

AVOCADOFURANE®, PENTAPEPTIDES AND SOY ISOFLAVONES : A CLINICAL STUDY AGAINST HORMONAL AGING

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Introduction

Cutaneous aging is characterized by modifications of the skin aspect and by appearance of wrinkles. This process accelerates after the menopause. The knowledge of the cellular mechanisms likely 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 pathways of synthesis and degradation of conjunctive molecules such as the collagen. Indeed, chronological aging [1], but also external factors (sun, tobacco) lead to [2, 3]:

- an activation of Matrix Metalloproteases (MMPs) enzymes responsible for the degradation of the collagen
- an alteration of TGF-β1 pathway (Transforming Growth Factor beta-1), which is involved in the synthesis of the collagen fibers [4, 5].

After the menopause, the fall of the rate of circulating estrogens explains the acceleration of the atrophy and the degradation of the dermis [6]. All these mechanisms contribute to a massive loss of collagen and result in a less elastic, less functional, and very fine skin strewn with wrinkles.

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 [7], 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 [8].

These two innovative molecules, were associated to soy isoflavones, or phyto-estrogens, which allow especially to stimulate the renewal of fibroblasts and to increase the synthesis of the collagen. They favor also cellular renewal of the epidermis. In this study, we present the evaluation of the efficiency of this association (Avocadofurane®, Pentapeptides and soy isoflavones : "verum") in the post-menopausal aging by means of several innovative and non invasive techniques

Protocole

This monocentric study implicated 30 volunteers who have applied the *verum* twice daily during 12 months. The evaluation of the treatment was performed according to a simple blind protocol on the face and to a randomized double blind one on the forearm (against excipient). Evaluations were performed at T0, T6 and T12 months. Only the 31P nuclear magnetic resonance spectroscopy (31P NMR) was the object of a particular protocol, with evaluations at T0, T3 and T7 hours, T14 days and T6 months on 10 volunteers.

Recruitment : The female volunteers were more than 50 years old (between 50 and 69 years, average age: 59 years), menopausal for at least 2 years, without any HRT. Their skins had to be of phototype II or III, without sign of photo-aging, or actinic keratosis and without evolutionary dermatosis of the face.

Treatment : normal conditions of use, with two daily applications. Two sites of applications were held : the face for the *verum* as well as the two forearms with randomized application of either the excipient or the *verum*.

Methods of evaluation :

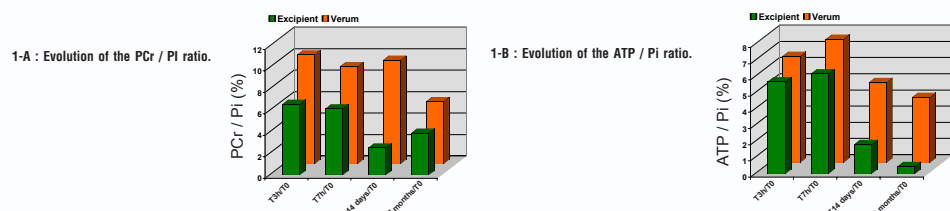
- Measure of the cellular energy (31P NMR spectroscopy) : forearms
- Measure of the cutaneous thickness and dermal echogenicity (20 MHz ultrasound imaging) : face & forearms
- Evaluation of the relief of the wrinkles of the crow's-foot and of the micro-relief (imprints) : face &/or forearms
- Measure of the biomechanical properties of the skin (cutometry) : face & forearms
- Clinical evaluation : face

Results

1- Replenishment of the skin cellular metabolism.

Human *in vivo* 31P NMR spectroscopy permits the evaluation of the cellular energy metabolism by the dosage of the main skin phosphometabolites : inorganic phosphate (Pi), phosphocreatine (PCr), adenosine tri-phosphate (ATP), phosphomonoesters (PME) and phosphodiester (PDE) [9]. The results of the 31P NMR are presented on the Figure 1 with "1-A" the evolution of the PCR/Pi ratio which reflects the energy reserves and "1-B" the evolution of the ATP/Pi ratio, the reflection of the directly useful energy by the cell. The results indicated significant efficiencies of the *verum* and of the excipient with regard to the initial state (T0). The comparison of the efficiency of the two products showed globally better performances for the *verum*, with a statistically significant superiority at T3h and T7h for the energy reserve. Thus the twice daily application of a daily care cream containing Avocadofurane®, Pentapeptides and soy isoflavones allows to improve cutaneous energy status.

