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"Synergistic Effects of Carnosine and Retinol in Inhibiting Melanogenesis in Human Melanoma MNT-1 Cells"

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1. Introduction

Melanogenesis, the complex biochemical process responsible for melanin synthesis, plays a pivotal role in determining skin pigmentation. Abnormal regulation or hyperactivation of melanogenesis frequently leads to dermatological concerns such as hyperpigmentation, melasma, and age-related skin discoloration, prompting significant interest within the cosmetic industry to discover effective strategies to control or mitigate these issues [1,2].

Retinol (ROL), a widely recognized derivative of vitamin A, has been extensively studied and employed in dermatological treatments and cosmetic formulations, primarily due to its potent anti-aging effects. ROL is known to stimulate collagen synthesis by activating fibroblasts in the dermis, leading to improved skin texture and elasticity. Additionally, ROL has been shown to increase the production of hyaluronic acid in epidermal keratinocytes, further contributing to enhanced skin hydration and reduced wrinkle formation [3,4]. Despite its known roles in anti-aging and skin rejuvenation, the direct effects of ROL on melanogenesis requires further elucidation.

Carnosine, a naturally occurring dipeptide composed of β-alanine and histidine, has received considerable scientific interest for its antioxidant, anti-glycation, and anti-inflammatory activities. Several studies suggest that carnosine can modulate oxidative stress and inflammatory responses, process often implicated in aberrant melanogenesis and pigment disorders [5,6]. However, the direct interaction between carnosine and melanin synthesis pathways remains largely unknown.

This study investigates the novel synergistic inhibitory effects of ROL and carnosine on melanogenesis in human melanoma MNT-1 cells. By evaluating the combined effect of ROL and carnosine on melanin production, we seek to provide valuable insights into their potential for addressing hyperpigmentation and enhancing cosmetic outcomes. This research not only expands our understanding of melanogenesis regulation but also offers innovative approaches to the development of advanced skincare products for improving skin tone uniformity and brightness.

2. Materials and Methods

Cell culture and chemicals

MNT-1 cells were cultured in minimal essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) (Gibco BRL, Grand Island, NY, USA), 10% AIM-V medium (Gibco BRL), 10 mM HEPES, 0.1 mM nonessential amino acids, and 1mM sodium pyruvate at 36°C with 10% CO_2. MNT-1 cells were passaged at 80-90% confluence. Carnosine and ROL were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Melanin content assay

ROL and carnosine were treated twice every 3 days, and cells were harvested on the 6th day after the treatment. Cells were harvested and centrifuged at 20,000 g for 2 minutes to obtain pellets. Cell pellets were washed with phosphate-buffered saline (PBS) twice and 1N NaOH was added to dissolve the cell pellets. After 5 minutes of incubation in an 80°C heat block, the dissolved melanin content was quantified by measuring absorbance at 490 nm.

Cell viability test

Cell viability test was conducted using the cell counting kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions. MNT-1 cells were seeded in 6-well plates and treated with carnosine, ROL, or their combination at various concentrations for 6 days. After the treatment period, CCK-8 reagent was added to each well and incubated for 1 hour at 37°C. Absorbance was measured at 450 nm using a microplate reader.

Statistical analysis

The experiments were independently replicated at least three times. The results were expressed as the mean value ± standard deviation (SD). Statistical analyses were conducted using Student's t-test or ANOVA

3. Results

To evaluate the effects of carnosine and ROL on melanogenesis, human melanoma MNT-1 cells were treated with each compound individually or in combination (Figure 1A). Carnosine alone exhibited no significant inhibition on melanin synthesis. In contrast, co-treatment with carnosine and ROL resulted in a notable reduction in melanin content compared to individual treatments. Specifically, co-treatment with 3 mM carnosine and 100 nM ROL resulted in a 52% inhibition of melanin synthesis relative to the vehicle (Figure 1B).

