A promising alternative to retinol as powerful anti-aging cosmetic ingredient

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Abstract (Maximum of 250 words)

The anti-aging offer on the cosmetic market is huge and it can be sometimes difficult to choose and/or to differentiate between the right ingredients. Retinol still remains the golden anti-aging standard, but it is not always well tolerated on the skin. Therefore, consumers have to play with multiple parameters like the frequency of use, the concentration, the moment of the day to apply the product etc.

The goal of this scientific work was to investigate the *in vivo* anti-aging performances of our ingredient, RCL (INCI: Sorbitol, Dihydroxy Methylchromone) vs retinol. RCL is a nature-identical, multifunctional active ingredient We therefore conducted a 28-days *in vivo* study in which multi parameters including notably wrinkles status, biomechanical properties of the skin and cutaneous barrier integrity were explored.

In this *in vivo* study we could show better performances for the emulsion containing RCL than for the end formulation containing retinol.

Keywords: potential retinol-alternative, natural-identical, anti-wrinkles, smoothing, firmness/elasticity, sustainability

Introduction

Quest for eternal youth remains like ever a crucial topic for women and more and more for men too.

The anti-aging offer on the cosmetic market is huge and it can be sometimes difficult to choose and/or to differentiate between the right ingredients. Retinol still remains the golden anti-aging standard. But it is also known for years that retinol is not always well tolerated by the skin. Redness, itching and stinging sensation may occur upon application of retinol-containing products [1]. Therefore, consumers have to play with multiple parameters like the frequency of use, the concentration, the moment of the day to apply the product etc... Furthermore, the stability of retinol in formulations still may be a challenge [2], so that manufacturing under specific and restrictive conditions (e.g., under inert gas) is necessary. The need to propose an alternative for retinol to the consumer is still justified. Our ingredient (RCL, INCI: Sorbitol, Dihydroxy Methylchromone) is a nature-identical, multifunctional active ingredient and a potent phyto-compound found e.g., in medicinal rhubarb. RCL works on 3 levels, supporting the skin barrier, reducing signs of inflammation, and protecting key components of epidermis and extra cellular matrix (ECM) to reduce signs of inflammaging. There are already multiple *in vitro*, *ex vivo* and *in vivo* data available for this ingredient (earlier generated). However, we so far had no comparison on an *in vivo* level between RCL and retinol.

The outcome of the new *in vivo* study is presented here.

Materials and Methods

Products tested

RCL: INCI: Sorbitol, Dihydroxy Methylchromone (2% in a o/w formulation corresponding to 0.1% of the active ingredient)

RCL is a natural-identical multifunctional active ingredient based on dihydroxy methylchromone (DHMC), a potent phytocompound found e.g., in medicinal rhubarb. Readily biodegradable, its production process has been carefully optimized to minimize the

carbon footprint. Sustainability is driven by e.g., optimized use of reaction materials, water consumption optimization and eliminating CO_2 producing steps. Our ingredient is obtained in high yield and purity.

RET: A market product (o/w day cream) containing 0.2% retinol

The end formulation was selected in order to have comparable concentrations of active (retinol and DHMC).

All-trans retinol, belongs to the family of endogenous natural retinoids It is a 20-carbon molecule consisting of a cyclohexenyl ring, a side chain with four double bonds, all in trans configuration, and an alcohol end group. It was recognized as an effective photoaging treatment by Kang *et al* even if it was used earlier [3],[1].

Placebo

Placebo formulation is based on the same chassis than for the formulation with RCL, but in the placebo formulation there are no active ingredients.

***In vivo* study**

A 28-days double-blind study was organized. It was an intra-individual study; each subject is her/his own control for the comparison from the baseline. 44 healthy Caucasian volunteers (24% male; 76% female) were analyzed, between 45 to 65 with phototype I to IV having visible wrinkles/fine lines on crow's feet, having visible underneath eyes wrinkles, having dry to very dry skin on face, having loose skin face and finally having cutaneous imperfections: blotches and diffuse redness. Volunteers were divided into 2 groups of 22 and applying the products on hemifaces. One group applied an emulsion containing 2% RCL (corresponding to 0.1% DHMC) vs placebo and the other group applied the emulsion containing 2% RCL vs a market end formulation containing 0.2% retinol (2% RET).

