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"Novel combination of compounds for skin regeneration and rejuvenation through retinoid and epigenetic pathways"

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1. Introduction

Retinoids, derivatives of vitamin A, are extensively utilized in dermatology for their efficacy in treating a range of skin conditions, including acne vulgaris, photoaging, and hyperpigmentation. These compounds function by modulating gene expression through retinoic acid receptors (RARs), leading to increased cell turnover, collagen synthesis, and normalization of keratinization processes. Topical retinoids, such as retinol and its derivatives, have demonstrated significant clinical benefits in improving skin texture, reducing fine lines, and diminishing hyperpigmented lesions [1].

Despite their proven efficacy, the use of retinoids is frequently associated with adverse effects, particularly during the initial phases of treatment. Common side effects include erythema, irritation, dryness, and pruritus, collectively referred to as "retinoid dermatitis". These reactions are often dose-dependent and can lead to decreased patient compliance . The underlying mechanisms involve the activation of inflammatory pathways, contributing to skin irritation [2].

Given these limitations, there is a growing interest in identifying alternative compounds that can replicate the therapeutic effects of retinoids while minimizing adverse reactions. Such alternatives should not only mimic the action of retinoids on RARs but also target additional pathways implicated in skin aging. These pathways include cellular senescence, the transforming growth factor-beta (TGF-β) signaling pathway, and epigenetic modifications, including the regulation of microRNAs (miRNAs). Cellular senescence contributes to the aging process by promoting a pro-inflammatory environment and impairing tissue regeneration [3]. The TGF-β pathway plays a crucial role in collagen synthesis and extracellular matrix remodeling, processes that are often dysregulated in aged skin [4]. Moreover, epigenetic factors such as miRNAs have been identified as regulators of gene expression involved in skin homeostasis and aging [5].

Here, we show a new combination of compounds that boosts retinol effects at low doses targeting retinoid-specific pathways and key markers involved in skin senescence, regeneration and rejuvenation.

2. Materials and Methods

In vitro assays were performed to prove the effect of compounds on retinoid and skin aging pathways. Based on previous internal research, retinol 5 µM was defined as the maximum viable dose in cells, and thus the highest dose of retinol to be applied *in vitro*. On the other hand, retinol 1 µM was chosen as the lower concentration of retinol to be combined with the other compounds. Human epidermal keratinocytes were treated for 24h with retinol 5 µM alone or the combination of compounds (retinol 1 µM + hydroxypinacolone retinoate 100 µM + protocatechuic acid 500 µM) and gene expression was quantified by qPCR (real-time polymerase chain reaction). Human dermal fibroblasts where irradiated with UVB several times to induce senescence. The irradiation protocol included two irradiations with 25 mJ/cm^2 in 4 days. Senescent fibroblasts were treated for 48h with retinol 5 µM alone or the combination of compounds (retinol 1 µM + hydroxypinacolone retinoate 10 µM + protocatechuic acid 500 µM) and gene expression and miRNA levels was quantified by qPCR.

3. Results

As shown in Table 1, human epidermal keratinocytes treated with retinol alone showed an expected increase in retinoid-induced genes such as CRABPII, RAR-G, TGF-b1, CTGF or CGRP. Compared to retinol alone, the combination of lower concentration of retinol with hydroxypinacolone retinoate (HPR) and protocatechuic acid (PA) increased to a higher extend the levels of CRABPII, RAR-G, TGF-b1 and CTGF compared to retinol alone. Besides, the combination upregulated c-jun, a keratinocyte proliferation regulator induced by retinoids [6], downregulated CCN1, an extracelular protein that boosts inflammatory cytokines, and slightly upregulated CGRP, a marker that has been associated to retinol-induced skin irritation.

Table 1. Gene expression changes in human epidermal keratinocytes treated with retinol or the combination of compounds for 24h. T-student analysis was performed to evaluate significant differences between the conditions, comparing control cells vs treated cells. * indicates $p < 0.05$; ** indicates $p < 0.01$; *** indicates $p < 0.001$.

Gene	Control	Retinol	Retinol + HPR + Protocatechuic acid
c-jun	1.00	0.98 (±0.09)	1,85 (±0.10) **
CRABPII	1.00	1,62 (±0.07) **	2,87 (±0.09) **
RAR-G	1.00	1,47 (±0.03) **	2,54 (±0.11) **
TGF-b1	1.00	1,48 (±0.03) **	2,01 (±0.06) **
CTGF	1.00	2,63 (±0.14) **	3,03 (±0.24) **
CCN1	1.00	2,01 (±0.10) **	0,83 (±0.18) **
CGRP	1.00	2,38 (±0.05) *	1,32 (±0.35) *

As retinoids have been described to regulate fibroblast activity [6], we tested the effect of retinol alone compared to the combination in senescent dermal fibroblasts. Retinol alone at the highest dose showed some beneficial effect, such as the reduction of *MME* and *MFAP5* or the upregulation of *KGF* and *FN1* (Table 2). However, retinol alone also showed some detrimental effects on senescent fibroblasts, such as the upregulation of *MMP14, ICAM1, IL-6* or *GDF15*.

