2-MERCAPTONICOTINOYL GLYCINE, A POTENT BRIGHTENING AGENT EXHIBITING A UNIQUE MODE OF ACTION INVESTIGATED *IN VITRO* & *IN VIVO*

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1 INTRODUCTION

Pigmentation disorders and skin unevenness often constitute aesthetic or psychological discomforts affecting the quality of life across all populations. The discovery of new ingredients able to effectively manage melanin production is crucial. 2-MNG (2-mercaptonicotinoyl glycine), is a new molecule with a thio-niacinamide backbone, offering high performance in inhibiting melanin production (1). The objective of this study is to determine the mode of action of 2-MNG, which was explored in tubo, in vitro and in vivo.

2 MATERIALS & METHODS

The structure of 2-MNG suggests that it reacts with o-quinones to form adducts through the thiol group. Mechanism of action studies were carried out *in tubo*, *in vitro*, and *in vivo* to confirm this assumption.

- **Tubo demonstration:** Oxidation reactions of melanin precursors by tyrosinase were carried out in the presence of 2-MNG: A solution of L-DOPA (solution 1), DHICA (solution 2) and DHI (solution 3) were were prepared at 100 µM, 100 µM and 200 µM respectively in 0.05 M sodium phosphate buffer, pH 6.8. 2-MNG was added to each solution at the same concentration as each of the melanin intermediates. The solutions were mixed with 25 U/ml mushroom tyrosinase in 1 ml buffer. The oxidation reaction was stopped by adding 1 ml of 1 M HCl (solutions 1 and 2), or 200 mg of Na2S2O5 and 4 ml of 1 M HCl (solution 3). The structures formed were analyzed by NMR and mass spectra

- **In vitro demonstration:** Normal human melanocytes were seeded in 6-well plates and incubated for 72h with increasing concentrations of 2MNG (7,4 to 200 µM). Supernatants were collected and frozen for analysis. Cell layers were frozen at -80°C. The formation of adducts between 2-MNG and Dopaquinone was monitored, in the cell lysates and in the supernatant using LC/MS system, rather than LC/UV system as reported previously. Similar experiment was carried out with 700µM of L-Tyrosine added in the culture medium.

- **UV induced pigmentation clinical study**: Formulae containing 2-MNG at 0,5 and 1 % were tested versus their vehicle in a double blinded and randomized clinical study detailed by De dormael and al (2). Briefly 33 female and male, aged 18 to 50 years with phototypes III/IV/V (-01°≤ITA°≤28°) were treated on minizones on the back, five days a week during seven weeks, with 4mg/cm² of each formula. During the second week, volunteers were exposed under 0.5MED of UV-daylight during 4 consecutives days. The primary evaluation criteria was colorimetry, using Chromameter (deltaE)

3 RESULTS & DISCUSSION

2-MNG conjugates with DOPA, DHICA, and DHI through the o-Quinones *In tubo*

The possibility that 2-MNG reacts with o-quinones was examined in tubo by tyrosinase-catalyzed oxidation of L-DOPA, DHICA and DHI in the presence of 2-MNG.

1H and 13C NMR & MS spectra were performed and confirmed the disapearance DOPA, DHICA and DHI starting compounds and the appearance of new products identified as 6-MNG-DOPA (92% yield), 4-MNG-DHICA (45% yield), 2-MNG-DHI (15% yield), 4-MNG-DHI (13%), and 2,4-diMNG-DHI (5%). This indicates that 2-MNG reacts effectively through the thiol group to o-quinones.

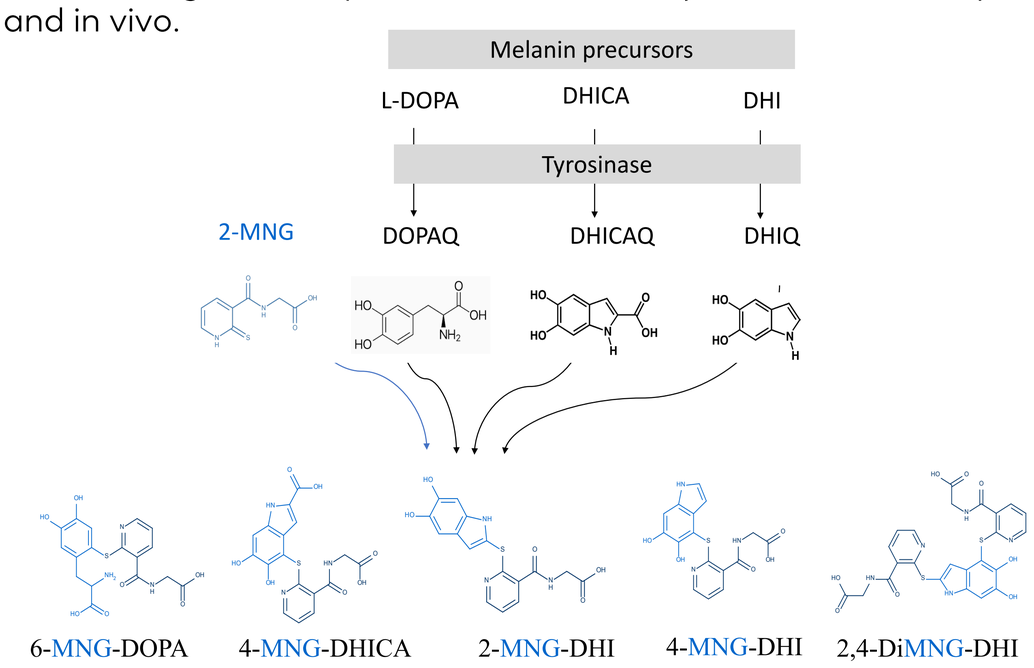


FIGURE 1 Structure of 2-MNG adducts to *o*-quinones

In vitro confirmation: 2-MNG conjugates with Dopaquinone

Dopaquinone being the first o-quinone formed during melanin synthesis process, the formation of 6-MNG-DOPA was monitored after incubation of melanocytes in presence of increasing concentrations of 2-MNG. 6-MNG-DOPA was detected both in cell lysates and supernatant showing the formation of 2-MNG and Dopaquinone adducts in the cell, and a release of the adduct out of the cell. Addition of L-Tyrosine in the culture medium, aiming at increasing melanin production increased 6-MNG-DOPA formation and release, showing a systematic conjugation of 2-MNG with dopaquinone.

