

A TOPICAL FORMULATION CONTAINING L-TYROSINE DOES NOT INTENSIFY PIGMENTATION IN HUMAN SKIN

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Summary

Topical tyrosine has been proposed as a tanning accelerator because tyrosine is a precursor of melanin. We tested formulations containing 0, 1, and 2% L-tyrosine topically on 14 individuals daily for 28 days. Percutaneous absorption using ¹⁴C tyrosine was 1.23% in 24 hr. No enhanced pigmentation was clinically evident in any of the subjects. In this study, we were unable to demonstrate that topical tyrosine enhanced pigmentation.

Riassunto

E' stato proposto di utilizzare la tirosina per uso topico quale mezzo per accelerare la pigmentazione, essendo questo aminoacido il precursore della melanina. Abbiamo controllato l'attività di alcune formulazioni cosmetiche contenenti dallo 0,1 al 2% di l-tirosina applicandole ogni giorno per 28 giorni su 14 soggetti volontari. Utilizzando Tirosina marcata ¹⁴C, l'assorbimento percutaneo è stato dell'1,23% in 24 ore. Su nessuno dei soggetti trattati è stato ottenuto aumento della pigmentazione rispetto ai controlli. Non è stato così possibile dimostrare che la l-tirosina incrementi la pigmentazione, se utilizzata topicamente.

BACKGROUND

Tyrosine is a well studied non-essential amino acid that has many roles which alter body functioning.

It is a precursor to several hormones such as norepinephrine, epinephrine, and thyrosine (1). Tyrosine has been reported to decrease blood pressure (2), help patients with depression (3), and decrease prolactin levels (4). Tyrosine is the major building block of melanin. The creation of melanin begins in melanosomes where tyrosinase oxidizes L-tyrosine to L-dihydroxyphenylalanine (5). Tyrosinase is the rate limiting enzyme in this process (6). Tyrosinase is activated by UV irradiation resulting in two pigment types: red-yellow pheomelanin and brown-black eumelanin. Eumelanin production correlates with darker skin and a greater ability to tolerate the damaging effects of ultraviolet radiation (7).

L-Tyrosine has been demonstrated to be a rate limiting substrate for melanin synthesis (8). When tyrosine is added to human melanocytes in culture, melanin synthesis is increased (9). Similar stimulatory effects of tyrosine have been observed in cultures of human (10) and hamster (11) melanoma cells.

Since tyrosine is a rate limiting substrate for melanogenesis, claims have been made that topical tyrosine administration to skin would increase melanogenesis. Indeed tyrosine containing cosmetic preparations are promoted to induce pigmentation.

In our study we have used human subjects to determine the effects of tyrosine on melanogenesis when applied topically to skin. Men and women were recruited of Fitzpatrick skin types II-V to test the hypothesis that tyrosine would augment melanogenesis. In this paper we conclude that L-tyrosine in the formulation described below when applied topically to human skin does not increase pigmentation.

METHODS

Solutions were prepared by one of us (MO) and contained 10% ascorbic acid, 2% zinc sulfate, 0,5% bioflavinoids in an aqueous solution (pH 2.3) and either 0,1% or 2% tyrosine. Solutions were coded so that neither subject nor investigator knew their identities.

14 subjects were recruited from the Duke Dermatology Division. They ranged in age from 25 to 56 (mean 49 ± 2.7 SEM). They were of Fitzpatrick skin type II (3), III (6), IV (3) and V (2). Subjects were instructed to apply one drop daily of each of the coded solutions to a designated spot on the volar forearm for 28 days. All subjects completed at least 21 applications except for three subjects who completed 6, 14, and 18 applications respectively.

In order to confirm cutaneous tyrosine delivery to the skin after topical dosing, *in vitro* percutaneous absorption studies were conducted. Product was formulated containing 10% ascorbic acid, 2% zinc sulfate, 0,5% tyrosine and 0,5 bioflavinoids. ^{14}C L-Tyrosine ($20\mu\text{Ci/ml}$) was added to the final formulation. This formulation was doses on to dermatomed ($500\mu\text{m}$) porcine skin ($n=4$ replicates) and perfused in a flow through diffusion cell system for 24 hours as described previously (12). Perfusate samples were collected during the experiment as well as the dosed skin at termination. Samples were combusted in a tissue oxidizer and then analyzed in a liquid scintillation counter for total ^{14}C determination.

RESULTS

Percutaneous Absorption

Radioactivity corresponding to tyrosine crossed the skin barrier at a level of $1.23\% \pm 0.17$ (SEM) of the applied dose.

Melanogenesis

None of the solutions produced any visual variation in skin pigmentation in any of the subjects in the study. Since the sensitivity of visual inspection is so great, spectrophotometric measurements were not undertaken.

CONCLUSIONS

In this study, a topical formulation containing tyrosine applied topically to the forearms of male and female subjects with Fitzpatrick skin types II-V failed to induce any evidence of hyperpigmentation. So far as we are aware this is the first study of topical tyrosine reported in human subjects.

It has previously been demonstrated the topical tyrosine administration to mouse skin daily for four weeks did not cause pigment enhancement as measured spectrophotometrically or histologically (5). Tyrosine ingestion in mice has also been investigated as a method for increasing skin tyrosine levels and subsequent pigment enhancement (6). No increased melanin synthesis was observed with or without irradiation.

It has been clearly demonstrated that L-tyrosine can enhance pigment production in human (10) and hamster (11) melanoma cells as well as human melanocytes (9) in culture. Moreover, L-tyrosine has been demonstrated to increase melanosome synthesis in hamster melanoma cells (11). High concentrations of tyrosine have been shown to preferentially induce pheomelanin synthesis in human melanocytes (9).

Why then does it appear that topical tyrosine does not stimulate pigment production in skin? In order for tyrosine to have an effect it must first traverse the stratum corneum barrier. Percutaneous absorption studies using dermatomed porcine skin indicated adequate delivery of tyrosine into skin. Once in skin tyrosine must get into melanocytes. Tyrosine is transported into

cells by both sodium dependent and sodium independent systems (13). Its uptake can be blocked by excess phenylalanine apparently accounting for the reduced pigmentation observed in persons with phenylketonuria (13). Excess tyrosine may even be toxic to pigment producing cells (14). During melanin synthesis production of the intermediate 5,6-dihydroxyindole can cause cytotoxicity. Finally, if cellular tyrosine levels are adequate in human skin, melanogenesis can proceed at optimal levels. Indeed, human epidermal melanocytes and keratinocytes adequately synthesize L-tyrosine from L-phenylalanine and do not depend on transport (15).

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