Figure 1 : The association Avocadofurane®, Pentapeptides and soy isoflavones is able to relaunch the cutaneous cellular metabolism of the forearm. Comparison versus excipient.



2- Improvement of skin thickness and dermal echogenicity (Table I) [10].

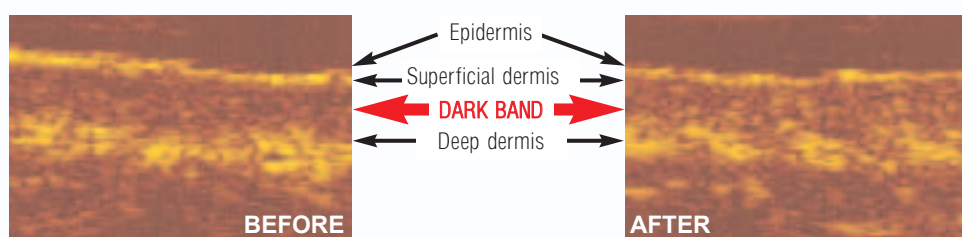
The used material was a DERMICUP 2020 (2MT, France) equipped with a probe operating at a central frequency of 20 MHz. Two images were acquired for every location. The evaluated parameters were the thickness of the skin (epidermis + dermis), and the differential quantification of the echogenicities of the superficial and the deep dermis ("hypochoegenic band" or "Dark Band"). Repeated applications of the *verum* on the face led to a significant increase in the average cutaneous thickness on the temple of the subjects, at T6 and T12 months. In addition, a significant reduction of the difference of echogenicity between superficial and deep dermis was observed at T6 months (-32.1 % ; p< 0.05), confirmed and stressed at 12 months (-36.1 % , p<0.01 vs T0). The reorganization of the dermis associated with the twice daily application of the *verum* during 12 months, on the face, is illustrated with the Figure 2.

On forearm, the *verum*, as well as the excipient led to significant and similar increases in the cutaneous thickness with regard to T0, after 6 and 12 months of application. Only the *verum* allowed a significant reduction of the difference of echogenicity between the superficial and the deep dermis after 12 months of use (-30.3 % ; p< 0.01). There is thus a significant improvement of this specific marker of the skin aging [11] after 12 months only due to the trio of ingredients. We can suggest that the three ingredients contained in the product are responsible for the reorganization of the dermis, probably by means of the stimulation of the synthesis of the constituents of the extracellular matrix.

Table I : Evolution of the skin thickness and echogenicity after 6 and 12 months of treatment as measured by 20 MHz ultrasound imaging. S = Statistically significant , NS = not significant.

FACE	Verum	THICKNESS		SKIN ECHOGENICITY	
		T6 month	T12 month	T6 month	T12 month
FOREARM	Excipient	+12.3% (S)	+16.6% (S)	+11.3% (NS)	-9.6% (NS)
	Verum	+11.6% (S)	+19.0% (S)	-5.9% (NS)	-30.3% (S)

Figure 2 : Illustration of the partial restoration of the echogenicity of the superficial dermis. After 12 months of twice daily application of the association Avocadofurane®, Pentapeptides and soy isoflavones on the face.



3- Improvement of the skin relief.

Imprints were realized on one crow's-foot (by random) as well as on an internal face of the two forearms by means of a resin of silicones (Silflo, Flexico Developments Ltd, Great Britain), and were analyzed by the method of projection of fringes. This profilometric method consists in determining the topography of the cutaneous surface from the modifications of an image resulting from interferences stemming from projections of networks of black and white bands on a relief. Measures were realized by means of a system of projection of fringes coupled with a high camera CCD resolution - Micropip system interfaced with the software Optocat (EOTECH, France). Three-dimensional reconstructions were then analyzed with the Toposurf software (EOTECH, France). On the wrinkles of the crow's-foot : the *verum* applied during 6 and 12 months entailed a reduction of the depth of wrinkles (Table II).

Table II : Variations in % of the profilometric parameters of the wrinkles of the crow's-foot after applications of the *verum*. *Significant with regard to T0 (p<0.05). Rt : maximal observed amplitude; Rq : average quadratic difference from the variations of amplitude of the relief ; Ra : average amplitude.

	Rt	Rq	Ra
T6 months	-9.4	-15.8	-19.0*
T12 months	-5.6	-14.9*	-16.2*

On the micro-relief of the forearm (Figure 3) one notes, at T6 month, a decrease in the studied parameters for the *verum* and significant augmentations of the studied parameters of the micro-relief under excipient. The *verum* behave in a significantly different way of the excipient in 6 months and limit the degradation of the relief. The significance with regard to T0 is reached after T12 month for the *verum* with a decrease in the depth of furrows, whereas no significant variation has been registered with the excipient (Figure 3).