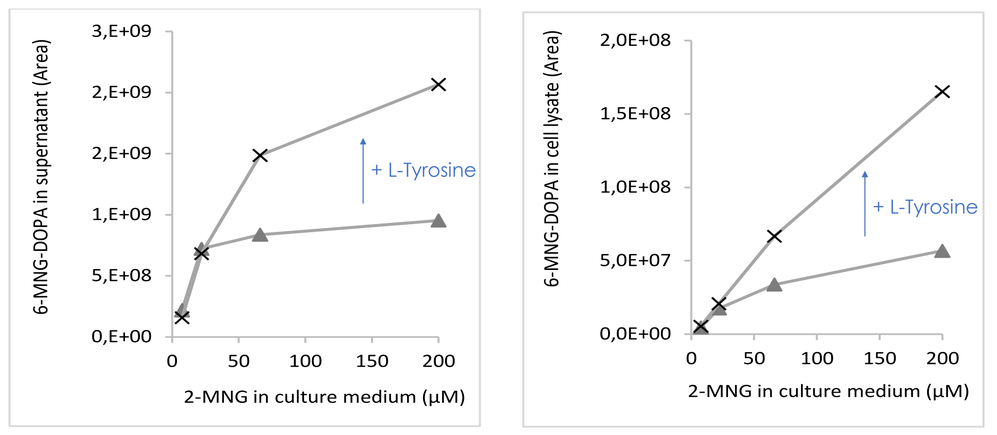
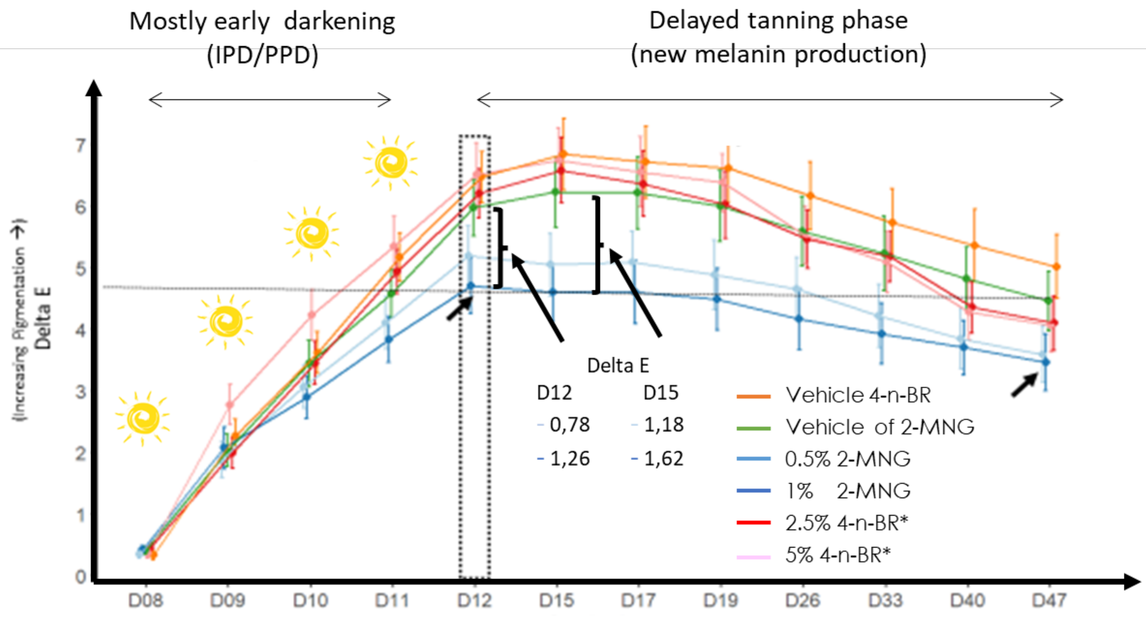


FIGURE 2: 2-MNG dose effect on melanocytes grown in presence of high concentration of L-Tyr (x) or without L-Tyr addition (△).

In vivo efficacy and mode of action of 2-MNG in a UV induced pigmentation study

Following UV exposures, an immediate pigmentation appears, resulting from the oxidation of preexisting melanin precursors, followed by a delayed tanning resulting from new melanin production. Melanin precursors being involved in both phenomena, a UV induced pigmentation study was performed to assess the efficacy of 2-MNG. The in vivo dose effect (0.5 & 1%) of 2-MNG was demonstrated, either to prevent the early darkening or to reduce delayed tanning.



4 CONCLUSIONS

2-MNG is an innovative technology for hyperpigmentation management based on a thiopyridinone backbone (1).

Experiments were performed to give evidence that 2-MNG conjugates with some melanin precursors preventing their integration into growing melanin, which may explain its potent efficacy in managing melanin production *(1)*.

In vivo, its unique mode of action led to a high clinical performance either to prevent UV induced immediate skin darkening or inhibit delayed tanning with no adverse effects.

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