IFSCC 2025 full paper (357)

"The curious case of the MC1R-inhibiting depigmenting peptide: new hypothesis for pathway link with sensitive skin"

Wagner Vidal Magalhães[1], Lilian Mussi* [1], Edson Katekawa[1], Cleverson Rogerio Princival[1], Flavio Bueno de Camargo Junior[1], Giovana Padovani[1], Mateus Biasoto de Mattos Santiago[1], Natália Mencacci Esteves[1], Suzana Marques de Jesus[1], Vinicius Kuchenbecker[1], Mariana Mitie Yamamoto M. Guimarães[2]

[1]Research, Development & Innovation Department, Chemyunion Ltda., São Paulo, Brazil.
[2] Chemyunion Inc., Manalapan, New Jersey, USA.

*Lilian Mussi, Avenida Independência, 1501 – Iporanga – Sorocaba – SP – Brazil – CEP 18087-101, +55 15 99672-9955, lilian.mussi@chemyunion.com

1. Introduction

Hyperpigmentation of the skin is considered a common dermatological manifestation and a source of discomfort for the individuals it affects, since it significantly affects psychological well-being, contributing to lower productivity, social function, and self-esteem, impacting an individual's mental health, leading to issues such as depression and anxiety. Conversely, mental health disorders can exacerbate skin conditions, creating a cycle that can be challenging to break [1,2]. Hyperpigmentation is characterized by increased production and accumulation of melanin in the skin. The main factors responsible for this alteration are endocrine disorders, exposure to solar radiation, pregnancy, genetic factors, aging, and skin inflammation due to acne or contact dermatitis [3,4].

Despite the availability of numerous therapeutic options, many conventional depigmenting agents are limited by safety concerns, suboptimal efficacy, or pharmacotechnical constraints. Various treatments exist for skin pigmentation disorders, yet many present safety, efficacy, or formulation limitations. Common depigmenting agents such as hydroquinone, arbutin, and kojic acid are widely used due to their melanin-inhibiting properties. Hydroquinone, in particular, has been linked to disruption of extracellular matrix components (collagen and elastin), adrenal and thyroid dysfunction, and reduced skin firmness. It may also cause exogenous ochronosis and paradoxical hyperpigmentation [5]. Similarly, kojic acid presents dermatological risks including contact dermatitis, erythema, burning sensations, and increased photosensitivity. Moreover, *in vitro* studies suggest genotoxic potential through the induction of DNA single-strand breaks and point mutations in human lymphoblastoid cells, raising concerns about long-term safety at elevated concentrations or prolonged use [6,7]

Given these limitations, alternative molecular targets for the modulation of melanogenesis are actively being explored. Among these, the melanocortin 1 receptor (MC1R) has emerged

as a promising candidate. The MC1R plays a crucial role in melanogenesis, the process of melanin production in melanocytes, which is essential for skin pigmentation and protection against ultraviolet (UV) radiation. MC1R is a G-protein-coupled receptor (GPCR) that, upon activation by its ligands such as α-melanocyte-stimulating hormone (α-MSH), triggers a cascade of intracellular signaling pathways leading to increased melanin synthesis. MC1R is highly polymorphic, with several variants associated with different pigmentary phenotypes and skin types. Variants that result in loss of function are linked to fair skin, red hair, and increased sensitivity to UV radiation, which may affect the skin's response to MC1R antagonists. The receptor is also involved in DNA repair and reducing oxidative stress, which are crucial for protecting against UV-induced damage. Its inhibition by selective antagonists presents a highly promising approach in the management of hyperchromia [8-10].

In this study, a novel peptide was developed through molecular modeling, capable of modulating this receptor in a specific way. Palmitoyl Tetrapeptide-112 (T112) stood out as a potential MC1R antagonist. Surprisingly, a consistently more pronounced depigmenting effect was observed in individuals with sensitive skin (SK), raising new hypotheses about the link between this target and skin sensitivity.

2. Materials and Methods

1.Selection of MC1R antagonist candidates and peptide synthesis.

The MC1R antagonist candidates were selected through in silico simulation employing homology modeling and structure-based virtual screening, followed by cell assay. Palmitoyl Tetrapeptide-112 was obtained by Solid Phase Peptide Synthesis.