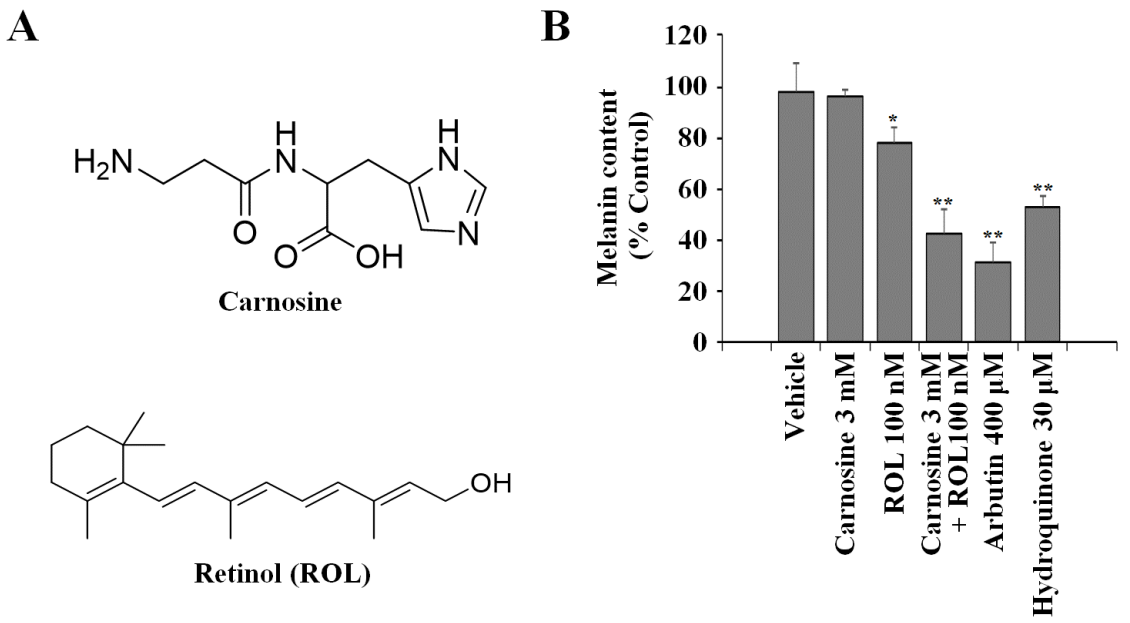

Figure 1. Evaluation of carnosine and ROL on melanogenesis in human melanoma MNT-1 cells. (A) Chemical structures of carnosine and retinol (ROL). (B) Melanin content in MNT-1 cells was quantified on the 6th day after the treatment. Values represent the mean expression ± standard deviation (SD) (n=3). *p≤0.05 vs. vehicle control; **p≤0.01 vs. vehicle control.

To further characterize the concentration-dependent effects of the combination treatment, MNT-1 cells were exposed to varying concentrations of carnosine and ROL. As a result, a significant and dose-dependent inhibition in melanin synthesis was observed (Figure 2A). Co-treatment with 1 mM carnosine and 100 nM ROL led to a 38% inhibition of melanin synthesis, whereas increasing the concentration of carnosine to 3 mM in the presence of 100 nM ROL resulted in a 52% inhibition. Furthermore, melanin content in co-treated cells was significantly lower than that observed in the vehicle (Figure 2B).

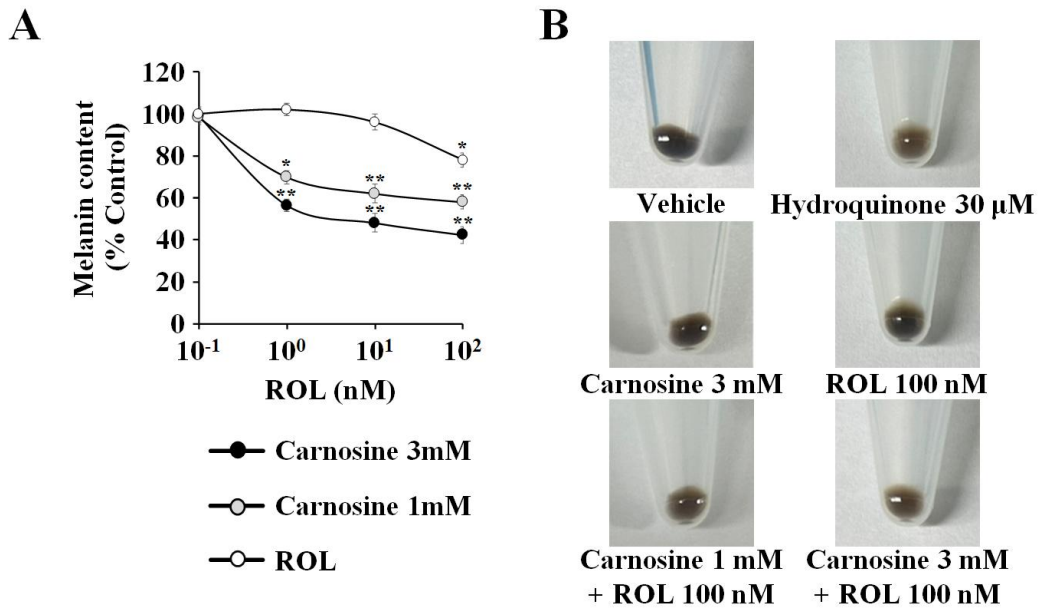

Figure 2. Concentration dependency of carnosine and ROL co-treatment on melanogenesis inhibition in human melanoma MNT-1 cells. (A) The concentration dependency of carnosine and ROL co-treatment on melanogenesis inhibition was determined. (B) Representative images of melanin content after treatments. Values represent the mean expression ± standard deviation (SD) (n=3). *$p \leq 0.05$ vs. vehicle control; **$p \leq 0.01$ vs. vehicle control.

