Sodium Riboflavin Phosphate

A new component in anti-ageing cosmetics

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Keywords

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Summary

In the present paper, we described an extensive testing by non-invasive methods on a recently marketed formulation containing Sodium Riboflavin-5'-phosphate, which led to substantiate its anti-ageing properties and, at the same time, evidenced an unexpected bleaching and lighting effect on skin. These findings pave the way to further studies about the use of Sodium Riboflavin-5'-phosphate as active ingredient in cosmetic products.

Introduction

From several years our group is involved in the study of pharmacokinetic and pharmacodynamics of riboflavin-5'-phosphate (RF), by studying both its penetration through corneal epithelium and its ability to increase corneal collagen cross-linking after UV-A irradiation, as a widely used keratoconus treatment procedure to avoid surgery (1,2). In keratoconus treatment, RF reacts with corneal collagen fibrils, creating covalent interfibrillar bonds that increase their mechanical resistance and restore corneal physiological curvature, otherwise altered by keratoconus' progression.

Since corneal collagen has a similar molecular packing to the skin (3), we decided to investigate the properties of a cosmetic with RF on a series of skin parameters, measurable by non-invasive in vitro methodologies.

Among the parameters, melanin and erythema indexes were considered, since the literature shows that RF interferes with melanogenesis (4) and is used in phototherapy (5).

In the cosmetic field, RF is used as dye (6) and as a co-factor to reduce C vitamin photodegradation in creams (7).

With those premises, a formulation with RF was considered (patent WO2015033316)(8) along with its effects on the skin, using an efficacy evaluation. The study protocol involved measurements on 20 sane volunteers, between 40 and 70 years old, who applied the cream twice a day for 8 weeks. At the start, during and at the end of the treatment a series of parameters was measured in different facial areas. Data were then analyzed and statistically compared. The formulation showed an increase in skin elasticity and firming effect, along with a depigmentation activity, caused by a significant reduction of melanin and erythema indexes.

Materials and Methods

This study evaluated in vivo the efficacy of cream cod.3A, manufactured by IROMED group, used to improve clinical signs induced by skin photo-ageing. The study consisted in 8 weeks of treatment. The product, delivered in anonymous package, was applied twice a day (morning and evening) on a specific facial area for the entire duration of the test. The application spot was alternated between the subjects, chosen by the operator depending on base parameters (wrinkles, spots), and intermediate

measurements (T1=2 weeks; T2=4 weeks; T3=6 weeks; Tf=8 weeks) were taken on the same area with maximum reproducibility.

All the volunteers, selected by a precise inclusion criteria, were subjected at the starting time (T0), at preset intervals (every 15 days) and at the end of the application period (Tf) to the following instrumental measurements:

- skin desquamation, sebum, pores by means of Visioscope ®PC35 (Courage & Khazaka) with the aid of corneofix and sebufix;
- skin elasticity by using a Cutometer ® DUAL MPA 580 (Courage & Khazaka) with standard probe (diameter Ø mm 2) for the measurement of the cutaneous visco-elasticity by suction;
- images of wrinkles, skin spots and related numerical rating by means of Visioscope® PC35 (Courage & Khazaka);
- skin thickness by the use of a Reviscometer, instrument for skin isotropy determination;
- skin color by using a Mexameter ® MX 18 (Courage & Khazaka).

The measurements were carried out on the cheek, close to the eye and on the forehead of the volunteer, depending on the parameter to be measured, in a reproducible way and they have been always supplemented by the respective skin images.

All instrumental measurements were performed in a room with T=25°C and controlled humidity (35-45% RH), after an acclimatization period of approximately 30 minutes.

The volunteers at the end of the time of use expressed a subjective judgment about the cosmetic pleasantness (fragrance, ease of absorption and application), the subjective product effectiveness (hydration, elasticity, roughness) and the tolerability of the cream, by filling in a form, in which each parameter is given a numerical index or liking.

Statistical considerations and mathematical processing

Having been considered the average of the measurements at T0, T1, T2, T3 and Tf, the Student's t-test was used to assess whether the difference between means was significant; the level of significance was set for $p \leq 0.05$ values.

