ROLE OF HYALURONIC ACID AND VITAMIN C IN PHOTOAGEING

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Synopsis

In order to influence the collagen content of the dermis, for ameliorating the wrinkling appearance of the facial skin, different active compounds are used, topically applied by cosmetic emulsions or by subdermal injection. Among these, recently, the more used are: ascorbic acid, beta-glucan, and hyaluronic acid, for their ability to retain large amount of water into intercellular space, to make jelly matrix, to protect cell structure, to hold cell and to defend it against external pollutants and bacteria on infections. These active compounds are used by topical application or by injection as corrective for wrinkles. The anti-wrinkle activity of two new products, HCG 1000® (vitamin C, beta-glucan, hyaluronic acid based) and HY 20® (Bio-technological and purified Hyaluronic Acid) have been controlled “in vitro” and “in vivo” by a double-blind study on 30 volunteer patients in an out-patient office. From the obtained results, it is possible to resume that HCG 1000® may be added to the patient’s skin care as a cosmetic routine, before and after the injection of different augmentation materials, to increase the efficacy of the implant.

Riassunto

Al fine di modificare la presenza di collagene nel derma e per migliorare l’aspetto delle rughe cutanee, si utilizzano generalmente diversi preparati, quali, ad esempio, acido ascorbico, betaglucano e acido jaluronico, sottoforma di cosmetici o di “dispositivi medici”.

Questi composti attivi, sempre più spesso, vengono utilizzati attraverso applicazioni topiche o attraverso iniezioni intradermiche come correttivi per le rughe. A tal proposito, con uno studio a doppio ceco su 30 volontari, è stata controllata, “in vitro” e “in vivo”, l’attività antirughe svolta da due nuovi prodotti, un preparato a base di vitamina C, betaglucano e acido jaluronico, denominato HCG 1000® e un preparato a base di acido jaluronico purificato di origine non animale, denominato HY 20®. Dai risultati ottenuti, è possibile desumere che l’HCG 1000® può essere utilizzato, quale coadiuvante dell’attività svolta dall’acido jaluronico o dal collagene, nella terapia topica o iniettiva.
INTRODUCTION

The skin is an organ in which aging changes are easily observed. The degree of change varies between individuals and on the different areas, and can be distinguished in intrinsic or chronologic ageing and extrinsic or photoageing. Generally in intrinsic ageing, collagen in the dermis is lost, and reduction of cellular activity and skin thinning occur (1-3). Conversely, photoaged skin results thickened and characterized by wrinkles, roughness, tangled degraded elastic fibers and matted hypo-pigmentation and hyper-pigmentation. Both photoaging and intrinsic ageing occur especially in facial skin results in wrinkling, but the ageing changes are also in depending of lifestyle, such as the amount of sunlight time exposure and quality and quantity of skin-care used. In order to influence the collagen content of the dermis, for ameliorating the wrinkling appearance of the facial skin, different active compounds are used, topically applied by cosmetic emulsions or by subdermal injection. Among these, recently, the more used are:

A) Ascorbic acid, which appears to exert a transcriptional or translational control of collagen synthesis and shows promise as a broad-spectrum photoprotectant. (4-6)

B) The biological response modifier Beta-glucan, which seems able to stimulate the skin own defence mechanism, enhancing the macrophage-mediated phagocytosis and the production of cytolytic and cytostatic factors. (7-9)

C) Hyaluronic acid for its ability to retain large amount of water into intercellular space, to make jelly matrix, to protect cell structure, to hold cell and to defend it against external pollutants and bacterial infection. (10-13)

AIM

The aim of this study was to evaluate “in vitro and in vivo” the effect of a balanced and stabilized combination of hyaluronic acid, vitamin C and beta-glucan (HCG 1000® active) in patients showing evidence of photoageing changes in the skin. It was also controlled the clinical effect of a new sterile hyaluronic acid of bio-technological culture origin, used by injection as corrective for wrinkles.

MATERIAL AND METHODS

Products

HCG 1000® (active): aqua, sodium hyaluronate, sodium carboxymethyl betaglucan, ascorbic acid, arginiane.

HCG 1000® (placebo): aqua, arginine.