To confirm whether the antimelanogenic effects were associated with cytotoxicity, cell viability test was assessed using the CCK-8 assay. MNT-1 cells maintained viability above 90% across all treatment conditions, including the highest test concentrations 3 mM carnosine and 100 nM ROL, with no statistically significant differences compared to the vehicle (Figure 3). These results confirm that the observed inhibition of melanin synthesis was independent of cytotoxic effects.

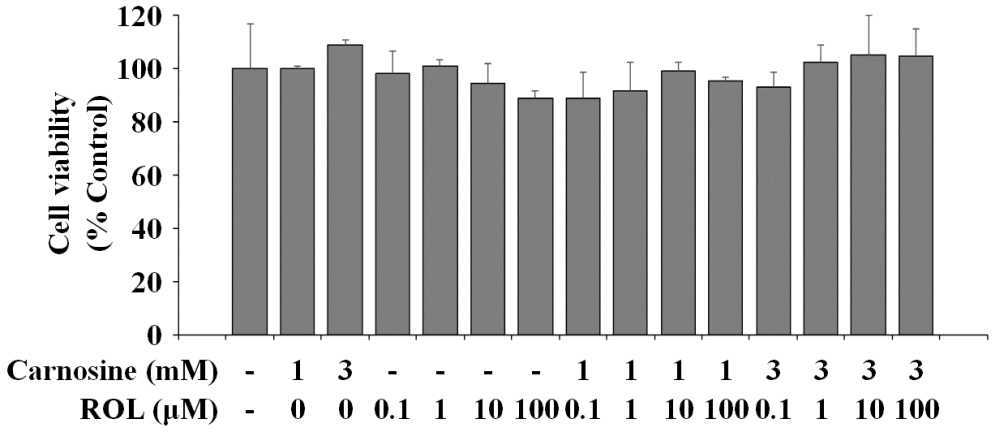

Figure 3. Evaluation of cell viability in MNT-1 cells. Viability of MNT-1 cells treated with various concentrations of carnosine and ROL was determined using the CCK-8 assay. Values represent the mean expression ± standard deviation (SD) (n=3).

4. Discussion

This study demonstrates the combined effects of carnosine and ROL on melanogenesis in the human melanoma MNT-1 cell line. While carnosine alone exhibited no significant effect on melanin production, its co-administration with ROL resulted in a concentration-dependent and synergistic inhibition of melanogenesis. Notably, the combination of 3 mM carnosine and 100 nM ROL led to a 52% reduction in melanin content, producing greater inhibition than either compound alone.

ROL is widely recognized for its ability to regulate keratinocyte proliferation and differentiation, as well as its inhibitory action on melanogenesis [7]. Carnosine has been reported to protect against oxidative stress-induced damage [8]. Although carnosine alone did not inhibit melanin synthesis in this study, its co-treatment with ROL potentiated the antimelanogenic effect, suggesting that carnosine may enhance ROL's efficacy by mitigating oxidative stress or modulating upstream signaling pathways involved in melanocyte activation.

The observed synergism may be resulting from the complementary mechanisms of action of the two compounds; ROL directly suppresses genes involved in melanin synthesis, while carnosine may modulate the cellular environment to favor the downregulation of melanogenesis, potentially through antioxidant mechanisms [9].

Moreover, cell viability test confirmed the absence of cytotoxicity across all treatment conditions, indicating that the observed anti-melanogenic effects were not the result of cytotoxicity.

Collectively, these findings highlight a novel combinatorial approach to melanogenesis inhibition, wherein ROL exerts direct transcriptional suppression of melanogenic pathways, while carnosine enhances cellular resilience and possibly modulates the microenvironment. Further studies, including transcriptomic and proteomic analyses, are needed to elucidate the specific molecular mechanisms involved.

5. Conclusion

This study demonstrates that co-treatment with carnosine and ROL exerts a synergistic, concentration-dependent inhibition of melanin synthesis in MNT-1 cells, without inducing cytotoxicity. Although carnosine alone did not affect melanogenesis, its combination with ROL significantly enhanced the anti-melanogenic effect. These findings suggest that the carnosine and ROL combination holds a promising, safe, and effective strategy for cosmetic formulations aimed at skin lightening and improving uneven skin tone.

References

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