PHOTOAGING : CLINICAL AND BIOMETROLOGICAL RESULTS OF A DOUBLE-BLIND RANDOMIZED TRIAL EVALUATING A NEW COSMETIC PRODUCT CONTAINING AVOCADOFURANE®+PENTAPEPTIDES AND RETINOL

Humbert Ph (1), Creidi P (1), Zahouani H (2), Chadoutaud B (3), Choulot JC (4) and Msika P (4)
(1) Dermatology, CHU Besançon, France - (2) UMR CNRS 5513, Lyon, France ;
(3) Clinical Research Consultant, Toulouse, France - (4) Laboratoires Pharmascience™, R&D Center, Epernon, France.
pmsika@expanscience.com

Introduction

Cutaneous aging is characterized by modifications of the aspect of the skin (firmness, texture, colour) and by appearance of wrinkles and results of the combination of both chronological and photo-aging. The knowledge of the cellular mechanisms susceptible to explain the visible signs of the cutaneous aging progressed a lot these last years. Especially, skin cells have to face an imbalance of the ways of synthesis and degradation of collagen and related molecules. These metabolic pathways are :

- an activation of Matrix Metalloproteases (MMPs) enzymes responsible for the degradation of collagen. The activity of these enzymes tends to increase spontaneously in different situations : with ageing [1] and in the non exposed skin of the smoker where MMP-1 is over-expressed [2]. Moreover, infra-erythemal UV doses induce a fast accumulation of the MMP-1 ,-3 and -9 [3].
- an alteration of TGF-β1 pathway (Transforming Growth Factor beta-1), which is involved in the synthesis of the collagen fibers. This factor appears as a new cellular intermediary, sensitive to the cellular senescence [4], as well as to the outside attacks [5], and susceptible to explain the decrease of the collagen synthesis characteristic of the cutaneous aging.

To answer in a specific way to the loss of dermal collagen, we developed and patented two original active ingredients :

- **Avocadofurane®**, a lipidic furane extracted by molecular distillation from the unsaponifiable fraction of a very specific avocado oil. Tested on human skin fibroblasts [6], this molecule stimulated the synthesis of TGF-β and increased in a significant and specific way the synthesis of collagen (X4).
- **Pentapeptides**, are obtained from sweet White Lupin seed according to a biotechnological process. These peptides, applied to human fibroblasts in culture inhibited significantly the production of the MMP-1, -9 and -3 further to UVA irradiation [7].

These two innovative molecules, were associated to palmitate retinol, whose action on the epidermis is clearly established. The efficiency of this formulation was tested in a double-blind randomized study versus excipient. The main criteria of judgment were the decrease of wrinkles and fine wrinkles, reorganization of the dermis and enfeeblement of the skin pigmentation.

Materials & methods

1- Inclusion of the volunteers and treatment.

30 non menopausal women, from 40 to 50 years old, were enrolled in this comparative (vs excipient, hemi-face) double blind study, for 6 months. The volunteers were non smoking and presented clinical signs of photo-aging (moderate to severe). The criteria of exclusion included : the application of a product with retinoïdes during a period of 2 months preceding the study, application of another anti-wrinkle cream or of a dermatologic treatment in the 30 previous days, per os administration of either retinoïdes, photo-sensitizer or anti-age nutraceuticals. The volunteers had to apply the product or its excipient to every right or left face, twice a day during 24 weeks, in normal conditions of use.

2- Clinical and biometrological evaluations.

Non invasive and non traumatic biometrological measurements, as well as clinical evaluations were made at T0, 8, 16 and T 24 weeks.

- **Biometrological tests :**
  - Measurement of the biomechanical properties of the skin at the level of the temple by means of the CUTOMETER SEM 575 (Courage + Khazaka, Germany). Cutometry is essentially used for the evaluation of the cutaneous elasticity, which decreases in a linear way in time (3 % by decade).
  - Analysis of the cutaneous relief at the level of the crow's-foot from imprints of skin realized by means of polymerized resin (SILFLO©) Measurements were then made by means of a defocalized laser microscope.
- **The clinical analysis** included a clinical observation of the investigator estimated according to 13 criteria noted from 0 to 9.

The treatment of results was of two orders : the first is the degree of improvement with regard to T0 after twice daily applications of the tested product. The second compares the performances of the product versus its excipient (randomized results).

Results

1- Clinical evaluations

Table I summarizes the evolution of the clinical parameters compare to T0. A statistically significant improvement was observed from the 8-th week for the majority of studied criteria. In the 16-th week, 3 other criteria improved significantly. After 24 weeks of application, clinical parameters evolved for the greater part significantly with regard to the initial clinical status. We can conclude to significant and quick efficiency of the cream containing the active ingredients, especially on wrinkles.