On the other hand, the combination of compounds outperformed retinol alone in most of the tested genes, increasing the levels of regeneration cytokines and growth factors (*KGF, IL-15*) while reducing matrix metalloproteinases (*MME, MMP9, MMP14*), propigmentary factors (*GDF15*).

Table 2. Gene expression changes in human senescent dermal fibroblasts treated with retinol or the combination of compounds for 48h. T-student analysis was performed to evaluate significant differences between the conditions, comparing no UVB control vs UVB control and UVB control vs UVB treated cells. * indicates $p < 0.05$; ** indicates $p < 0.01$; *** indicates $p < 0.001$.

	No UVB	UVB		
Gene	Control	Control	Retinol	Retinol + HPR + Protocatechuic acid
MME	1.00	2.89 (±0.23)**	1.94 (±0.16)*	2.17 (±0.07)*
MMP-14	1.00	1.70 (±0.07)**	2.18 (±0.12)*	1.96 (±0.10)**
MMP-9	1.00	2.39 (±0.31)**	2.22 (±0.31)	1.28 (±0.13)*
ICAM1	1.00	4.13 (±0.31)**	35.83 (±5.07)**	4.58 (±0.04)**
IL-6	1.00	8.51 (±0.39)***	22.85 (±1.34)***	4.28 (±0.41)*
MFAP5	1.00	1.83 (±0.10)**	1.57 (±0.22)*	1.19 (±0.05)**
GDF15	1.00	2.92 (±0.31)**	6.33 (±0.81)**	1.89 (±0.05)*
IL-15	1.00	1.35 (±0.03)**	2.41 (±0.05)***	2.97 (±0.08)**
KGF	1.00	1.43 (±0.14)*	1.86 (±0.05)*	2.61 (±0.18)**
FN1	1.00	1.70 (±0.11)**	2.20 (±0.01)**	4.72 (±0.10)***

Finally, we studied the effect of retinol alone and the combination of compounds on several miRNAs that are involved in skin aging processes [7, 8]. Specifically, these miRNAs control cell adhesion, extracellular matrix remodelling and senescence features in dermal fibroblasts. As shown in Figure 1, some miRNAs were upregulated in senescent dermal fibroblasts, and retinol alone only reduced the levels of one of them, miR-23a-3p. On the other hand, the combination of compounds downregulated the levels of the 3 tested miRNAs, inducing a higher effect than retinol alone.