HY 20®: hyaluronic acid mg.20, sodium chloride and distilled water to 1ml.

CREAM BASE®: aqua, glycolic acid, propylene glycol, glycerin, lactic acid, stearyl alcohol, cetearyl octanoate, cera alba, cetyl palmitate, cetearyl heptanoate, glycine, gelatin, cetyl alcohol, sodium PCA, stearyl caprylate, steareth-10, steareth-7, isopropyl myristate, paraaffinum liquidum, mirtystil alcohol dimethicone, titanium dioxide, parfum, imidazolidinyl urea, tocopheryl acetate, arginine, methylparaben, hydrolyzed collagen, alumina, silica, sodium polya crylate, propylparaben, diazolidinyl urea, isopropyl paraben.

CLEANSING LOTION®: aqua, isopropyl myristate, ceteareth-6, stearyl alcohol, dimethicone, glycolic acid, octyl stearate, sorbitol, propylene glycol, glycine, arginine, tocopheryl acetate, parfum, imidazolidinyl urea, methylparaben, lactic acid, linoleic acid, linolenic acid, retinyl palmitate, hydrolyzed collagen, disodium EDTA, propylparaben.

Patients

All the 30 volunteer patients (age range 45-68 years) and of all both sexes (15 males and 15 females), had attended the dermatology out-patient office, according to Lever et al. (14). The only criterion for entry in the study was the presence of one or more signs of photoageing affecting the face, such as fine wrinkling around eyes , crease lines around mouth and cheeks, telangiectasia, wrinkling of skin.

(1) Trade name: QM IDRATANTE 10:MAVI ROMA DAYTIME
(2) Trade name: QM LOZIONE DETERGENTE, MAVI ROMA GENTLE CLEANSING LOTION
on the backs of the hands etc, the state of their skin corresponding to degree 3 to 5 on photodigital scale described by Larnier et al. (15). All the subjects gave their written informed consent in accordance with the ethics of cosmetic experimentation.

**Study design “in vitro”**

It was investigated the activity of the HCG 1000\(^\circ\) solution on human keratinocytes obtained by biopsy from the skin of some volunteers of the study group.

**Keratinocytes**

The Keratinocytes culture medium was added with the HCG 1000\(^\circ\) active at two different concentrations of 0.01 and 0.02% (w/v) and controlled at 24, 48, 72 and 120 hours according to Zulli et al. (9). The obtained results are reported in figure 1.

**Fibroblasts**

The fibroblasts culture was added with the HCG 1000\(^\circ\) active for 20 hours before being exposed to UV-B radiation at two different doses, 30 and 60 mj/cm\(^2\), according to Mori et al. (16) and to our own experience (17). The obtained results on the influence of human keratinocytes growth, and on the level of the cyclic pyrimidine dimers (photo-immuno-protection effect), are reported in figure 2.

**Study design “in vivo”**

The study was enrolled as a placebo-controlled, double-blind trial in which the HCG 1000\(^\circ\) active and HCG 1000\(^\circ\) placebo were randomly applied to the right of left sites of the face and the dorsum of the hands together with the Cream base. The patients were given a 15 ml dosable bottle glass container for each site and instructed to apply 3/4 drops of each product to the back of the hand, right or left, and the same amount to the ipsilateral half of the face. The Cream base was applied on the total surface of both face and hands. Each glass-bottle contained the active HCG 1000\(^\circ\) or its vehicle and all the bottles were identical. Monthly checks were made to ensure that the patients understood the need to avoid contamination of one side by the solution allocated to the other side and the preparations were applied regularly.

![INFLUENCE OF HCG 1000\(^\circ\) ON THE GROWTH OF HUMAN KERATINOCYTES](image_url)

**Fig 1.**
Treatment

Patients were instructed to apply the drops twice a day in the morning and just before retiring in the evening at least 30 min. after cleansing with the specific product supplied. The treatment period lasted 12 weeks during which no other cosmetics or drugs were to be used with the exception of the special cream base and the cleansing lotion supplied. Exposure to strong sunlight was avoid.