The mean value and the standard deviation were calculated for the initial instrumental values, intermediate and final, through the aid of a spreadsheet using:

Tf-T0= varied mean value

    T0= mean value at the beginning of treatment

    T1= mean value after 2 weeks of treatment

    T2= mean value after 4 weeks of treatment

    T3= mean value after 6 weeks of treatment

    Tf= mean value at the end of treatment after 8 weeks.

This difference is also reported as a percentage of variation. The difference between the initial and final values was considered significant when the probability was $p \leq 0.05$.

Results and Discussion

The following are the results listed by test type and skin effect.

Desquamation

The skin desquamation in all volunteers showed a mean decrease of 16% (statistical significance of p= 4,4 E-09) within 8 weeks (**Fig.1**), with a significant decreasing trend starting from the second week of use of the cream and during the whole subsequent period of use.

From this we can deduce that the decrease of the stratum corneum loss is correlated to a greater hydration and firmness of the skin hydrolipidic film in all subjects during the use of the cream.
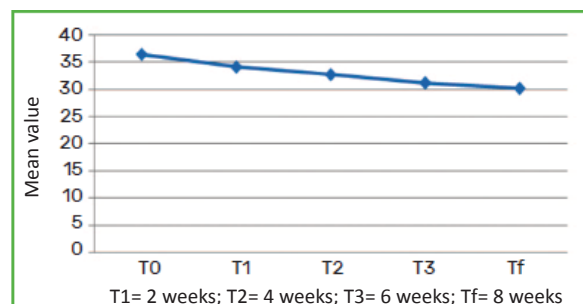

T1= 2 weeks; T2= 4 weeks; T3= 6 weeks; Tf= 8 weeks

Figure 1: Desquamation progress over time, from time zero (T0=36,44±2,61) for 8 weeks (Tf=30,25±2,58).

Depigmenting effect

Both the erythema and melanin index decreased for the 20 subjects with a mean percentage of respectively 10% and 15% (**Fig.2**) compared to the base value; the percentage of variation was considered significant as the probability was respectively p=0.00212 and p=0.029. More precisely, the erythema index significantly decreased during the first month of treatment and then remained constant, whereas the melanin index decreased especially during the first two weeks of treatment, after which it remained constant in the next period.
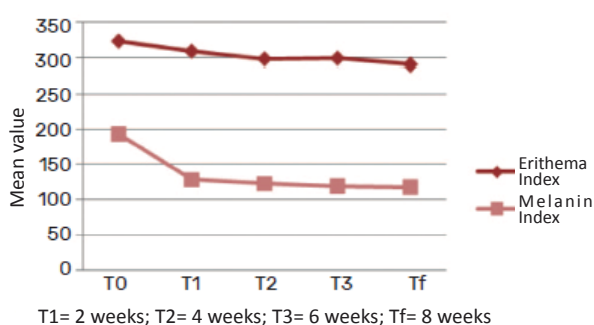

T1= 2 weeks; T2= 4 weeks; T3= 6 weeks; Tf= 8 weeks

Figure 2: Erithema and Melanin indexes progression from time zero (T0=323,52±31,96; T0=139,61±34,10) for 8 weeks (Tf=291,18±25,12; Tf=117,82±26,16).

Simultaneously, the evidence showed that the skin spots pigmentation registered a net decrease of 33% (statistical significance of p=0.004) at the end of treatment (**Fig.3**). The application of the cream in the first two weeks of use showed a good depigmenting effect on the skin, descending trend that was maintained throughout the whole period of use.
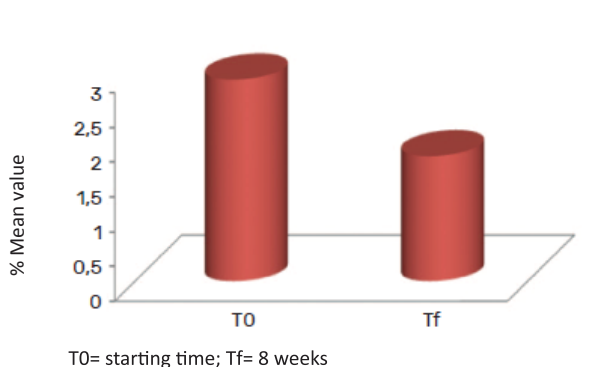

T0= starting time; Tf= 8 weeks

Figure 3: Numerical comparison between percent mean values of skin spots at time zero (T0=0,025±0,005) and after 8 weeks (Tf=0,017±0,003).

