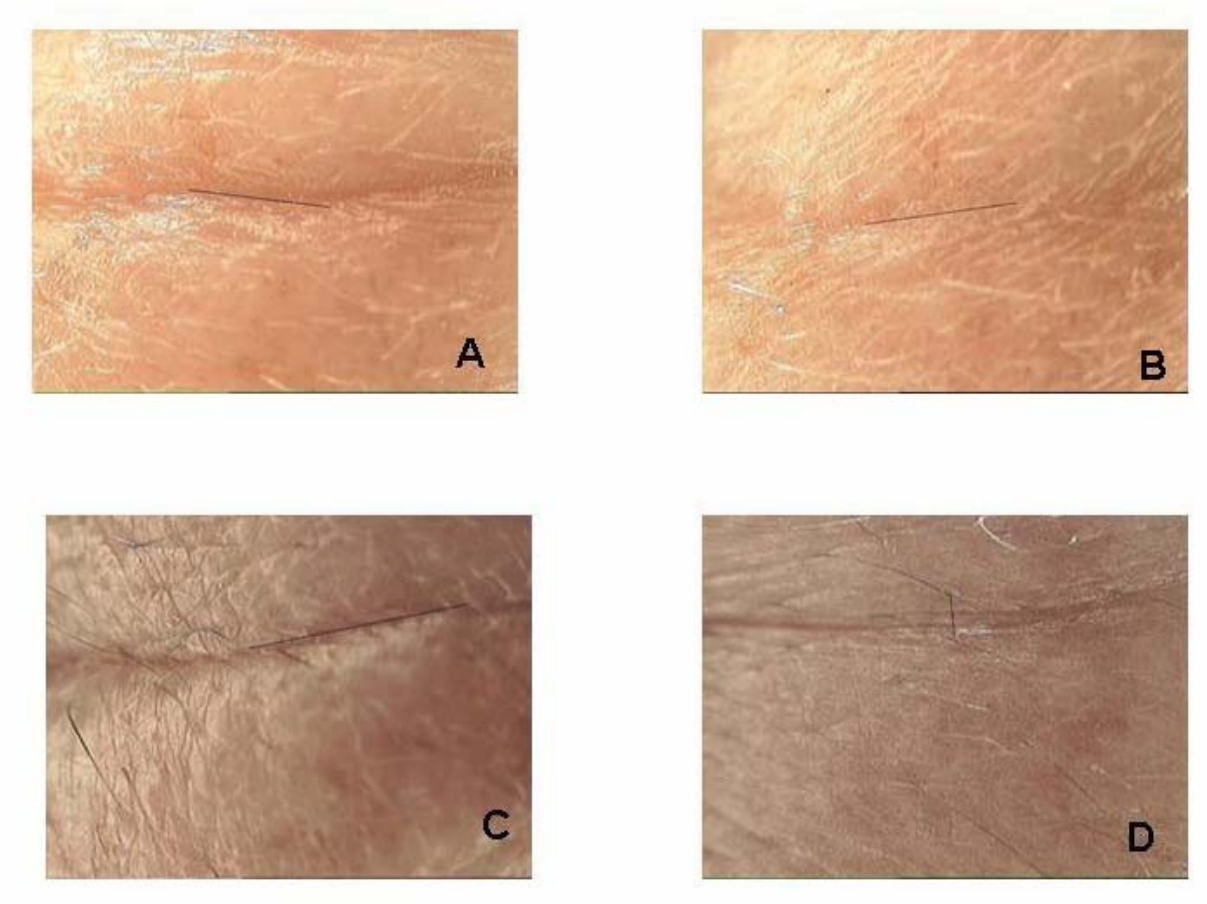


Figure 4. Visual evaluation of average variation in wrinkle depth.

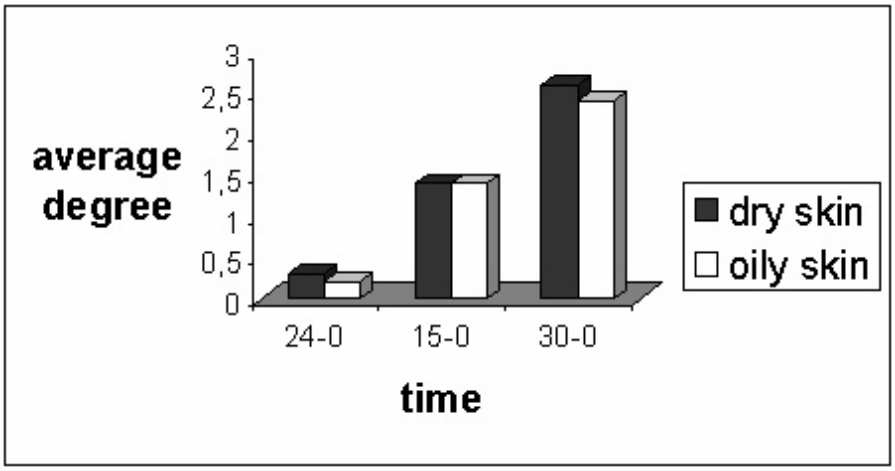


Table I. Visual evaluation of variation in wrinkle depth.

Time (days)	0		1		15		30	
volunteers	Dry skin	Oily skin	Dry skin	Oily skin	Dry skin	Oily skin	Dry skin	Oily skin
degree								
1	1	1	1	2	2	4	3	4
2	1	1	1	1	2	2	3	3
3	1	1	1	2	1	2	4	4
4	1	1	1	1	2	2	3	3
5	1	1	3	1	4	2	4	4
6	1	1	2	1	3	3	4	4
7	1	1	1	1	2	2	4	3
8	1	1	1	1	3	2	4	3
9	1	1	1	1	3	3	4	3
10	1	1	1	1	2	2	3	3
Average degree	1	1	1.3	1.2	2.4	2.4	3.6	3.4

Table II. Visual evolution of wrinkle depth.

time	Dry skin	Oily skin
t_{24}-t_0	0.3	0.2
T_{15}-t_0	1.4	1.4
T_{30}-t_0	2.6	2.4

Conclusions

In accordance with the visual analyses of the photographs and the studies of depth and width, it was verified that there was a notable reduction in the dimensions of the wrinkle studied in each patient during the month of treatment. Likewise, the level of moisturisation increased in all cases.

The differences observed between the untreated and treated skin were always significant. The results demonstrate that, over the course of the trial, there was a progressive increase in the level of moisturisation, as well as a progressive reduction in the depth and width of the wrinkle compared to day 0.

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