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Multicenter study for the evaluation of tolerance and efficacy of a new integrated aminoacidic treatment on the aging face

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Summary

Multicenter study for the evaluation of tolerance and efficacy of a new integrated aminoacidic treatment on the aging face

Age-related changes in the dermis mainly consist of reduced thickness and flattening of the dermo-epidermal junction. In aging skin less efficient biosynthetic activities make the newly formed collagen more easily attacked by collagenases and metalloproteinases. We have evaluated the activity of a specific treatment based on an injectable aminoacid mixture plus low molecular weight hyaluronic acid along with food and cosmetic supplementation of the same aminoacids. In the used mixture the aminoacids glycine, proline, leucine and e lysine are in a stechiometric ratio specifically studied to improve collagen neo-synthesis. The aminoacidic treatment produced a very statistically significant reduction of skin roughness evaluated both clinically and instrumentally; in particular, profilometric parameters were significantly decreased after 1 month and dramatically decreased after 4 months. Our results demonstrate that specific aminoacid formulations as substrates to collagen synthetic pathways may have a positive role in improving the ageing signs of the skin.

KEY WORDS: *Aminoacidic treatment, Aging face*

Introduction

Age-related changes in the dermis mainly consist of reduced thickness and flattening of the dermo-epidermal junction (1). This is particularly true for sun-exposed skin and during menopause in women (2, 3). The alterations of the dermal connective tissue, corresponding mainly to a reduction of the extracellular matrix, are highly responsible for the wrinkling and sagging of the skin, since they determine deep modifications in the mechanical properties of ageing skin. Several studies have demonstrated that ageing unbalances the enzymatic activities related to the synthesis, remodelling and catabolism of the extracellular matrix components in the dermis: collagen, elastin and glycoaminoglycans. As a result, not only do ageing processes induce a reduction of the extracellular matrix, but its quality is also affected. Moreover, in aging skin less efficient biosynthetic activities make the newly formed collagen more easily attacked by collagenases and metalloproteinases. Nowadays, skin can be stimulated to improve quali-quantitative ageing alterations by the intradermal injection of biological substances able to induce a revitalization of the dermis. The most frequently used substance is natural, not cross-linked, low molecular weight hyaluronic acid. In fact, with ageing there is a decrease in the content of glycoaminoglycans in the dermis, in particular of hyaluronic acid, the major non-sulphated glycoaminoglycan of connective tissue ground substance. The reduction in hydrophilic glycoaminoglycans leads to a direct reduction in water content and skin turgor. Interestingly, not only can the injected simple hyaluronic acid molecules

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provide ground substance enrichment and deep hydration of the skin, but they also strongly stimulate fibroblasts to sinthetize new ground substance (4). Moreover, recent publications have suggested the possible positive role of the current greater availability of specific aminoacid mixtures as substrates to collagen synthetic pathways in human organs (5-7). Collagen has a complex molecular structure, due to its particularly regular aminoacidic content. Although there are several different collagen types, in each type every third aminoacid one is glycine, the smallest of all aminoacids. The quite monotonous composition of collagen peptides is not only limited to the highly regular recurrence of the glycine residue, but is also present in the following two positions, called positions X and Y, where position Y is hydroxyproline (OH*pro*) in 50% of the cases, and hydroxylisine (OH*lys*) in most of the remaining sequences. These aminoacids are very rarely found in proteins other than collagen. They are fundamental in a cascade of events allowing a reaction forming inter- and intra- molecular bonds strongly entangling three peptides into fibrils and firmly tightening them into the complex collagen units. Having one glycine every third aminoacid allows peptides to bend regularly and form a left-handed helical structure, entwined with two other peptides in a super-helical structure twisted to the right, greatly similar to the structure of a rope. This gives extraordinary tensile strength and flexibility to collagen fibers. Based on the above, a new integrated treatment was prepared, consisting of a specific "*aminoacidic functional cluster*" assembled in order to physiologically promote local collagen synthesis through chemotactic stimulus (see Table 1 for the composition) and which includes intradermal injections (injectable aminoacidic mixtures plus low molecular weight hyaluronic acid), cosmetic treatment and food supplementation. The present study was performed in order to evaluate the activity of this integrated treatment on the aging face.