	T8 weeks	T16 weeks	T24 weeks
	Comparison vs T0		
Skin hydration	17%	27%	30%
Number of spots	-3%	-6%	-8%
Size of the spots	-3%	-8%	-11%
Intensity of spots	-18%	-21%	-22%
Telangiectasia	-4%	-6%	-8%
Sebacceous hyperplasia	-9%	-18%	-17%
Skin complexion	5%	15%	16%
Regularity of complexion	5%	10%	12%
Smoothness of the skin	13%	24%	24%
Number of wrinkles	0%	-2%	-7%
Depth of the wrinkles	-5%	-13%	-15%
Number of fine wrinkles	-1%	-9%	-12%
Depth of the fine wrinkles	-8%	-14%	-19%

Table I : Clinical efficiency of the cream containing the active ingredients ("open results"). In red : statically significant at T8 weeks ; in green at T16 weeks and in orange at T24 weeks.

	T16 weeks	T24 weeks
	Comparison vs excipient	Comparison vs excipient
Skin hydration	16%	17%
Number of spots	-5%	-8%
Size of the spots	-8%	-9%
Intensity of spots	-8%	-10%
Telangiectasia	-5%	-12%
Sebacceous hyperplasia	-16%	-13%
Skin complexion	12%	15%
Regularity of complexion	4%	8%
Smoothness of the skin	16%	15%
Number of wrinkles	-3%	-7%
Depth of the wrinkles	-3%	-8%
Number of fine wrinkles	-7%	-8%
Depth of the fine wrinkles	-6%	-13%

Table II : Comparison of the clinical efficiency of the active ingredients versus the excipient. The yellow box mean statically significant compare to the excipient.

For randomized results, the association of principles was statistically superior to its excipient from the 16-th week for 2/3 criteria (in particular for the number of wrinkles and fine wrinkles). In the 24-th week, all the clinical criteria were statistically improved. The most significant differences between the tested product and the excipient are listed in Table II.

2-Biometrological evaluations

2-1-Cutometry (Figure 1).

After 24 weeks, the anti-aging product provoked an earning of elasticity statistically superior to the excipient at all time points.

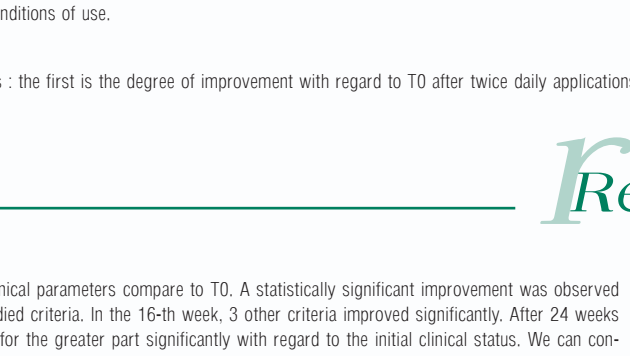

Figure 1 : Improvement of the skin elasticity register with the active ingredient containing cream is statically significant superior versus the excipient.

2-2-Analyses of imprints

The analysis of the surface parameters, depth and volume of wrinkles confirmed the data of the clinical exam and even showed better performances at T24 weeks (Figure 2). These results are also illustrated with the Figure 3 showing a reconstruction in 3 dimensions of a wrinkle before and after treatment by the anti-aging formulation.

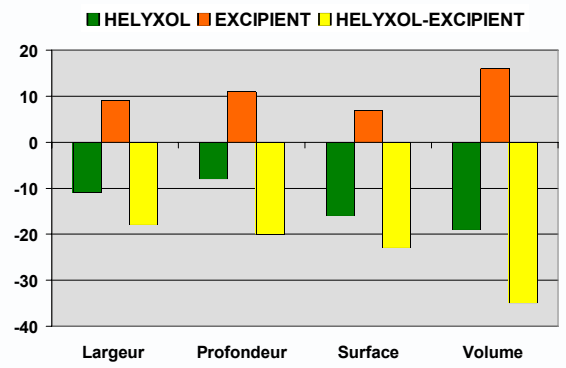

Figure 2 : Imprints analysis showing the evolution of the wrinkles parameters for all the subjects after 24 weeks of twice daily application of the anti-aging product or the excipient.