Read Out parameters and instrumental methods

The following endpoints were explored at day 0 and day 28

- Anti-wrinkle effects on crow's feet by studying directly *in vivo*, the cutaneous relief parameters (average roughness Ra, maximum amplitude Rt and average relief Rz) using DermaTOP® (EOTECH – France)
- The skin biomechanical properties (firmness and elasticity, described in this study by the parameters R0, R1 and R5, R7 using Cutometer® (COURAGE & KHAZAKA).
- The effects on cutaneous barrier by measurements of the trans epidermal water loss (TEWL) using Aquaflux® AF200 (BIOX)
- A subjective evaluation questionnaire filled by the volunteers.
- Clinical grading of skin irritability (signs observed like erythema, edema, dryness, desquamation, roughness). The sum of the 5 grades is performed to highlight skin irritability observed by the clinician. The signs felt by the subjects were also recorded (tightness, stinging, itching, warm, burning sensation, redness/erythema, edema, dryness and desquamation roughness). by clinical grading. The sum of the 9 grades is performed to highlight skin irritability reported by the subjects.
- Illustrative pictures were taken using Colorface®

Statistical analyses were done using an ANOVA model

Results

Smoothing and anti-wrinkle effects

As shown in Figure 1 and on Figure 2, 2% RCL (corresponding to 0.1% of the active ingredient DHMC) showed a significant smoothing and anti-wrinkle effect. Indeed, average roughness Ra and average relief Rz values both decreased in a significant way ($p = 0.0315$ and $p= 0.0436$ respectively) by 5%.

Neither placebo nor the market product containing 0.2% retinol showed any significant decrease of Ra and Rz values (for 0.2% RET limit of significance for Ra, $p=0.0756$).

The maximum relief amplitude Rt value decreased by 5% for 2% RCL and for 0.2% RET nonetheless at the limit of significance (p = 0.0736 and p= 0.0678 respectively, results not depicted).
Visual effects (pictures and 2D/3D illustrations were taken and exemplary results are shown on Figure 3. Pictures correlate well with objective measurements.

Biomechanical properties of the skin

The cutaneous firmness (R0) is linked to the maximal final deformation amplitude and a decrease in R0 characterizes a skin firming effect.
As depicted in Figure 4, in the case of 2% RCL the R0 parameter decreased by 7% in a very significant way (p=0.0013) and showed a significant firmness effect of 2% RCL. It is the only test product showing performance: neither the placebo nor the market product containing 0.2% retinol did show any significant decrease in R0 value.
Moreover, the comparative statistical analysis highlighted a greater firming effect with 2% RCL compared to the market product RET 0.2% (p=0.0067).
The parameter describing skin elasticity, and its capacity to return to its initial state (R1), net elasticity (immediate retraction) (R5) and raw elasticity (R7) are also shown here (see Figure 5).
A decrease of R1 describes a more elastic skin. R1 values decreased in a significant way only for 2% RCL (-11%; p=0.0184). In the case of 0.2% RET the p value = 0.4784 and for the placebo p= 0.5240. The comparative statistical analysis highlighted a greater skin elasticity of 2% RCL in comparison to the placebo (p=0.0256).
An increase of R5 describes a more elastic skin and R5 values only increased in a significant way for 2% RCL (+9%, p= 0.0258) (for RET 0.2%, p=0.0993 and for placebo p= 0.7713).
Finally, an increase of R7 is associated with a more elastic skin and the raw elasticity R7 values increased by 7% and 10% for respectively 2% RCL and 0.2% RET. However, it was at the limit of significance (p= 0.0794 and p= 0.0688 respectively). The comparative statistical analysis highlighted a greater effect with 2% RCL compared to placebo at D28 (p = 0.0385).

Cutaneous barrier

The cutaneous barrier was investigated via the measurement of the TEWL. A low TEWL is associated with good skin barrier integrity [4]. The cutaneous barrier is strengthened in case of TEWL decrease and maintained when TEWL value does not change.
Results depicted in Figure 6 showed that there was no significant change in the TEWL value for 2% RCL (p= 0.3170) and for 0.2% RET (p=0.4795) after 28 days therefore showing that the skin barrier integrity was kept in the case of the treatment with 2% RCL and 0.2% RET. The TEWL value for the placebo significantly increased (+16%, p=0.0172) after 28 days and the comparison with 2% RCL was significant (p=0.0049).