2.Total Depigmenting Capacity by *ex vivo* Fontana-Masson Technique

Eyelid fragments from elective blepharoplasty surgeries were obtained from healthy patients aged 35 to 70 at the Banco de Olhos do Hospital Oftalmológico de Sorocaba (BOS-HOS). The acquisition and utilization of these human skin explants were sanctioned by the HOS Ethics Committee, referenced under report number 3.065.484. The fragments, measuring 0.5 cm^2, were cultured for 72 hours prior to treatment with T112 (50 ppm) and a placebo. The application rate of the products to the skin was 12 mg/cm^2. The control group, fragments of human skin incubated in a culture medium, was assessed in parallel. Sections of 10 µm of the fragments were collected for silanized slides and then stained using the Fontana-Masson technique with a specific kit (Fontana-Masson Kit 4X, FM07223SO, Scientific Exodus). Melanin levels were analyzed using the Optical Microscope (Leica, DM6000B), accompanied by a 2.8 MP camera (Leica, DFC7000T). Images were captured using LAS v.4.12 software (Leica Application Suite). The images obtained were processed with ImageJ® software to semi-quantify the pixels generated by melanin pigment.

Variance analysis (ANOVA) was used for statistical analysis. The Dunnett's test is used when the analysis of variance detects significant comparative differences among groups. In all groups studied, it is considered statistically substantial those whose P values are equal to or less than 0.05.

3.Clinical assessment

A single-blind clinical trial was conducted with 41 Asian female participants, considering 20 volunteers/group, with half of the population affected by SK and equally distributed in the groups. The volunteers aged between 40 and 54, with Fitzpatrick skin phototype IV–V, self-

perceived dull skin, visible signs of pigmentation related to skin aging on both sides of the face, and moderate signs of periorbital aging. These subjects evaluated a formulation containing T112 (50 ppm) or a placebo over 84 days. The formulations were administered to the face region, bi-daily.

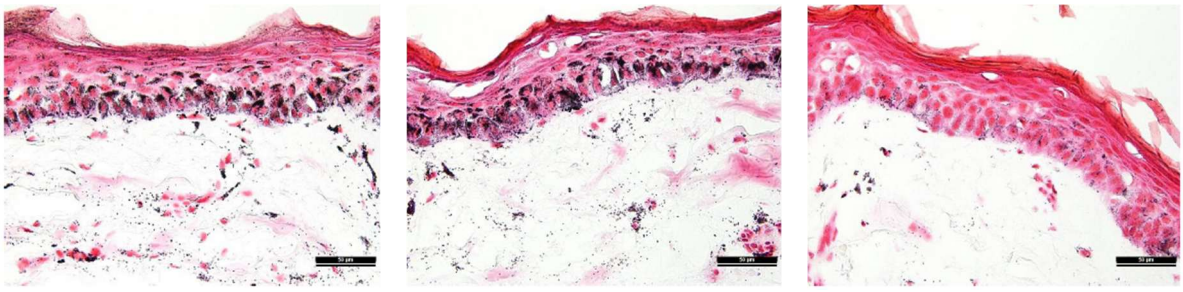
Assessments were conducted at baseline (Day 0) and after 84 days (D84) of product application. Facial pigmentation (L* value) and individual typology angle (ITA°) for skin tone were measured on the forehead (non-pigmented area) and cheeks (pigmented areas) using the SkinColorCatch™ device. Melanin index and erythema index were quantified using the Mexameter®. Standardized facial imaging photographs was taken using the VISIA-CR system. Data collected from each participant at the designated timepoint (D84) were normalized against baseline values (D0) for the entire cohort and subjected to statistical analysis for each measured parameter. Data normality was assessed using the Shapiro–Wilk test. Statistical differences between groups were evaluated using paired Student's t-test and independent samples t-test, or the Wilcoxon signed-rank test and Mann–Whitney U test, depending on the normality of the data distribution. Results were considered statistically significant when $p<0.05$.

The clinical protocol received ethical approval from The Ethics Committee of the Faculty of Medicine, University of Indonesia – Cipto Mangunkusumo Hospital (Jakarta, Indonesia). The study protocol was conducted in accordance with the principles of the Declaration Helsinki 64th WMA General Assembly (Brazil, 2013) and ICH Good Clinical Practice (GCP). All participants provided informed consent prior to their involvement in the study.