Skin elasticity and firming effect

Of all the parameters given by the Cutometer curves were selected as the most representative the parameters R2 and R5, that is, the total elasticity (resistance to the ability to return) and net elasticity (elastic portion of suction compared to elastic portion of the relaxation). Their increase, significant from time zero to the end of use (8 weeks), was on average 83% for R2, and 56% for R5, with a statistical significance of respectively p = 3.7 E-11 and p = 4.2 E-10. The increasing trend during the use of the cream for both parameters within 8 weeks is shown in **Figure 4**. From that we can deduce that the skin elasticity parameters increase during the whole period of use of the cream, but more significantly after the first four weeks of treatment.
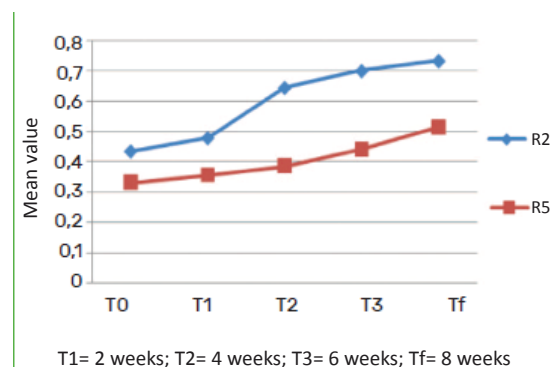

T1= 2 weeks; T2= 4 weeks; T3= 6 weeks; Tf= 8 weeks

Figure 4: Progression of R2 and R5 parameters, representing skin elasticity, from time zero (for R2: T0=0,44±0,10; for R5: T0=0,33±0,08) for 8 weeks (for R2: Tf=0,73±0,09; for R5: Tf=0,52±0,06).
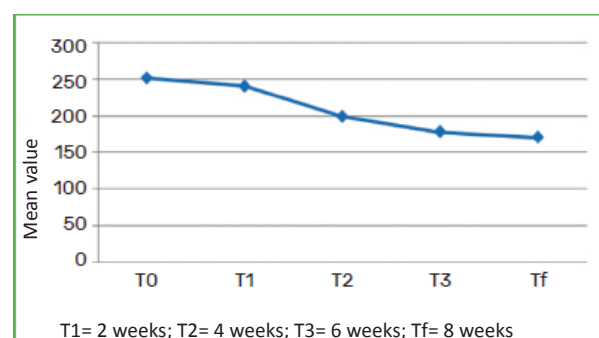

T1= 2 weeks; T2= 4 weeks; T3= 6 weeks; Tf= 8 weeks

Figure 5: Skin Isotropy values, expression of skin firming effect, progression from time zero (T0=252,02±100,32) for 8 weeks (Tf=170,84±90,55).

Moreover, the decrease of the Reviscometer values (skin isotropy), which occurs in the same way noticeably after 4 weeks and after 8 weeks

of treatment reaches a value of -33% (**Fig.5**), with statistical significance of p = 0.0106, points out a contemporary firming effect of all volunteers skin. All of this results in a more elastic and firm skin.

Wrinkles analysis

The analysis of the images and the related numerical parameters of the wrinkles near the eye and forehead of the volunteers showed an average decrease of 35% respectively and 28% (**Figure 6**) at the end of 8 weeks of treatment, with a percental variation considered significant as p≤0,05. The trend, as can be seen in **Figure 7** , is decreasing during the whole period of the application of the cream.