Subjective evaluation questionnaire

A subjective evaluation questionnaire is filled in by the subjects at the end of the study to subjectively evaluate the properties of the studied products and their global efficacy.
To evaluate the significance of the answers, the 95% confidence interval is determined according to the Wilson method and compared to the theoretical proportion of 50%.
The test product 2% RCL was found to be more effective in improving multiple skin parameters compared to the placebo and the market product containing 0.2% retinol Significant answers are depicted in bold (see Figure 7).

Clinical grading of skin irritability

A decrease in the clinical grading represents an improvement in the skin state and therefore a decrease in the skin irritability (soothing effect).
A significant decrease in the total irritability grading (observed by the clinician and reported by the subjects) was observed after 28 days of use for 2% RCL, 0.2% RET and for the placebo and the comparative statistical analysis showed a greater soothing effect of 2% RCL compared to the placebo. There was no statistical difference between the formulation with 2% RCL and the 0.2% RET (see Figure 8).

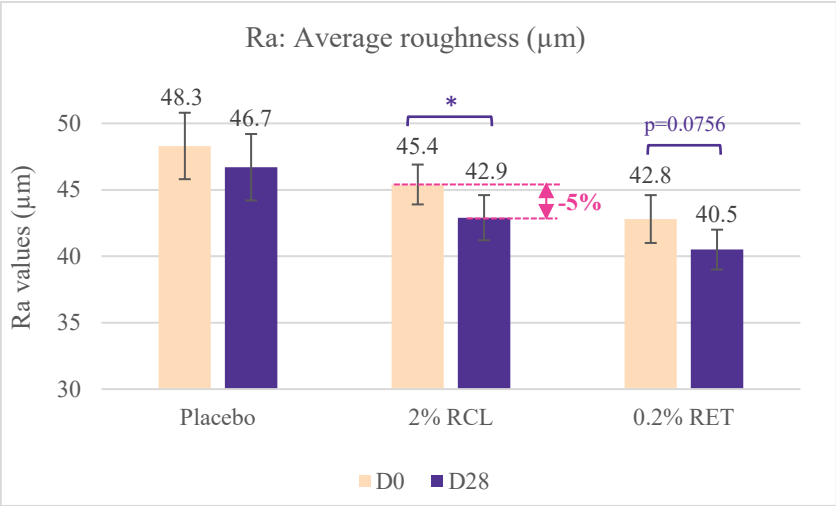


Figure 1: Smoothing effect. Evolution of Ra values for 2% RCL, 0.2% RET and placebo between D0 and D28.