Control assessment and evaluation

Control visit and measurements were performed on 1st day (baseline) and after 15 (D15), 30 (D30), 45 (D45), 60 (D60), 75 (D75) and 90 (D90) days of treatment with a follow up visit at 120 (D120) always from the same dermatologist, using a clinical score method. The individual signs of photoaging were scored on 0-10 visual analogue scale with separate scores for each site of the face and each hand. Subjects were also evaluated objectively for the signs and symptoms of skin irritation and subjectively by asking them if there was any itching, stinging or burning sensation.

After the 1st, the 2nd and 3rd month of treatment, the subjects evaluated their satisfactory or unsatisfactory scoring the firmness, softness and the wrinkles appearance, using a scale of 0 to 4 for each criterion (0: unsatisfactory; 4: satisfactory) according to Berardesca et al. (18) The obtained results are reported on fig. 3-4 and tab. 1-2).

Hyaluronic acid (HY 20°) treatment

The injections of HY 20° were performed only on 10 people of the studied group. Because of the nature of the product, composed from hyaluronic acid of no-animal origin, skin testing was not required. Evaluated by “in-vitro” and by preclinical “in-vivo” studies, HY 20° was found to be biologically compatible, stable in the dermal tissue and persistent in the skin longer than the collagen control.

The viscosity/concentration curve was also another fundamental parameter controlled (Fig 5)(19). Identified the wrinkles suitable for inclusion, HY 20° was injected to 4 sites per subject into the dermal tissue through a 30-gauge needle by the new Di Pietro methodology (20). All the subjects treated gave their informed consent prior to entering the study and were free to withdraw at any time. HY 20° was admi-
mistered at the first visit (week 1), and touch-up injections were permitted at the next 2 visits (weeks 2 and 4), when necessary.

**Clinical evaluation**

Evaluations and assessment were performed at follow-up visits at weeks 2, 4, 6, 12, 18, and 24. At each visit the dermatologist and the patient, separately, evaluated the degree of correction for each treatment site, using a 0-10 visual analog scale (0 = no correction, 10 = total correction). The values from each site were added together and the mean value was determined. The obtained results are reported on fig. 6.

**RESULTS OF 3-MONTH TREATMENT BY HCG 1000º AND CREAM BASE ON PHOTOAGED SKIN**

n = 30

(DEGREE OF SATISFACTION FROM THE TREATMENT)

![Graph showing results of 3-month treatment by HCG 1000º and cream base on photoaged skin.](image)

*Fig 3. All p values are significant as to baseline (p<0.05)*

**SUBJECTIVE EVALUATION AFTER 3-MONTH TREATMENT BY HCG 1000º AND CREAM BASE ON PHOTOAGED SKIN**

n = 30

(DEGREE OF SATISFACTION FROM THE TREATMENT)

![Graph showing subjective evaluation after 3-month treatment by HCG 1000º and cream base on photoaged skin.](image)

*Fig 4. All p values are significant as to baseline (p<0.05)*
Role of hyaluronic acid and vitamin C in photoageing

**Statistical analysis**

A two tailed student’s test for paired series was used to analyze the differences between the values obtained before and after the treatments.

**Product safety**

The use of both HY 20* and HCG 1000* did not lead to any unwanted skin reaction, demonstrating their complete safety.

<table>
<thead>
<tr>
<th>CLINICAL SIGNS</th>
<th>TREATMENT</th>
<th>PERIOD</th>
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<th>AFTER</th>
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<tbody>
<tr>
<td>Fine wrinkling around eyes</td>
<td>vehicle</td>
<td>3 month</td>
<td>6.4</td>
<td>4.5</td>
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<td></td>
<td>HCG 1000*</td>
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<td>6.7</td>
<td>3.2</td>
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<td>4.3</td>
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<td></td>
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<td>HCG 1000*</td>
<td></td>
<td>6.0</td>
<td>4.2</td>
</tr>
<tr>
<td>Telangiectasia on the cheeks</td>
<td>vehicle</td>
<td>3 month</td>
<td>3.3</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>HCG 1000*</td>
<td></td>
<td>2.8</td>
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**Table I.** Mean results of the 3 month-treatment by HCG 1000* and a cream-base on photoaged skin. Mean results: visual analog score (1-10) for clinical signs. (n=30)