3. Results

Starting from an initial universe of about 50 thousand molecules, the Palmitoyl Tetrapeptide-112 *(*palmitoyl-L-glutaminil-L-histidyl-L-tryptofil-L-valine emerged as a potential antagonist of MC1R as a result *in silico* simulation employing homology modeling and structure-based virtual screening, followed by cell assay. Palmitoyl Tetrapeptide-112 was obtained by Solid Phase Peptide Synthesis - a protocol well described in the literature that involves a series of amino acid coupling reactions where the final residue is covalently bonded to a solid support. This synthesis strategy allows the obtaining of peptides of very high purity.

Palmitoyl Tetrapeptide-112 was submitted to an ex vivo evaluation to assess its depigmenting capacity. When applied at 50 ppm in a cosmetic formulation, T112 (50ppm) was able to significantly decrease the deposition of melanin (66%; $p<0.01$) compared to placebo (Figure 1).



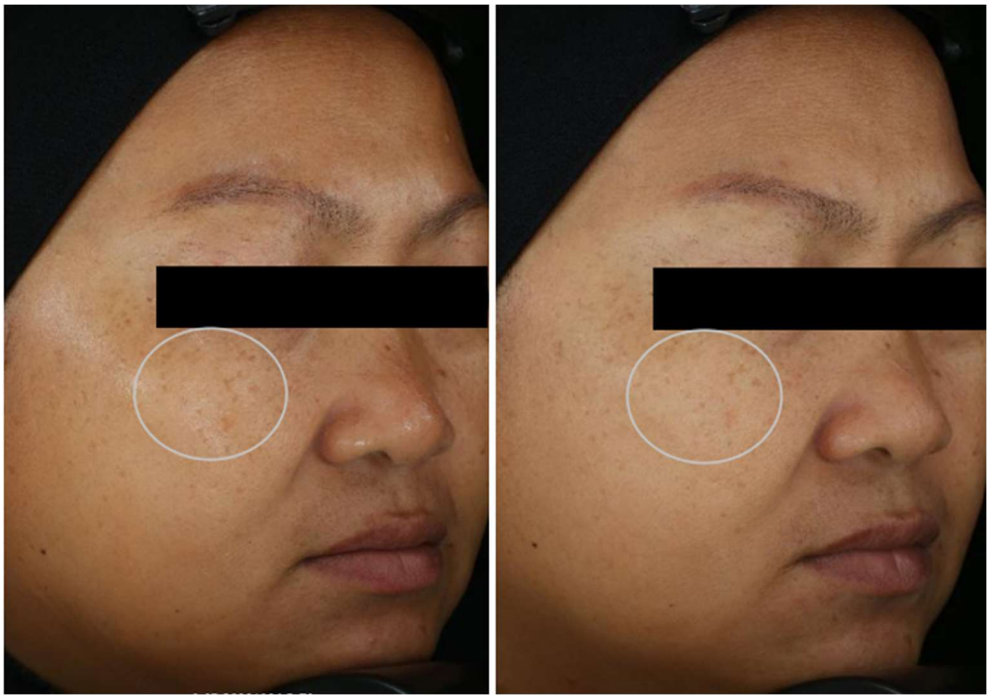
Control **Placebo** **T112 (50 ppm)**

Figure 1. Evaluation by Fontana-Masson, microscope images at 400x magnification. Human skin fragments (*ex vivo*) incubated in culture medium (Control), treated with Placebo, and test product T112.

Clinical findings, following an 84-day regimen applying T112 (50 ppm) cosmetic formulation, revealed noteworthy enhancements on the skin tone parameters ΔL* and ΔITA°, as well as, in Melanin index and Erythema index, in both non spotted area (NSA) and spotted area (SA), of the complete group of volunteers and also, more significantly, in the group of volunteers with sensitive skin (Table 1). The results suggest that T112 is a powerful alternative for treating skin dyschromias, as shown in Figure 2.

Table 1. Improvement in skin tone parameters, melanin index, and erythema index. (*p<0.05, **p<0.01; ***p<0.001; ****p<0.0001, related to D0).