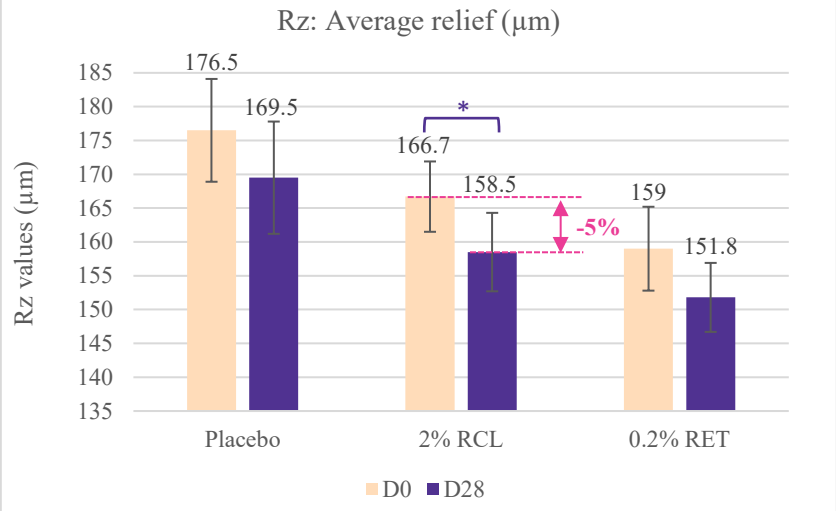
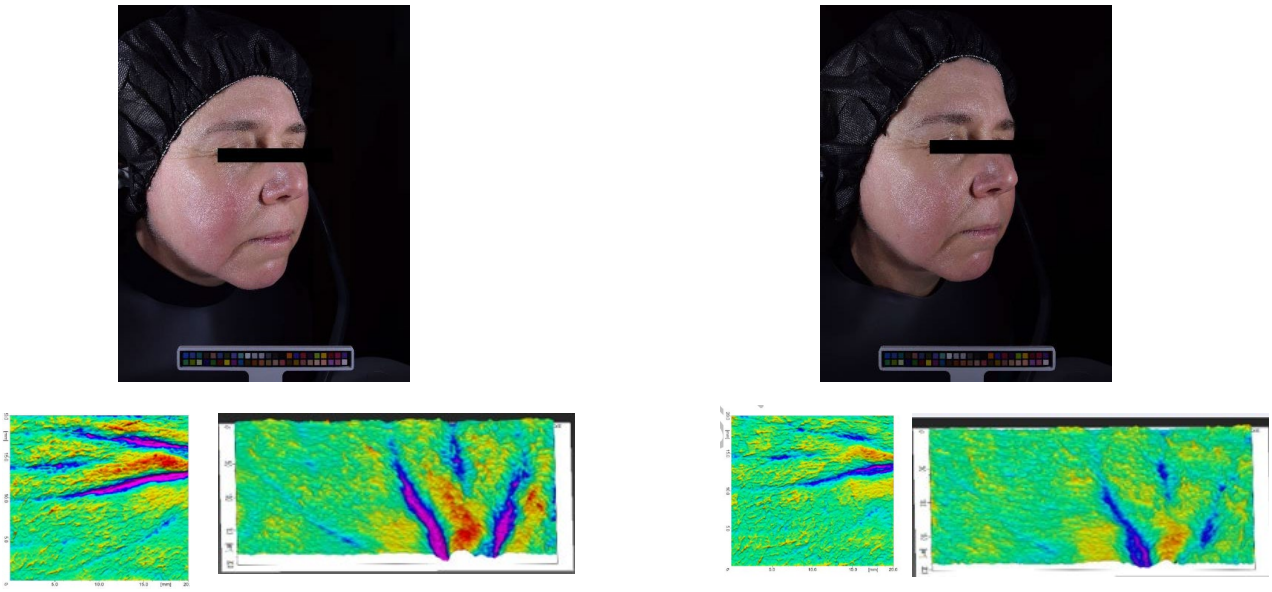


Figure 2: Anti-wrinkle effect: Evolution of Rz values for 2% RCL, 0.2% RET and placebo between D0 and D28.



(a) (b)

Figure 3: Exemplary pictures and corresponding 2D/3D illustrations for one subject at (a) D0 and (b) D28.

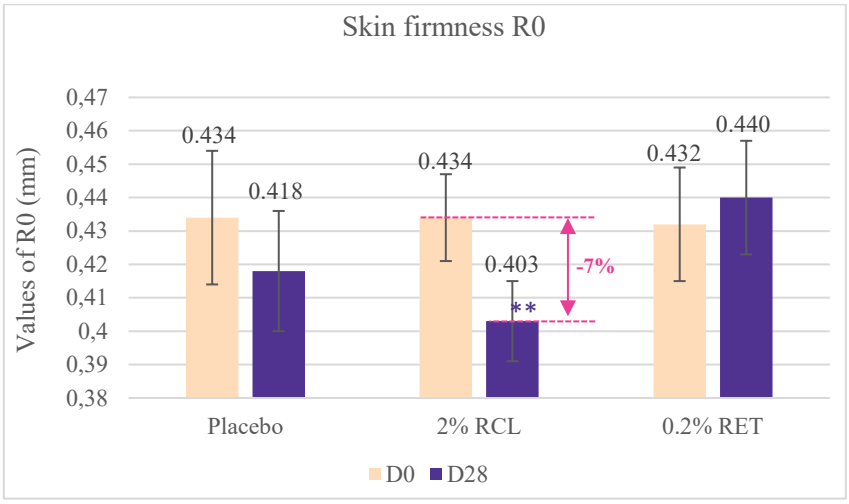


Figure 4: Skin firmness R0 values for 2% RCL, 0.2% RET and placebo at D0 and D28. The comparative statistical analysis highlights a greater effect with 2%RCL compared to 2%RET (p=0.0067)