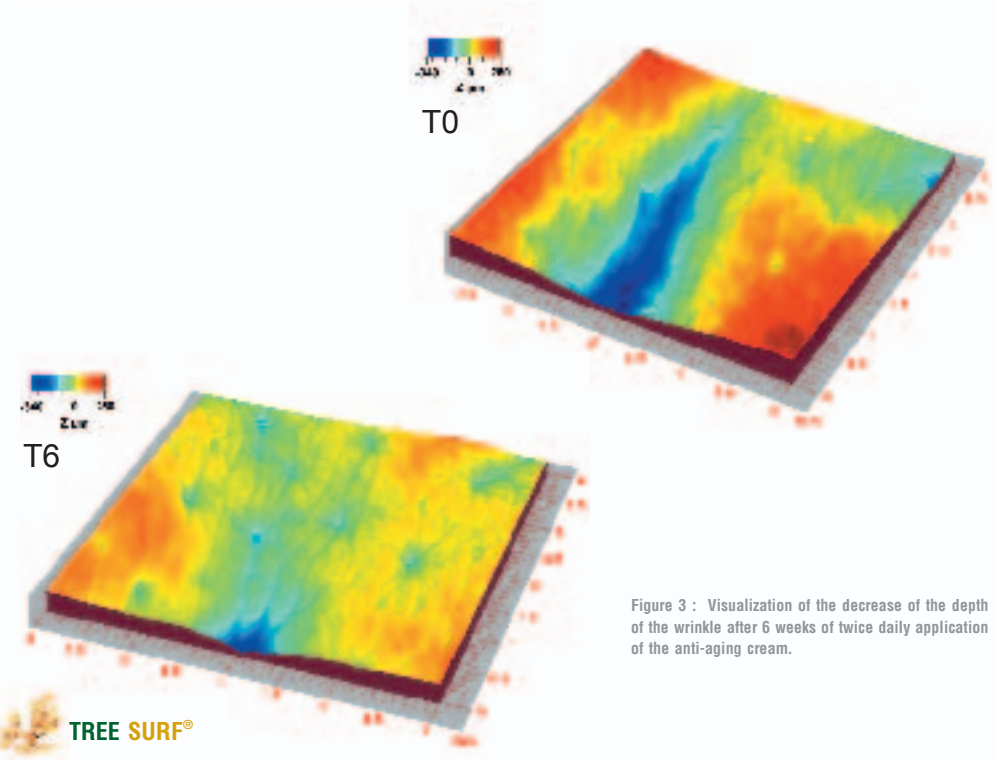

Figure 3 : Visualization of the decrease of the depth of the wrinkle after 6 weeks of twice daily application of the anti-aging cream.

Conclusion

Randomized double blind studies versus excipient are rare in the field of the beauty care. It is nevertheless about the only method capable of evaluating the properties of active principles incorporated into a formulation. The efficiency of our anti-aging product containing both Avocadofurane®, Pentapeptides and palmitate retinol was so verified.

This study stresses the fact that our formulation containing specific active ingredients acts in a significant way on the visible signs of photo-aging this as soon as 8 weeks of application. All the clinical parameters are improved significantly, versus excipient, after 24 weeks of twice daily application, including the decrease of the number and depth of wrinkles and fine wrinkles. These clinical observations are correlated to biometrological parameters. Indeed, the analysis of the imprints of the crow's-foot allowed to put in evidence a decrease of the surface and the volume of wrinkles.

Helyxol®, Noviderm, Laboratoires Pharmascience™

References

1- Khorramizadeh MR, Tredget EE, Telasky C, Shen Q and Ghahary A, *Aging differentially modulates the expression of collagenase in dermal fibroblasts.* Mol Cell Biochemistry 99-108, 1999.
2- Lahmann C, Bergemann J, Harrison G, Young AR, *Matrix metalloproteinase-1 and skin aging in smokers.* Lancet, 24, 935-936, 2001.
3- Fisher GJ, Wang ZQ, Datta SC, Varani J and coll, *Pathophysiology of premature skin aging induced by ultraviolet light.* New England J of Dermatol, 337, 1419-1428, 1997.
4- Mori Y, Hatamochi A, Arakawa M, Ueki H, *Reduced expression of mRNA for transforming growth factor beta (TGFβ) and TGFβ receptors I and II and decreased TGFβ in in vitro aged fibroblasts.* Arch Dermatol Res, 290, 158-162, 1998.
5- Quan T, He T Voorhees JJ and Fisher GJ, *Ultraviolet ray blocks radiotherapy cellular responses to transforming growth factor-β by down-regulating its type-II receptor and inducing Smad 7.* J Biol Chem, 28, 26349-26356, 2001.
6- Ghayor C, Chadjichristos C, Msika P and Pujol JP, *Avocado unsaponifiables (AU) enhance transforming growth factor-β1 (TGF-β1) and collagen expression in cultured dermal fibroblasts.* JID, 113, 452, 1999 (abstract).
7- Piccardi N, Piccirilli A and Msika P, *Effect of Pentapeptides one the regulation of matrix metalloproteinases pathway.* JID, 115, 562, 2000 (abstract).

LABORATOIRES PHARMASCIENCE™

EXPANSCIENCE GROUPE