		All Volunteers		Sensitive Skin Volunteers	
		Placebo	**T112**	**Placebo**	**T112**
ΔL*	NSA	+0.56%*	+1.28%****	+0.32%	+1.77%****
	SA	+0.35%	+0.80%****	+0.56%	+1.15%**
ΔITA°	NSA	+4.60%	+15.40%****	+6.80%	+23.60%****
	SA	+5.60%****	+9.90%****	+5.40%****	+13.60%****
Melanin index	NSA	-2.10%****	-4.40%****	-2.10%**	-5.50%****
	SA	-2.70%****	-5.50%****	-2.60%***	-6.20%***
Erythema index	NSA	-1.40%	-4.30%**	-1.10%	-6.00%**
	SA	-2.00%	-7.20***	+2.00%	-7,20%***



D0 **D84**

Figure 2. Photographic records showing the depigmentation effect at initial time (D0) and after 84-day treatment (D84) with a formulation containing T112 (50 ppm)

4. Discussion

The MC1R plays a crucial role in regulating melanogenesis, which is influenced by various factors, including genetic variations, environmental stimuli, and the presence of antagonists. The melanogenesis process mediated by the MC1R may differ between normal and sensitive skin, but this difference is not fully elucidated. In normal skin, MC1R is functionally active, responding to α-melanocyte-stimulating hormone (α-MSH) by promoting eumelanin synthesis, thereby providing effective photoprotection [11,12]. In contrast, individuals with sensitive skin may harbor MC1R variants with partial loss of function or altered α-MSH binding affinity, leading to reduced eumelanin production and diminished UV protection [13–15].

In normal skin, MC1R activation contributes to melanogenesis without inducing significant basal inflammation. Furthermore, α-MSH–MC1R interaction supports a balanced anti-inflammatory response [13,15]. Sensitive skin, however, often exhibits elevated baseline inflammation due to heightened immune reactivity. In this context, MC1R expressed in immune-related cells such as keratinocytes and fibroblasts may be more actively involved in modulating both pigmentation and inflammation. Chronic or exacerbated inflammation can dysregulate melanogenesis and increase susceptibility to MC1R antagonists. Therefore, while normal skin displays a balanced response to UV exposure and to cosmetic agents targeting melanogenesis (e.g., MC1R agonists or antagonists), sensitive skin tends to exhibit amplified or dysregulated responses due to its heightened reactivity.[11,15].

In this study, a MC1R antagonist was developed through molecular modeling. The Palmitoyl Tetrapeptide-112, a novel peptide, showed a significant reduction in melanin deposition when compared to placebo in an *ex vivo* study based on the Fontana-Masson Technique. Clinical assessment in Asian volunteers proved this potential to treat skin discromia by presenting outstanding improvement in L* value and ITA°, parameters related to skin tone, mainly in the SK group. Also, better results in the Melanin Index and Erythema Index were observed in the SK group compared to the complete group of volunteers.

The antagonism of MC1R represents a novel and targeted approach to managing hyperpigmentation. By inhibiting the MC1R pathway, melanin synthesis can be selectively downregulated without triggering cytotoxic or inflammatory responses typically observed with traditional depigmenting agents. This strategy may be particularly beneficial for populations with increased susceptibility to pigmentary disorders, such as Asian individuals, who often present with post-inflammatory hyperpigmentation and heightened cutaneous sensitivity [16-18]. As such, the development of MC1R antagonists holds significant promise as a safer and more physiologically compatible alternative to classical agents in the treatment of hyperchromia.

5. Conclusion

This study highlights Palmitoyl Tetrapeptide-112 (T112) as a promising MC1R antagonist for the treatment of skin dyschromias. The peptide demonstrated significant depigmenting effects both *ex vivo* and in clinical trials, particularly among individuals with sensitive skin—a group often unable to tolerate conventional lightening agents. This report associates MC1R antagonism with improved outcomes in sensitive skin within an Asian population, positioning T112 as a safe, effective, and targeted alternative to traditional depigmenting agents, offering a novel and physiologically compatible approach to hyperpigmentation management.