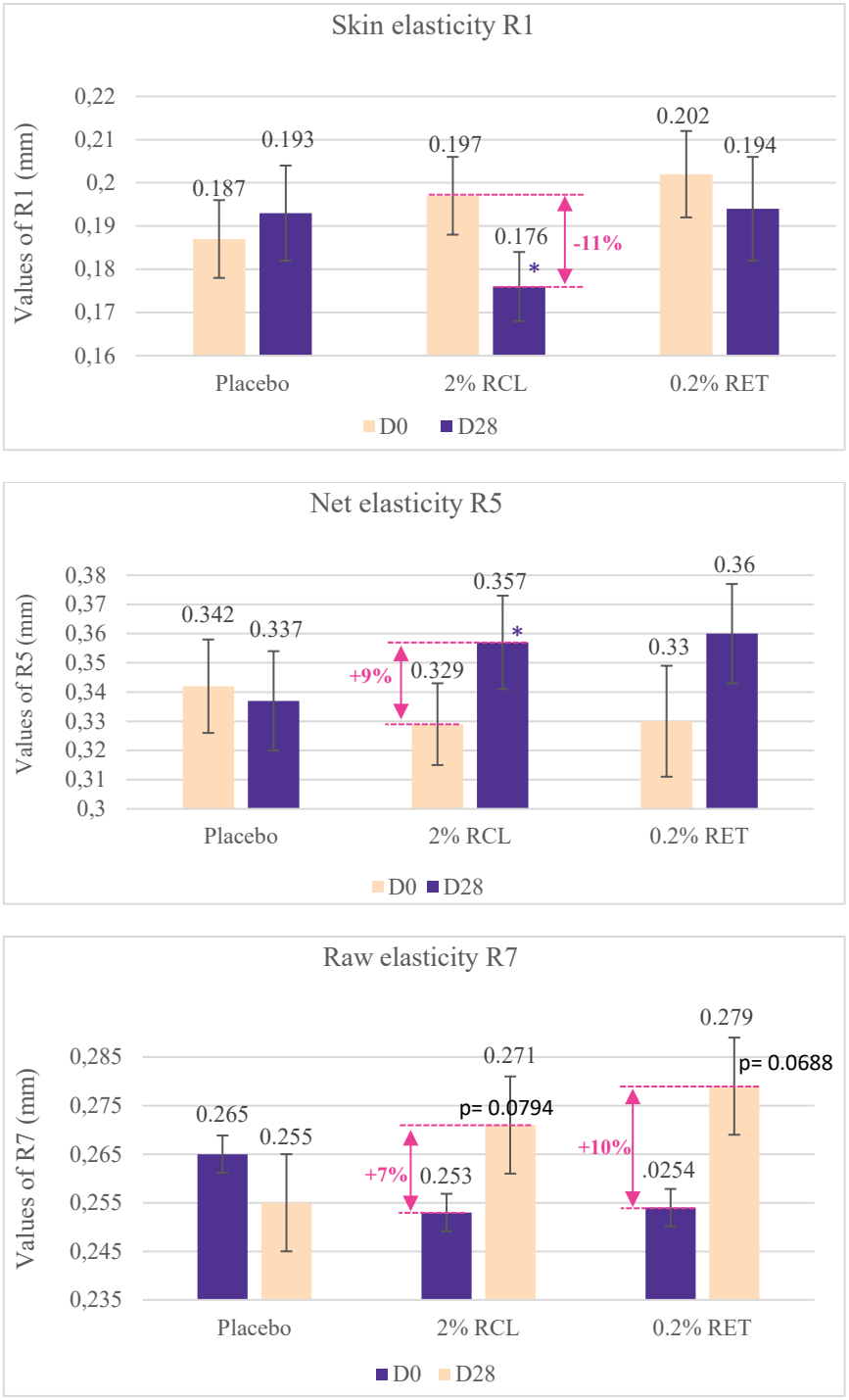


Figure 5: Skin elasticity R1, net elasticity R5 and raw elasticity R7 for 2% RCL, 0.2% RET and placebo at D0 and D28. The comparative statistical analysis highlights in the case of R7 and R1 a greater effect with 2% RCL compared to placebo (p=0.0385 and p= 0.0256 respectively.)

