Novel synergistic combination of active ingredients for melasma topical therapy

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INTRODUCTION

Melasma has a complex pathophysiology with different cell types and signaling pathways involved.[1,2] Paracrine factors secreted by keratinocytes but also fibroblasts and endothelial cells act on melanocytes and stimulate melanogenesis by modulating **several pathways including WNT, EDN1, α-MSH and SCF.[3]** Oxidative stress and inflammation also participate to the regulation of pigmentation.

OBJECTIVE

The aim of this research was to **find a novel combination of compounds to effectively treat skin hyperpigmentation** conditions such as melasma by targeting the main melanogenic pathways.

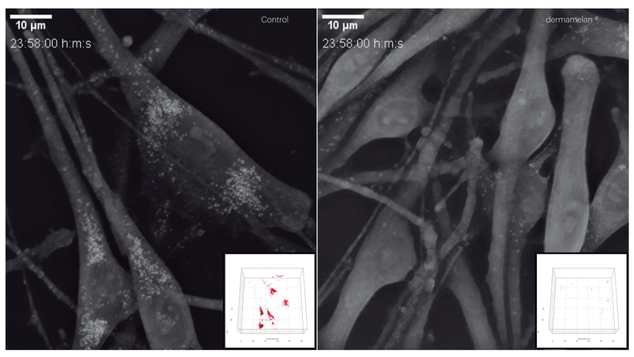
MATERIALS AND METHODS

12 compounds with proven depigmenting efficacy were analyzed using Artificial Neural Networks (ANNs)-based approach. This algorithm generates a model that simulates the skin pigmentation process considering all the molecular information available from reliable scientific databases, and gives the best combinations of depigmenting compounds. The proposed combination of actives (retinol, diosmin, ferulic acid) was further tested *in vitro* to assess the synergistic effect in tyrosinase activity extracted from melanocytes and to evaluate the inhibition of the key pathways regulating melanogenesis through the quantification of specific markers by qPCR. The synergistic complex was also tested *ex vivo*, and semiquantitative 2D melanin analysis performed using the ImageJ software.

RESULTS

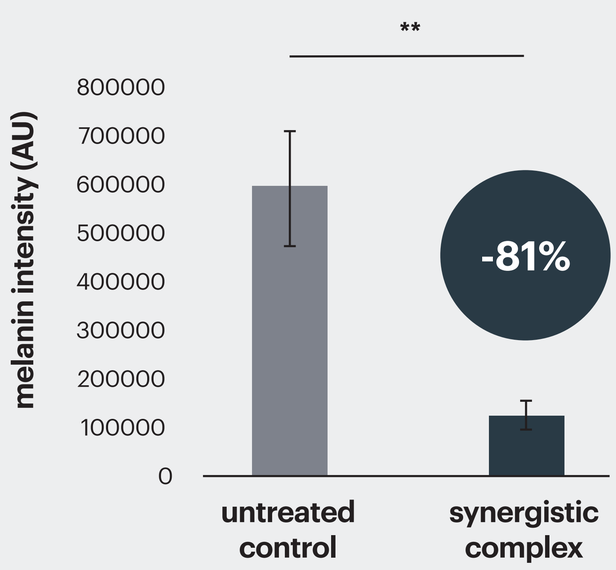
A **synergistic combination was found for 3 compounds, retinol, diosmin and ferulic acid**, and proved by *in vitro* studies that act on the key cellular processes of melanogenesis: inflammation, vascularity and the hormonal α-MSH pathway.

Regulation of the hormonal α-MSH pathway



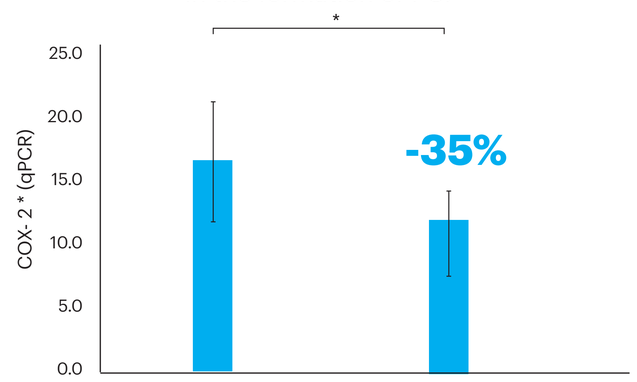
control with the synergistic complex

Inhibition of accumulation of melanin granules in melanocytes stimulated for 24 h with IBMX, an activator of the α-MSH pathway plus L-tyrosine, and treated with the synergistic complex.



Regulation of the inflammatory pathway

Reduction of COX-2, enzyme involved in the formation of PG.