Materials and methods

Five dermatological centers participated in the study. Investigations were carried out in 103 healthy female volunteers, aged 35 to 60 (average age: 47). All the enrolled subjects had low to moderate skin ageing/photo-ageing, according to a reference photographic scale, and had given their informed consent. Exclusion criteria were: pregnancy or lactation, use of permanent fillers in the past, presence of other systemic pathologies, like autoimmune disease, diabetes, liver and renal insufficiency, heart failure. Subjects who for any reason had taken drugs like aspirin or other NSAID's, or systemic corticosteroids during the three month preceding the present study, were also excluded.

The objective of the study was to evaluate the tolerance and efficacy of an intradermal injectable product associated to cosmetic treatment and food supplementation (*Jalupro® and Proglyme®, Professional Dietetics s.r.l., Milan, Italy*) over a treatment period of twenty-two weeks. The study was performed according to a multicenter open study protocol, and the treatment under investigation was administered as follows:

4 intradermal injections - done directly by participating dermatologists using the injectable product (*Jalupro®*); injections were done once a week, and the first one was done two weeks after the basal visits (subjects had already started to take cosmetic and food supplements two weeks before).

Food supplement - one ampoule/day of aminoacid food supplement (*Proglyme®*, in water or in any other drink) was taken by the subjects from week T-2 to the last intradermal implant, for a total of six weeks.

Cosmetic treatment - from T0 to T4: aminoacid cream, applied on the face twice a day (morning and evening) and liquid patch applied twice a week (30 minutes of setting).

Three evaluation visits were performed during the study: at baseline (T0), 1 month after the last

	L-Proline	L-Glycine	L-Lisine	L-Leucine
Injectable vials	37,6	50	5,4	7
Oral solution	43,8	50	5,2	-
Cream	37,6	50	5,4	7
Liquid patch	37,6	50	5,4	7

Table 1. *Aminoacidic composition (%) delivered by tested products.*

intradermal implant (intermediate visit, T1) and at the end of the study period (final visit, T4, four months after the last intradermal implant).

Clinical evaluations

The tolerance of the study treatment was evaluated by recording and monitoring any adverse event and by dermatological assessment of objective symptoms as erythema, oedema, papules, pustules or other symptoms at baseline and on each successive visit. During the visits the following clinical evaluations (visual score) were performed on the right or left face side, according to a previously completed randomization list: skin roughness (at the level of nasolabial folds and periocular area according to reference photographic scales, skin smoothness (surface microrelief), skin tonicity and skin brightness. The efficacy of the study treatment was assessed by visual evaluation conducted at baseline, at T1 and at the end of the study. Wrinkles grade (at the level of nasolabial folds and in the area around the eyes) was determined using a reference clinical and photographic scale (0= no wrinkles, 1= very mild, 2= mild, 3= quite evident, 4= evident, 5= very evident, 6= marked, 7= very marked. 0, 1 and 7 are exclusion criteria). Surface microrelief evaluation was performed according to a cheek surface photographic scale (1= very regular, 2= regular, 3= irregular, 4= very irregular). Skin tone and skin brightness were evaluated using a visual score (0= very mild, 1= mild, 2= medium, 3= marked, 4= very marked for skin tone and 1= very opaque, 2= opaque, 3= normal, 4= luminous for skin brightness).
At the end of the treatment (final visit) each volunteer gave her personal judgment on the efficacy of the study treatment on deep and superficial wrinkles, as well as on elasticity, smoothness and brightness of the skin.
In this paper, we have arbitrarily decided to define as antiageing activity on the face the possible positive influence on skin aging evaluated and measured locally through any eventual improvement in the thickness of nasolabial skin folds, as described in *Instrumental Evaluations*.