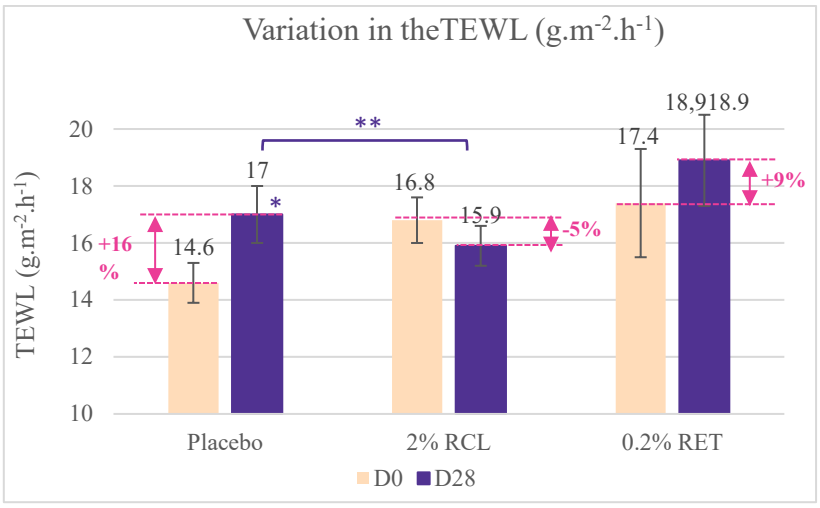


Figure 6: Skin barrier integrity: TEWL values for 2% RCL, 0.2% RET and placebo at D0 and D28. The comparative statistical analysis highlights a very significant difference between 2% RCL and the placebo (p=0.0049).