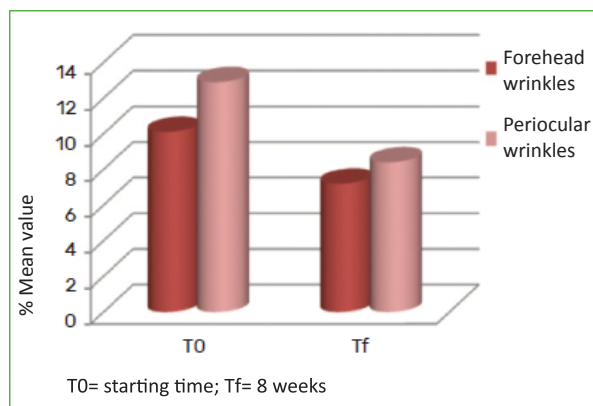T0= starting time; Tf= 8 weeks

Figure 6: Skin roughness values percent mean decrease progression from time zero for 8 weeks (-35% and -28%).
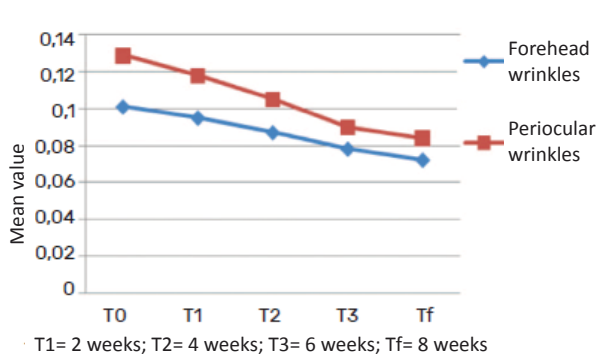

T1= 2 weeks; T2= 4 weeks; T3= 6 weeks; Tf= 8 weeks

Figure 7: Skin roughness values progression from time zero (forehead wrinkles: T0=0,13±0,02; periocular wrinkles: T0=0,10±0,02) for 8 weeks (forehead wrinkles: Tf=0,083±0,02; periocular wrinkles: Tf=0,07±0,01).

Sebometry

Finally, the sebum determinations on the cheek and forehead, as well as the amount of skin pores on the cheek of the volunteers, have shown a steady trend and in a "normal" range for almost all volunteers, so there was evidence that the cream did not increase the production of sebum (oily skin) and, in cases where the volunteer showed at T0 a high or poor value of sebum, it has been normalized at the end of the 8 weeks; in fact, already at the first measurement (after 15 days) values were normal, so it follows that the cream is sebum-balancing on different types of skin (dry or oily, data not shown).

Conclusions

During the period of use of the cream, 18 volunteers out of 20 considered the smell of the tested cream very pleasant, its texture excellent, as well as its spreadability. All reported a softening and firming effect and, above all, experienced a brighter and lightened face after two weeks of treatment. None presented intolerance to the cream. In conclusion, by the set of analyzed parameters and the data obtained in vivo in this study, it can be deduced that the cream containing riboflavin-5'-phosphate used by the volunteers was able to induce an improvement in elasticity and firming effect , attributable to the presence of the same riboflavin, able to form cross-linking with collagen even at the skin level. Further studies are currently in progress to confirm these early encouraging results and the actual contribution of the RF in the anti-ageing efficacy of the cream subjected to the study.

Bibliography

[1] Ostacolo C, Caruso C, Tronino D, Troisi S, Laneri S, Sacchi A et al (2013) "Enhancement of corneal permeation of riboflavin-5'-phosphate through vitamin E TPGS: A promising approach in corneal trans-epithelial cross-linking treatment" Int Journal of Pharm (Amsterdam, Netherlands) 440(2):148-153.

[2] Caruso C, Ostacolo C, Epstein RL, Barbaro G, Troisi S, Capobianco D (2016) "Transepithelial corneal cross-linking with Vitamin E-enhanced Riboflavin solution and abbreviated, low dose UV-A: 24 months clinical outcome"
Cornea 35(2):145-150.

[3] Yamauchi M, Chandler GS, Tanzawa H, Katz EP (1996) "Cross-linking and the molecular packing of corneal collagen" Biochem Biophys Res Commun 219(2):311-315.

[4] Takenouchi K, Aso K, Utsumi K (1960) "Participation of thiamine and riboflavin in the formation of tyrosine melanin" J Vitaminol 6(4):308-320.

[5] Sato K, Sakakibara N, Hasegawa K, Minami H, Tsuji T (2000) "A preliminary report of the treatment of blue nevus with dermal injection of riboflavin and exposure to near-ultraviolet/visible radiation (ribophototerapy)" J Dermatol Sci 23(1):22-26.

[6] Otterstatter G (1999) "Coloring of Food, Drugs and Cosmetics" Food Sci Technol (N.Y.) 91:385.

[7] Ahmad I, Sheraz MA, Ahmed S, Bano R, Vaid FH (2012) "Photochemical interaction of ascorbic acid with riboflavin, nicotinamide and alpha-tocopherol in cream formulations" Int J Cosmet Sci 34(2):123-131.

[8] Caruso C, Santangelo R (2015) "Cosmetic composition containing a combination of natural active ingredients" Patent WO20150333.