Instrumental evaluations

A part of the enrolled subjects (n= 21) was also submitted to instrumental evaluations using the optical profilometry technique previously described in the literature (12-14). These subjects were also administered an additional intradermal injection at T4 and were re-evaluated at T6 months. At baseline and subsequent visits (T1 and T4) skin replicas at the level of nasolabial folds were taken (right or left side, according to a randomization list). Replicas were obtained using silicone rubber (*Optosil, Heraeus Kulzer GmbH & Co, KG*) and allowed the evaluation of the dimension of the wrinkles by computerised image analysis. Image analysis of the replicas (optical profilometry): this evaluation was conducted through a computerised image elaboration (*Image Pro Plus, Media Cybernetics Inc., USA*). Replicas were illuminated with a 45° incident light, which creates shadows behind crests that can be photographed, digitised and analysed. The shadows were transformed into a grey scale, where grey intensities were directly proportional to shadow intensities and therefore to wrinkle depth. Shadows were detected by thresholding. By defining an area within the image, and by tracing a segment of known length in a defined position across the wrinkle and perpendicular to it, it was possible to calculate the following profilometric roughness parameters:
R_a= roughness average parameter which is the arithmetic mean of all ordinates from the mean line of the profile.
R_t= maximal depth of wrinkles.
At baseline (T0), T1, T4 and T6 frontal and profile standard pictures of the 21 volunteers were also taken.

Self-evaluations

During the final visit, each volunteer filled in a questionnaire regarding the efficacy of the treatment under study on deep and superficial wrinkles, as well as on the elasticity, smoothness and brightness of the skin of their faces (using the same score for each item: very marked; marked; medium; mild; absent).

Statistical analysis of data

The statistical evaluations of the visual and instrumental data (adjusted means and standard deviation) and their relative graphs were performed at the times required by the protocol.
The statistical analyses of the clinical and self-evaluation data were performed using the Wilcoxon and Friedman tests. The analysis of all parametric data was done using the *Student's t test*.

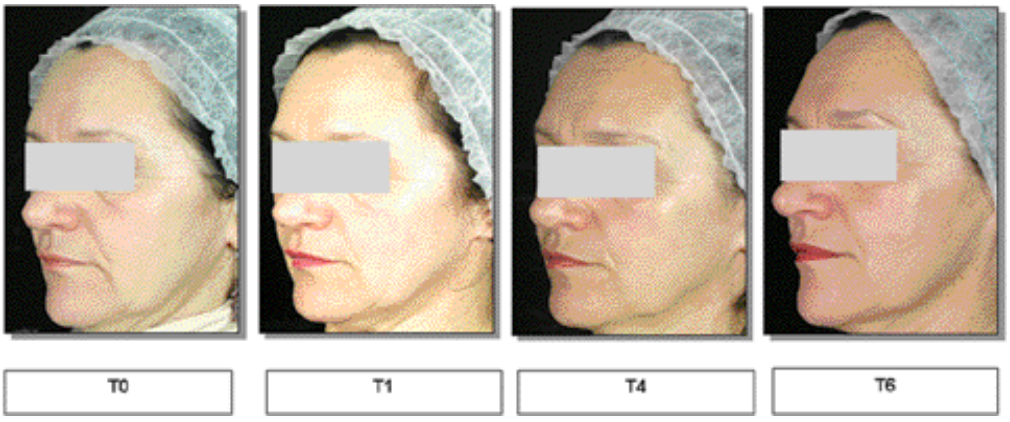

Figure 1.

Results

Clinical evaluations

Tolerance - The tolerance of the tested products was judged as very good and no adverse event occurred during the study period (final investigators' opinion: 81%= excellent, 18%= good).

The good tolerance was confirmed by the volunteers' opinion; as a matter of fact, only few subjects underlined the appearance of a slight erythema with a burning sensation following the first cream and/or liquid patch application and lasting few minutes.

Clinical evaluations performed during the entire treatment period demonstrated the absence of relevant clinical signs as erythema, oedema, papules, pustules or the like.