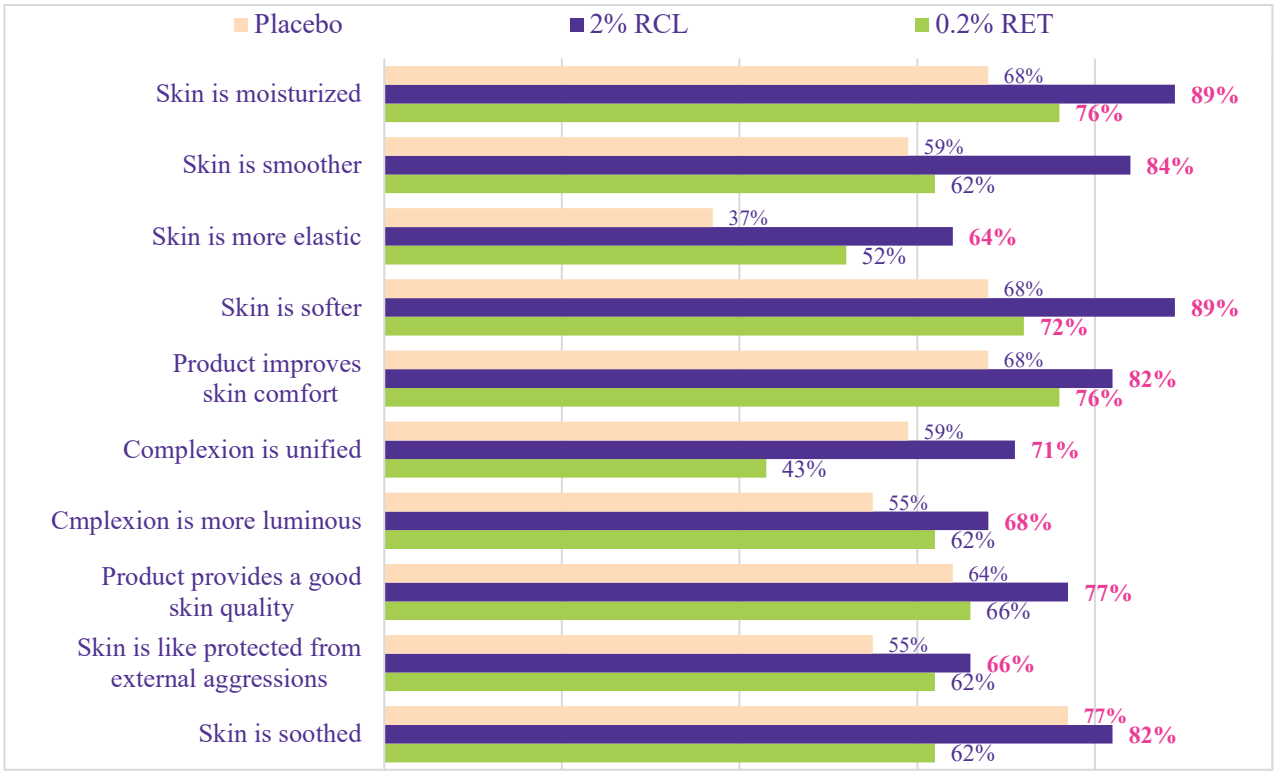


Figure 7: Subjective evaluation survey after 28 days of use for 2% RCL, 0.2% RET and placebo at D0 and D28.

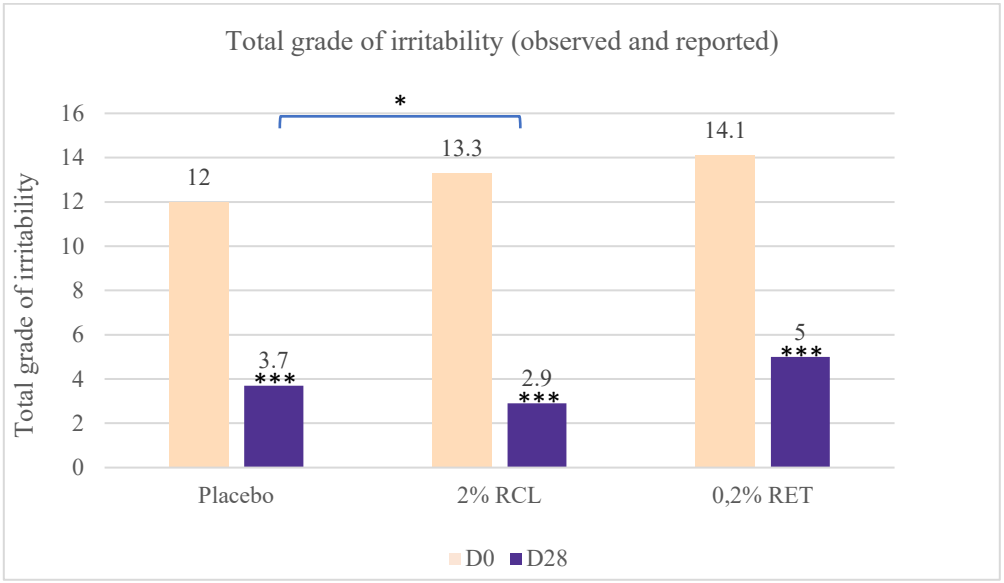


Figure 8: Clinical grading of skin irritability at D0 and D28 for 2% RCL and 0.2% RET

Discussion

Our *in vivo* study demonstrates that the anti-aging performance of an emulsion containing 2% RCL (corresponding to 0.1% DHMC) is better than retinol formulated in a market product (0.2% retinol) in this 28-days study.

Smoothing and anti-wrinkle effects and biomechanical properties of the skin

Smoothing/Anti wrinkles measurements are done directly *in vivo*, using the fringe projection system.

A decrease of Ra value characterized a decrease of roughness and therefore a smoothing effect. A decrease in Rz value characterizes a decrease in average relief and therefore an anti-wrinkle effect. A decrease in Rt value characterizes a decrease of the maximum amplitude and therefore an anti-wrinkle effect.

Our results (significant decrease of Ra and Rz values) showed that only the test product 2% RCL did show a significant smoothing and anti-wrinkle effect.

Several skin biomechanical parameters varied significantly in the direction of improvement of skin quality (R0, R1, R5 describing firmness, elasticity, net elasticity) for 2% RCL only. These results are especially valuable keeping in mind that the concentration of active in RCL is two times lower than the concentration of retinol in the market formulation.

The strong anti-wrinkles performance of RCL is not surprising. It has been shown (internal data) that DHMC, the active ingredient of RCL up-regulates collagen and ECM genes, like elastin and laminin and strongly down-regulates hyaluronidase-1 and MMP-1 genes (internal data).