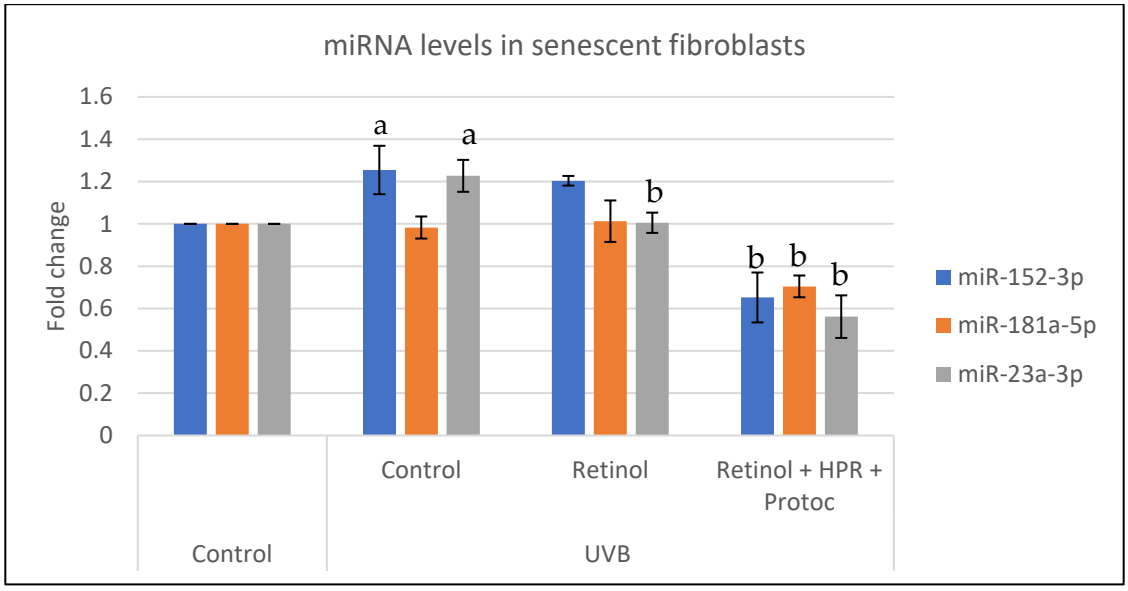

Figure 1. miRNA level changes in human senescent dermal fibroblasts treated with retinol or the combination of compounds for 48h. T-student analysis was performed to evaluate significant differences between the conditions. "*a"* indicates $p < 0.05$ comparing no UVB control vs UVB control, while "b" indicates $p < 0.05$ comparing UVB control vs UVB treated cells

4. Discussion

Retinoids, vitamin A derivatives, effectively treat acne, photoaging, and hyperpigmentation by boosting cell turnover and collagen production. However, they often cause irritation, dryness, and inflammation, reducing patient compliance. As a result, interest is growing in gentler alternatives that mimic retinoids and target other aging pathways like TGF-β signaling, cellular senescence, and epigenetic factors such as microRNAs. Here we describe a combination of compounds including low levels of retinol, hydroxypinacolone retinoate and protocatechuic acid to effectively target both retinoid and skin aging-regulated pathways.

We first tested the effect of retinol alone and the proposed combination of compounds on retinoid-induced genes in epidermal keratinocytes [6]. As expected, retinol alone at the highest viable dose upregulated most of the genes (Table 1). Some of these genes have a beneficial effect on skin regeneration and epidermal thickness, including *CRABPII, RAR-G, TGF-b1* or *CTGF*. On the other hand, retinol alone upregulated CCN1 and CGRP, two genes that favor proinflammatory and damaging environments, which could explain the observed adverse effects of retinol at high doses. When using lower doses of retinol combined with HPR and PA, the expression of retinoid-induced genes responsible for skin regeneration and epidermal thickness was higher compared to retinol alone, while the genes responsible for retinol adverse effects (CCN1 and CGRP) were significantly lower compared to retinol alone. This results suggest that the compounds HPR and PA are boosting the retinoid pathway but without increasing proinflammatory and irritation markers, and thus offers a good alternative to high doses of retinol for cosmetic use.

Subsequently, we tested the effect of retinol alone and the combination of human dermal senescent fibroblasts. As expected, genes associated to the senescence-associated secretory phenotype (SASP) were upregulated in senescent fibroblasts compared to non-senescent fibroblasts, including *MME, MMP14, MMP9, ICAM1, IL6, MFAP5* and *GDF15* (Table 2). When senescent cells were treated with retinol alone, some benefitial effects were observed, including the decrease in *MME*, while detrimental effects were also exerted by retinol alone, including the upregulation of ICAM1 and IL-6, which contribute to a proinflammatory environtment. This result highlights the limitations of retinol when used alone, and warrants the search for novel ingredients or combinations that can boost the efficacy of retinol without increasing the expected adverse effects for topical retinoids. In this sense, our proposed combination of ingredients showed a better profile in senescence regulation in dermal fibroblasts comparted to retinol alone, as the combination downregulated all the tested markers for senescence pathway and SASP, including the proinflammatory markers that were upregulated by retinol alone. This results suggest that the combination is a promising one for targeting the key features of cell skin aging without the adverse effects of high doses of retinol.

Finally, several miRNAs (miR-152-3p, miR-181a-5p and miR-23a-3p) that are involved in skin aging processes were quantified [7, 8]. Particularly, miR-152-3p and miR-181a-5p are increased in human dermal fibroblast senescence, which causes a decrease in key proteins for cell adhesion and extracellular matrix remodelling, such as integrin α5 and collagen XVI. On the other hand, miR-23a-3p is also involved in cellular senescence by decreasing the levels of hyaluronan synthase 2 (HAS2). As shown in Figure 1, retinol alone only downregulated one of the miRNAs, while the combination of compounds significantly downregulated the 3 tested miRNAs. This result confirms the anti-senescence effect of the combinatio as observed in the quantification of genes involved in SASP (Table 2). Besides, this result describes a complementary mechanism of action to that of the specific retinoid-signature, which is based on the regulation of key epigenetic markers that are involved in skin aging [9].

5. Conclusion

The proposed combination of compounds (low dose retinol, hydroxypinacolone retinoate and protocatechuic acid) shows promising effects as a substitution of high doses of retinol in dermatological therapy. Besides, epigenetic markers associated with skin aging are regulated by the combination but not by retinol alone. A formulation with such ingredients could be ideal for combination with other medical-aesthetic treatments including devices or chemical peelings that aim to reduce pigmentation, rejuvenate and/or regenerate skin..

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