Clinical evaluation of efficacy - The evaluation of the efficacy of the treatment under study took into account the above mentioned aging signs. An example of treatment efficacy is shown in Figure 1. The statistical evaluation of the clinical data was performed using the *Wilcoxon test*. One month after the last intradermal implant (T1), a significant and important improvement of nasolabial folds ($p < 0.001$) in 74% of the treated cases was obtained; in particular, in 59% of the subjects the visual score improved by 1 degree, in 12% of the subjects by 2 degrees and in 3% of the subjects it improved by 3 degrees. Data obtained at the end of the test (T4), confirmed T1

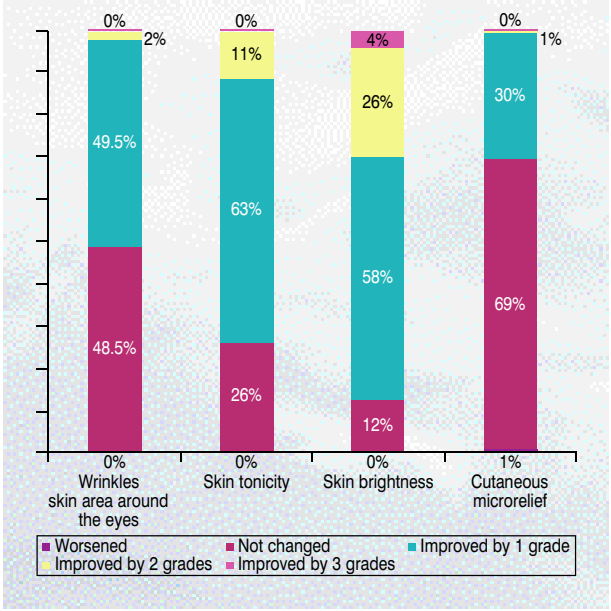

Figure 2. *Visual evaluation one month after the last intradermal implant (T1).*

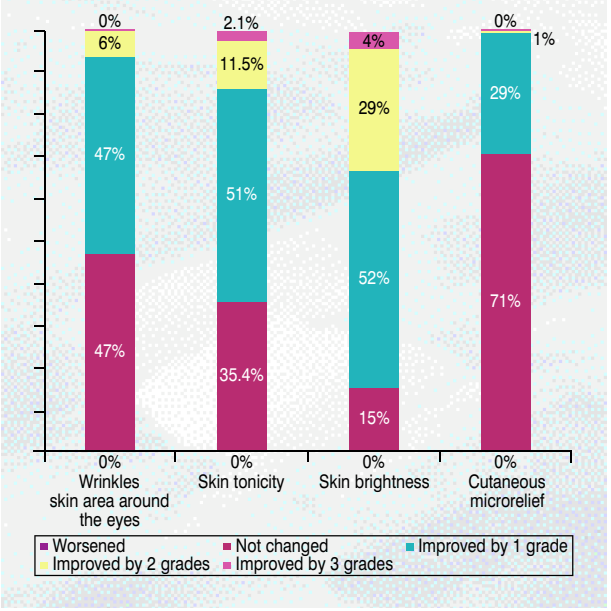

Figure 3. *Visual evaluation at the end of the treatment period (T4).*

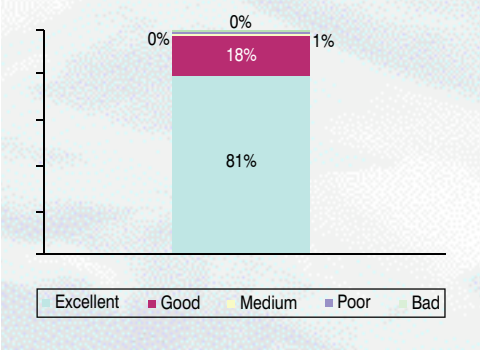

Figure 4. *Tolerance evaluation of the tested product by the investigators at the end of the study.*