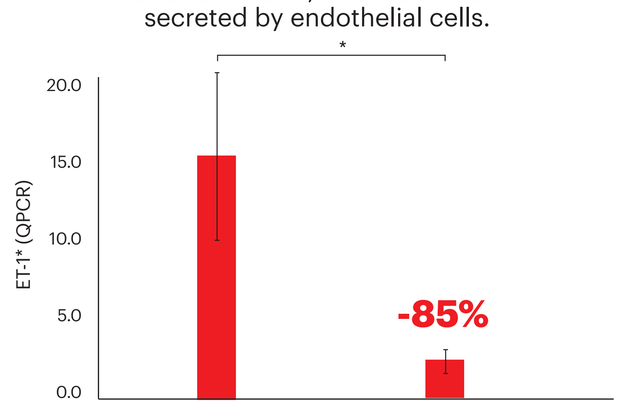


control with the synergistic complex
Fibroblasts induced with IL-1α after 24 h

*COX-2 enzyme involved in the formation of PG, main inflammatory marker.

Regulation of the vascular pathway

Reduction of ET-1, a vascular marker secreted by endothelial cells.

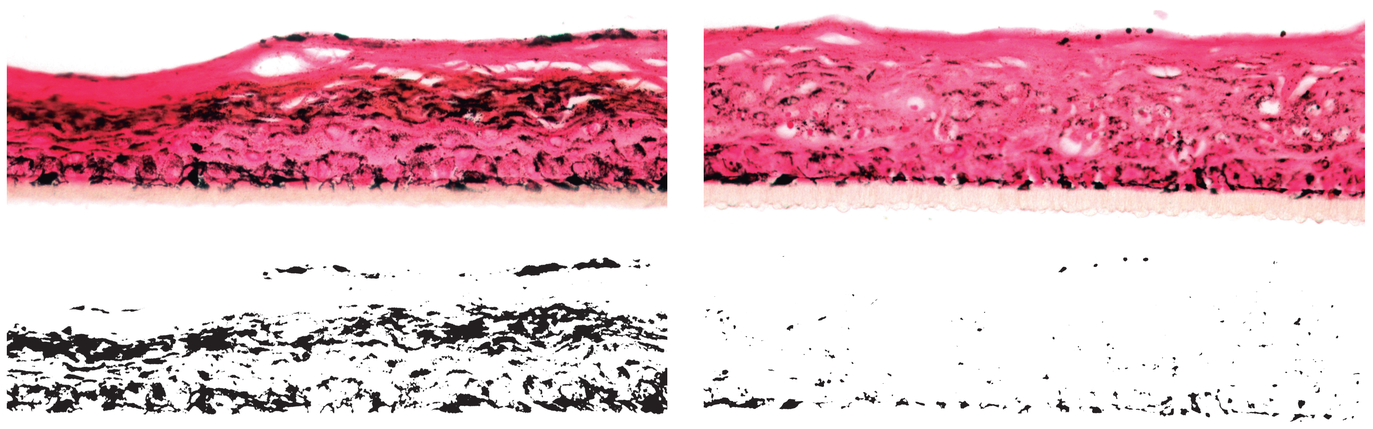


control with the synergistic complex
Fibroblasts induced with IL-1α after 24 h

*ET-1 is a vascular marker secreted by endothelial cells

Efficacy of topical active ingredients in a 3D skin model

Significant reduction of the accumulation of melanin after topical treatment of an epidermal model of phototype VI with the complex of active ingredients. (Fontana Masson staining; optical microcopy, 20x magnification)



Untreated control (D8) **Synergistic complex (D8)**

CONCLUSIONS

- The **complex of retinol, diosmin and ferulic acid** at specific concentrations inhibit synergically the key signaling pathways of the hyperpigmentation process.
- This leads to a **significant depigmenting efficacy on *in vitro* and *ex vivo* models**
- The active ingredients in the **topical formula** are **able to penetrate the skin to reach their target cells** and are therefore effective in the clinical treatment of hyperpigmentation.

1.Thierry Passeron, Mauro Picardo. Melasma, a photoaging disorder. Pigment Cell Melanoma Res. 2018;31(4):461-465. **2.** R Buscà, R Ballotti. Cyclic AMP a key messenger in the regulation of skin pigmentation. Pigment Cell Res. 2000;13(2):60-9. **3.** Yoshinori Miyamura, Sergio G Coelho, Rainer Wolber, Sharon A Miller, Kazumasa Wakamatsu, Barbara Z Zmudzka, Shosuke Ito, Christoph Smuda, Thierry Passeron, Wonseon Choi, Jan Batzer, Yuji Yamaguchi, Janusz Z Beer, Vincent J Hearing. Regulation of human skin pigmentation and responses to ultraviolet radiation. Pigment Cell Res. 2007;20(1):2-13.