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CONCISE COMMUNICATION

Cytidine decreases melanin content in a reconstituted three-dimensional human epidermal model

Sudhir M. Baswan[1] · Sunghan Yim[1] · Jesse Leverett[1] · Jeff Scholten[1] · John Pawelek[2]

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Abstract

The process of melanin biosynthesis and its distribution throughout the skin is regulated by complex processes involving several enzymes in melanocytes. Recently, Diwakar et al. demonstrated that cytidine—a sialyltransferase inhibitor, 6′-sialyllactose (6′-SL) and 3′-sialyllactose (3′-SL) inhibited melanogenesis and melanosome transfer process. In this study, we have furthered this research, considering cytidine as a commercially viable and safe option over 6′-SL and 3′-SL. The efficacy of 2% w/v cytidine was studied in MelanoDerm™ skin equivalents in comparison with the positive control 1% w/v kojic acid and the vehicle control. Both the positive control and cytidine demonstrated a significant reduction in melanin content relative to the vehicle control. These experiments conclude that cytidine can effectively reduce melanin content in a skin equivalence assay and suggests that cytidine may be a good candidate for a skin lightening agent for human skin.

Keywords Cytidine · MelanoDerm™ · Skin lightening · Melanin · Pigmentation · Reconstituted three-dimensional human skin equivalent

Introduction

In 2015, Diwakar et al. reported the significance of the role of protein glycosylation in melanogenesis and melanosome transfer, and each was shown to be inhibited by the nucleoside cytidine, a sialyltransferase inhibitor [1]. In this study, we aimed to test the melanin inhibition efficacy of cytidine in a reconstituted three-dimensional human skin equivalent at a clinically relevant concentration.

Materials and methods

Cytidine was obtained from Shanghai Sharing Technologies Co. Ltd (Shanghai, China), with a purity of ≥ 99%. The MelanoDerm™ skin equivalents (Mattek Corp, Ashland, MA, USA) derived from African American skin were procured from Mattek Corp (Ashland, MA, USA). The MelanoDerm™ tissues reconstructed from the neonatal cells of African American donors were used because of its ability to produce more melanin relative to other skin type donors (Asian or Caucasian), therefore, generating a higher sensitivity for the test method. The tissues were placed in an incubator containing 5% CO_2 at 37 °C. After 3 h, 25 µL of the vehicle control (90% PBS + 5% EtOH + 5% propylene glycol), the compound for testing (2% cytidine in vehicle solution), and positive control (1% Kojic acid in vehicle solution) were added topically to the skin equivalents. Media was changed every other day for 2 weeks. The tissues were then collected for melanin extraction and a cytotoxicity assay. The melanin was extracted via a chloroform/methanol method [2]. The melanin content was measured in a spectrophotometer at 405 nm using the standard linear curve of melanin [3]. Cytotoxicity was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay in duplicates [4].

Results and discussion

The effect of cytidine on melanin production was compared with both vehicle control and kojic acid, which is widely used as a de-pigmenting agent. Topical application of 2%

✉ Sudhir M. Baswan
sudhir.baswan@amway.com

[1] Amway Corporation, 7575 Fulton St E, Ada, MI 49355, USA

[2] Department of Dermatology, Yale School of Medicine, New Haven, CT 06520-8059, USA

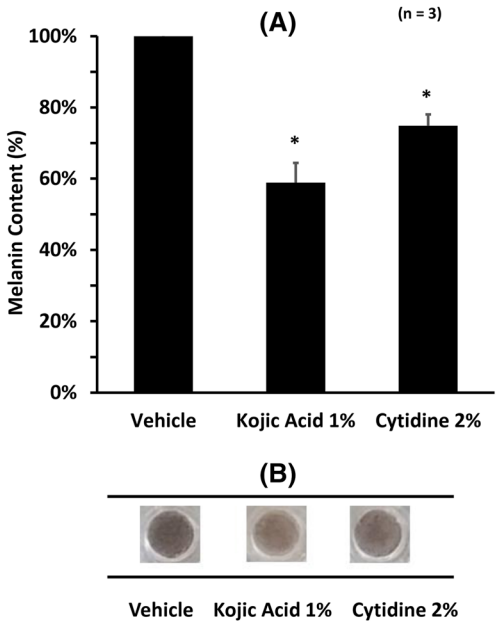

Fig. 1 MelanoDerm™ tissues were treated with vehicle control, 1% kojic acid, and 2% cytidine for 14 days in triplicates. **A** Graph demonstrating changes in melanin content. Data are presented as the percentage control (*$p < 0.05$), **B** Macroscopic images of the MelanoDerm™ on day 14

cytidine significantly decreased the melanin content by about 25% relative to the vehicle control ($p < 0.05$) (Fig. 1A). As expected, application of 1% kojic acid significantly decreased the melanin content by about 41% ($p < 0.05$). There was a visible difference in pigmentation after the treatments, compared with the vehicle control (Fig. 1B). This was consistent with the melanin content results, suggesting that cytidine could be an effective de-pigmenting agent. No cytotoxicity was observed with either with cytidine or kojic acid (data not shown).

Beyond the synthesis of melanin in melanosomes, cutaneous pigmentation is regulated by several other processes, including the transfer of melanosomes from melanocytes to keratinocytes and subsequent processing of melanosomes in keratinocytes [4]. It was shown that cytidine is not a tyrosinase inhibitor; however, it can reduce melanin content by interfering with post-tyrosinase pathways and melanosome transfer to keratinocytes [1]. In conclusion, this study confirmed that cytidine can effectively reduce melanin content in a skin equivalence assay, and suggests that cytidine may be a good candidate for a skin lightening agent for human skin.

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Compliance with ethical standards

Conflict of interest The authors SMB, SY, JL, and JS are employees of Amway Corporation which has applied for patents for the commercial applications of cytidine.

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