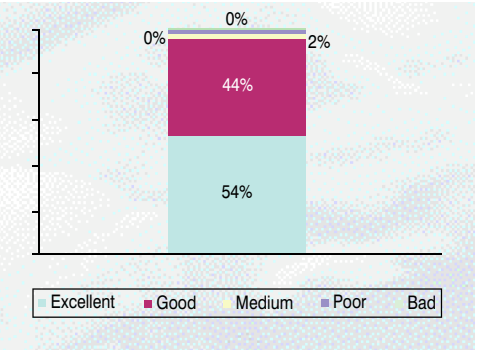

Figure 5. *Efficacy evaluation by the investigators at the end of the study.*

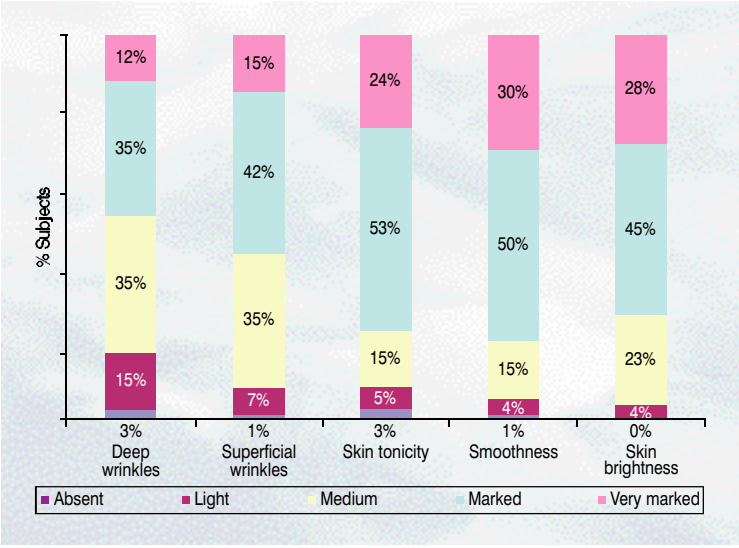

Figure 6. *Efficacy evaluation by the volunteers at the end of the study.*

results; in fact, in 72% of the subjects a statistically significant improvement ($p < 0.001$), at least 1 degree, was still present (in 62% of the subjects it improved by 1 degree; in 10% of the subjects by 2 degrees). No worsened case was observed. 1 month after the last intradermal implant (T1), a very significant improvement in skin roughness in the periocular area was found in 51.5% of the subjects ($p < 0.001$); in particular, in 49.5% of the subjects the visual score improved by 1 degree and in 2% of the subjects it improved by 2 degrees. Moreover, at T1 one could see a very significant ($p < 0.001$) improvement in skin tonicity in 74% of the subjects (improved by 1 degree in 63% of the subjects and by 2 degrees in 11% of the subjects), in skin brightness in 88% of the subjects (improved by 1 degree in 58% of the subjects, by 2 degrees in 26% of the subjects and by 3 degrees in 4% of the subjects), and in cutaneous micro relief in 30% of the subjects (improved by 1 degree in all subjects), as regards all enrolled subjects. Evaluations performed at the end of the study ($p < 0.001$ T0 vs T4 for all considered visual scores) confirmed the T1 results, showing the bio-revitalizing activity of the study treatment. In fact, a statistically significant improvement ($p < 0.001$) in the scores of wrinkles around the eyes, skin tonicity, skin brightness and cutaneous micro-relief was found respectively in 53% (improved by 1 degree in 47% of the cases and by 2 degrees in 6% of the cases), 64.6% (improved by 1 degree in 51%, by 2 degrees in 11.5% and by 3 degrees in 2.1% of the cases), 85% (improved by 1 degree in 52%, by 2 degrees in 29% and by 3 degrees in 4% of the cases) and 29% (improved by 1 degree in all cases) of all studied cases. No worsened case was noticed.

Volunteers' judgment on efficacy - 82% of the volunteers appreciated the efficacy of the treatment on deep wrinkles (12% as very marked, 35% as marked, 35% as medium) while 92% of the subjects noticed the effect on superficial wrinkles (15% as very marked, 42% as marked, 35% as medium).

Moreover, 92% of the subjects reported an improvement in skin tonicity (24% as very marked, 53% as marked, 15% as medium), 95% in skin smoothness (30% as very marked, 50% as marked, 15% as medium) and 96% in skin brightness (28% as very marked, 45% as marked, 23% as medium). The significance of the self-evaluation data was demonstrated by a *Friedman test*. A further assessment of global efficacy performed by the investigators confirmed these results (54%= very good, 44%= good, 2%= medium).