6. References

1. Rainieri GV, Machado MCL, Yonemoto ACF, Verona AV, Sartorelli VP, Silva SR da, Zanoni RD (2024) "The connection between mental health and dermatology: Exposing the

links between psychiatric disorders and dermatopathies". https://doi.org/10.56238/isevjhv3n2-018
2. Paucar Lescano PK (2018) "Relación bidireccional de las patologías cutáneas con los trastornos mentales". "Revista Científica Ciencia Médica" 21(1):84–89. https://doi.org/10.51581/RCCM.V21I1.96
3. Nandraj O, Gajanan A, Karande KM (2024) "A Concise Review on Contemporary and Novel Treatments Addressing the Prevention and Control of Hyperpigmentation". "Asian Journal of Pharmaceutical Research and Development". https://doi.org/10.22270/ajprd.v12i2.1363
4. Nouveau S, Agrawal D, Kohli M, Bernerd F, Misra N, Nayak CS (2016) "Skin Hyperpigmentation in Indian Population: Insights and Best Practice". "Indian Journal of Dermatology" 61(5):487–495. https://doi.org/10.4103/0019-5154.190103
5. Ishack S, Lipner SR (2021) "Exogenous ochronosis associated with hydroquinone: a systematic review". "International Journal of Dermatology". https://doi.org/10.1111/IJD.15878
6. Nakagawa M, Kawai K (1995) "Contact allergy to kojic acid in skin care products". "Contact Dermatitis" 32(1):9–13. https://doi.org/10.1111/J.1600-0536.1995.TB00832.X
7. Kawaguchi S, Nakamura T, Honda G, Sasaki YF (2007) "Evaluation of in vitro Genotoxic Potential of Kojic Acid in Human Lymphoblastoid Cells". "Genes and Environment" 29(4):153–158. https://doi.org/10.3123/JEMSGE.29.153
8. Herraiz C, Martínez-Vicente I, Maresca V (2021) "The α-melanocyte-stimulating hormone/melanocortin-1 receptor interaction: A driver of pleiotropic effects beyond pigmentation". "Pigment Cell & Melanoma Research" 34(4):748–761. https://doi.org/10.1111/PCMR.12980
9. García-Borrón JC, Abdel-Malek ZA, Jiménez-Cervantes C (2014) "MC1R, the cAMP pathway, and the response to solar UV: extending the horizon beyond pigmentation". "Pigment Cell & Melanoma Research" 27(5):699–720. https://doi.org/10.1111/PCMR.12257
10. Nasti TH, Timares L (2015) "MC1R, Eumelanin and Pheomelanin: Their Role in Determining the Susceptibility to Skin Cancer". "Photochemistry and Photobiology" 91(1):188–200. https://doi.org/10.1111/PHP.12335
11. Böhm M, Luger TA, Steingräber AK, Goerge T (2013) "Is MC1 dispensable for regulation of cutaneous inflammatory and immune responses?". "Experimental Dermatology" 22(12):792–794.
12. Jackson E, Heidl M, Imfeld D, Meeus L, Schuetz R, Campiche R (2019) "Discovery of a highly selective MC1R agonists pentapeptide to be used as a skin pigmentation enhancer and with potential anti-aging properties". "International Journal of Molecular Sciences" 20(24):6143.
13. Chen J, Li H, Liang B, Zhu H (2022) "Effects of tea polyphenols on UVA-induced melanogenesis via inhibition of α-MSH-MC1R signalling pathway". "Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii" 39(2):327–335.
14. Chen W, Li J, Qu HE, Song Z, Yang Z, Huo J, Zhang Q (2013) "The melanocortin 1 receptor (MC1R) inhibits the inflammatory response in Raw 264.7 cells and atopic dermatitis (AD) mouse model". "Molecular Biology Reports" 40:1987–1996.

15. Mun Y, Kim W, Shin D (2023) "Melanocortin 1 receptor (MC1R): Pharmacological and therapeutic aspects". "International Journal of Molecular Sciences" 24(15):12152.
16. Chan IL, Cohen S, Gonzaga da Cunha M, Maluf LC (2019) "Characteristics and management of Asian skin". "International Journal of Dermatology" 58(2):131–143. https://doi.org/10.1111/IJD.14153
17. Ho SGY, Chan HH (2009) "The Asian dermatologic patient: review of common pigmentary disorders and cutaneous diseases". "American Journal of Clinical Dermatology" 10(3):153–168. https://doi.org/10.2165/00128071-200910030-00002
18. Mar K, Khalid B, Maazi M, Ahmed R, Wang OJ, Khosravi-Hafshejani T (2024) "Treatment of Post-Inflammatory Hyperpigmentation in Skin of Colour: A Systematic Review". "Journal of Cutaneous Medicine and Surgery". https://doi.org/10.1177/12034754241265716