Additional earlier *in vitro* tests showed significant elastase inhibition on and MMP-1 inhibition on human fibroblasts.

Moreover, DHMC significantly increased the synthesis of hyaluronic acid by keratinocytes, thus improving the hydration status of skin's upper layers. It also inhibited the hyaluronidase activity significantly helping to preserve the levels of hyaluronic acid in the skin [5].

These results conform to earlier *in vivo* tests on the active ingredient DHMC 0.1% [5]. At this time retinol was not tested as a benchmark.

Keeping in mind that retinol represents the gold standard in anti-aging ingredients, when we started the *in vivo* study, our goal was to get comparable performance with the emulsion containing 2% RCL and we were positively surprised to discover while interpretating the results that 2% RCL emulsion outperformed the formulation with 0.2% retinol.

The market product contains 0.2% retinol and represents a moderate percentage of retinol. Stronger results are often seen with higher retinol concentrations (1%). Even if 4 weeks are enough to observe the positive effects of retinol, longer treatments demonstrate further increasing wrinkle scores [6]. Finally, the study was constructed into 2 groups, one testing 2% RCL vs placebo and the one testing again 2% RCL vs 0.2% RET, in all cases on hemi faces. RCL was tested on 44 volunteers while 0.2% RET on 22 which may explain mitigated aging properties of retinol.

Cutaneous barrier

The cutaneous barrier was investigated via the measurement of the TEWL. The cutaneous barrier acts as a regulator in skin water balance. When this is damaged, the water exchange regulation system becomes destabilized. This means that water migrates more easily to the outside environment, increasing Trans Epidermal Water Loss (TEWL). However, if the condition of the cutaneous barrier improves, water loss decreases as the water exchange

regulation mechanism recovers its balance. TEWL is therefore related to the state of the skin barrier. The cutaneous barrier is strengthened in case of TEWL decrease or maintained if there is no variation.

Our results showed that 2% RCL fully compensates the significant TEWL increase induced by the placebo (+16%, p=0.0172). Both test products 2% RCL and 0.2% RET maintained the skin barrier integrity.

These *in vivo* results correlate well with earlier *in vitro* results done on the active of RCL DHMC. It strongly up regulated the production of m-RNA of involucrin in a gene expression profiling using cDNA microarrays (internal results, not shown). Involucrin is a protein precursor of the epidermal cornified envelope. Its expression is initiated early in the epidermal differentiation process. Ultimately it becomes cross-linked to membrane proteins, helping in the formation of an intact skin barrier. Retinol is usually known to induce skin fragility [7].

Moreover, DHMC increased in a dose dependent way Transglutaminase 1 (TGM1) [5] activity on keratinocytes, up to 120%. In contrary, retinol reduces the activity of TGM) [8] TGM1 is a crucial enzyme involved in the keratinization process through crosslinking of cornified envelope proteins including involucrin, loricrin, and SPRs (small proline-rich proteins). Transglutaminases play an essential role in maintaining the barrier function of the skin [9].

Subjective evaluation questionnaire

The perceptions and self-evaluation of the subjects very well correlate with the objective measurements done and show that 2% RCL was better perceived that 0.2% RET.

Grading of the skin irritability

Our results show that the 3 test products have a soothing effect on the skin.
The results for RCL are in phase with earlier results showing how DHMC was able to reduce signs of inflammation (internal results). Results are less common for 0.2% RET, but as mentioned above, the 0.2% use level of retinol represents a moderate concentration of retinol, which may explain these results.

Conclusion.

With this *in vivo* study we could demonstrate very good and better anti-aging performance of an emulsion containing 2% RCL (corresponding to 0.1% of the active DHMC) vs an end formulation containing 0.2% retinol. Indeed, significant anti-wrinkles and smoothing effects as well improved biomechanical properties of the skin were demonstrated for 2% RCL. The cutaneous barrier was maintained for both test products RCL and RET. Finally, also in the self-evaluation questionnaire, RCL was better perceived than the end formulation with 0.2% retinol. These results of this *in vivo* study show a clearly better performance of RCL (INCI: Sorbitol, Dihydroxy Methylchromone) than the end formulation containing 0.2% retinol.

Conflict of Interest Statement. NONE.

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