Instrumental evaluations

An instrumental evaluation of the profilometric parameters was conducted on 21 of the 103 enrolled subjects. The statistical evaluation of the profilometric data was performed with a *Student's t test*. The image analysis of nasolabial folds at T0, (baseline), T1 (intermediate visit) and T4 (final visit) provided the following results:

- statistically significant reduction, at T1 and T4, of average R_a ($p < 0.001$ from mean basal value of 17.53 to 13.27 at T1 and 10.5 at T4, corresponding to 24% of reduction at T1 and 40% at T4);
- statistically significant reduction, at T1 and T4, of R_t ($p < 0.001$ from mean basal value of 72.21 to 54.07 at T1 and 49.12 at T4, corresponding to 25% of reduction at T1 and 32% at T4). Moreover, regarding the comparison between data at T1 and T4, a progressive and statistically significant improvement of the R_a parameter ($p < 0.05$ T1 vs T4 for R_a) corresponding to a 26% reduction was demonstrated.

T6 evaluations (clinical scores of nasolabial folds, wrinkles around the eyes, skin tonicity, skin brightness, cutaneos microrelief and optical profilometry on nasolabial folds skin replicas) were performed two months after the additional intradermal implant. For all considered parameters, the results obtained from the comparison of T6 and T4 did not show any statistically significant variation, underlining how the "*bio-revitalizing*" effect (i.e., using physiological drives for promoting improvement of biological activities) of the study treatment seen at T4 was still present at T6, although during this period (from July to September) all subjects had exposed their face to strong sun irradiation for a long time and had not regularly taken the cosmetic and food supplements of the treatment under investigation.

Discussion

The activity of intradermal injections of hyaluronic acid as a biorevitalizer is already well-known: several studies in the literature report a good antiage efficacy due to extracellular matrix augmentation, deep hydration and even antioxydizing activity of hyaluronic acid. Adequate aminoacids refueling of the skin is evidently indispensable for the maintenance of its integrity or repair. One of the first studies on this matter observed the negative incidence of protein-energy malnutrition effects on infection rate, healing, and mortality in elderly burn patients (8). Later, some other papers dealt with alterations of macronutrients balance and skin, especially regarding diabetes (9), anorexia nervosa (10) and postmenopausal women (11). All those papers linked poor availability of nutrition substrates and damage or atrophy of the skin. More recent studies have suggested the possible positive role of the current greater availability of specific aminoacids formulations as substrates to collagen synthetic pathways in other organs (5-7). Food supplementation with aminoacids has represented the first approach to the problem of atrophy in aging skin. This is certainly useful for the systemic treatment of the entire tegumentary apparatus. Ageing faces require stronger and more localized treatment, and in this case an intradermal injection of aminoacids could be a more effective choice, even more so if accompanied by the use of skin care cosmetics containing similar aminoacids formulations. For this reason, we have decided to evaluate the activity of a specific treatment based on an injectable aminoacid mixture plus low molecular weight hyaluronic acid along with food and cosmetic supplementation of the same aminoacids. In the used mixture the aminoacids glycine, proline, leucine and e lysine are in a stechiometric ratio specifically studied to improve collagen neo-synthesis. The aminoacidic treatment under study produced a very statistically significant reduction of skin roughness evaluated both clinically and instrumentally; in particular, profilometric parameters were significantly decreased after 1 month and dramatically decreased after 4 months. In particular, regarding the profilometric parameters, an important and significant decrease of average and maximum depth of nasolabial folds (filling efficacy) was demonstrated, while the reduction of the visual score of periocular wrinkles showed how the study product had an important "*lifting*" activity on the wrinkles (antiage activity). Clinical evaluations and efficacy judgements by the volunteers significantly confirmed the efficacy of the treatment. In conclusion, our results demonstrate that specific aminoacid formulations as substrates to collagen synthetic pathways may have a positive role in improving the ageing signs of the skin. The treatment protocol defined for the study was found effective. It remains to be established

whether this treatment can be used for "*combination*" antiageing therapies, that is if it would be possible to combine it with other treatments like chemical peeling, laser resurfacing and fillers and, if so, using which protocols.

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Politi G.R. (*Medical Center, Albenga*)

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