Figure 3 : Variation of profilometric parameters of the cutaneous micro-relief of the forearm after application of the *verum* or the excipient. * Significant with regard to T0 (p < 0.05).

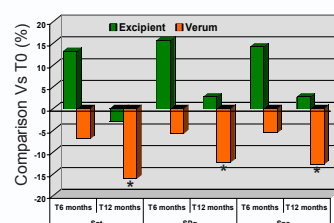
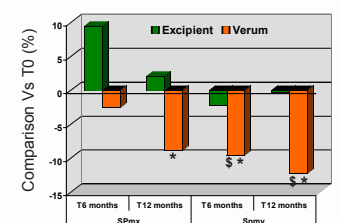


Figure 4 : Variations of the parameters of periodicity of the cutaneous micro-relief of the internal face of the forearm treated by *verum* and by excipient. * Significant with regard to T0 (p < 0.05), \$ significant *verum* versus excipient.



With regards to the orientation of the micro-relief, estimated from the parameters of periodicity Spmx and Spmy, one notes a reorganization of the micro-relief from 6 months of use of the *verum*, result confirmed at T12 months, while the excipient exhibited no beneficial effect (Figure 4).

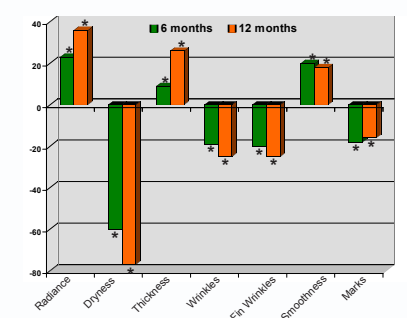
4- Improvement of the biomechanical properties of the skin (data not shown).

The cutometry allows to study the biomechanical properties of the skin and in particular the cutaneous elasticity. Measures were realized by means of the CUTOMETER SEM 575 (COURAGE and KHAZAKA, Cologne, Germany). The *verum* as well as the excipient inferred modifications of the biomechanical properties of the skin. After 12 months of use, average pure biologic elasticity, average elasticity rate of recovery are significantly superior on the side treated with the *verum* compared to the site treated with the excipient. At T12 months, the skin of the forearm treated by the *verum* shows significantly more elasticity than the skin of the forearm treated by the excipient.

6- Clinical evaluation on the face.

Very significant improvements were observed after 6 and 12 months for all the studied criteria. Note particular performances for the specific parameters of the hormonal cutaneous aging: luminosity of the complexion (+ 18 % at T12 months), cutaneous dryness (-77 %) and cutaneous thickness (+ 26 %). Effect on wrinkles and fine wrinkles was also very clear with a decline of 25 % at the end of one year. So, on the face, almost all the types of facial wrinkles are gradually and significantly eased, confirming clinically the set of biometrological data demonstrated previously.

Figure 5 : Evolution of clinical parameters after twice daily application of the *verum* on the face during 6 and 12 months. * Statistically significant with regard to T0.



This randomized double blind study against excipient on the forearm and in simple blind on the face of 30 menopausal women (59 years on average) without HRT, during one year, allowed to put in evidence that the use of this specific cream entails a reorganization in depth of the skin by way of probably the reflation of the energy and the cellular metabolism which results in a smoothing of the relief, a reorganization of the micro-relief and a benefit of cutaneous elasticity. These significant instrumental results confirm the data of the clinical examination. This new anti-age care product, specially conceived for the treatment of the hormonal cutaneous aging, whose 3 major active principles complement each other to play each on the mechanism of the aging : Avocadofurane restores an optimal synthesis

of collagen, patented Pentapeptides fights against the excessive destruction of collagen fibers and the soy isoflavones, which belong to the family of phyto-estrogens, aim to try to compensate locally for certain cutaneous effects connected to the fall of circulating estrogens. This study tends to confirm the concept based on a reorganization of the connective tissues of support by the stimulation of the synthesis of the collagen by way of the TGF-β associated to the protection of the macromolecules of the extracellular matrix by way of the inhibition of the MMPs and in profits brought with phyto-estrogens. This specific product* demonstrated objectively its capacity to treat the cutaneous signs of the hormonal aging. *HELYX-®, Noviderm, Laboratoire Pharmascience™

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