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<tr>
<td></td>
<td>HCG 1000*</td>
<td>2.8</td>
<td>1.7</td>
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**Table II.** Follow-up assessment at week 16 after the HCG 1000* and the Cream base treatment. Mean results: visual analog score (1-10) for clinical signs. (n=30)
RESULTS AND COMMENTS

In vitro studies

About "in vitro" studies culture experiments showed that HCG 1000® active seems to protect from 50
to 75% (p<0.01 (fig.2) skin fibroblasts against DNA damage on level of cyclic pyrimidine dimers upon
UV-B irradiation and promotes the growth of keratinocytes up 200% (fig.1) in depending of the HCG
1000® concentration used.
These results give the chance to think that HCG 1000®

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**THE VISCOSITY OF HYALURONIC ACID "HY 20®" SOLUTION RAPIDLY INCREASES WITH RAISE IN CONCENTRATION**

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**IMPROVEMENT OF SCARS AND WRINKLES TREATED BY "HY 20®"**

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Fig 5.

Fig 6.
may have an “in vivo” efficacy to protect skin against oxidative stress induced by UV irradiation with an immuno-protecting effect, and may be able to enhance the renewal rate of the stratum corneum improving skin firmness.

**In vivo studies**

As it is showed on tab.I, the studied HCG 1000® solution caused a progressive reduction of the clinical signs of photoaged skin, both on the face and on the dorsum of the hands of the treated subjects. This amelioration of the general aspects had a positive follow up at week 16 also.(tab.II)

Although all the clinical signs started to reduce just after the first 2 weeks of treatment, (fig.3) the differences did not reach statistical significance until after 6 weeks.

The differences between treatment with the HCG 1000® or its vehicle became really pronounced after 12 and 16 weeks of treatment (tab.I-II).

Moreover the clinical signs from the baseline decrease from about 50% (p<0.005) for the fine wrinkles around eyes and mouth to 21% (p<0.005) for the wrinkles on the dorsum of the hands or to 29% (p<0.005) for telangiectasias on the cheeks. The positive mean results obtained with the vehicle treatment from 30% (p<0.05) to 6% (p<0.05), was in our opinion due exclusively to the antiwrinkling activity of the cosmetic base used, rich in glycolic acid, vitamin C, and in other active compound.

High satisfaction (p<0.05) has been obtained directly from the patients’ response (fig.4) only after using active HCG 1000® in addition to the base, whereas not very impressive results have been mentioned after using the control applied in addition to the base cream. We have considered, then, useful and maybe essential, the contemporary usage of the base cream and the HCG 1000®, mostly in order to facilitate the transcutaneous penetration of the last one.

As a matter of fact, if we compare the control activity, represented by pure sterile water and arginine used in addition to the base cream, to the HCG 1000® activity, we’ll find that the last one shows more than doubled values, at the same time significant among them (p<0.005) (tab.I-II).

**HY 20® injections**

More significant appears the data obtained from a sub-group of the same patients treated also by HY 20® hyaluronic acid injections in order to try to eliminate or to reduce the deeper wrinkles.

As it is seen at week 12, patients with 85% of scares and wrinkles saw them improved at least moderately or better (from 40 to 85%) (fig.6).

Moreover all the patients received an initial treatment at week 0, and 1 touch-up treatment at week 2 or 4 and approximately 50-55% of the treated sites, maintained marked or complete correction (75-80%) until week 18 and 24, as compared to baseline (fig.6).

In terms of overall level of patient satisfaction with HY 20® treatment, 90% of the patients reported higher satisfaction at week 12 and no complications were observed.

**FINAL CONCLUSION**

From the obtained results both “in vitro” and “in vivo” it is possible to deduce that the balanced combination of Beta-glucan, Vitamin C and Hyaluronic acid in HCG 1000® active solution may be added to the patient’s skin care as a cosmetic routine, both before and after the injection of collagen or hyaluronic acid, such as HY 20® or other skin augmentation material, to increase the efficacy of the implant. In addition HCG 1000® may be dispensed together with other selected cosmeceuticals to promote firmer even toner skin with diminished appearance of fine lines and wrinkles.

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