D-penicillamine, a potent melanogenesis inhibitor, lacks any depigmenting effect on black guinea pig skin: The first randomized, evaluator-blinded, vehicle-controlled, in vivo study

M. Sharifian, F. Sari-Aslani, B. Hemmatinejad, M. K. Fallahzadeh, B. Kasraee, M. J. Khoshandish, R. Miri, S. Mohammadi-Samani, F. Jowkar, M. R. Namazi

KEY WORDS

D-penicillamine, depigmentation, tyrosinase, melanin

ABSTRACT

D-penicillamine is a melanogenesis inhibitor. This in vivo study on ten black guinea pigs using a 5% D-penicillamine ointment showed its lack of any skin-lightening effect. The potential reasons for this ineffectiveness are discussed in the paper, which could be very helpful for researchers exploring new skin-lightening agents.

Eumelanin derives from the precursor dopaquinone (ortho-quinone of 3,4-dihydroxyphenylalanine), which is formed via the oxidation of L-tyrosine by the melanogenic enzyme tyrosinase. Dopaquinone is a highly reactive ortho-quinone that plays pivotal roles in the chemical control of melanogenesis. Dopaquinone undergoes intramolecular cyclization to form cyclodopa, which is then rapidly oxidized by a redox reaction with dopaquinone to give dopachrome (and dopa). Dopachrome then gradually and spontaneously rearranges to form 5,6-dihydroxyindole and to a lesser extent 5,6-dihydroxyindole-2-carboxylic acid, the ratio of which is determined by dopachrome tautomerase (tyrosinase-related protein-2). Oxidation and the subsequent polymerization of these dihydroxyindoles leads to the production of eumelanin (1).

Penicillamine is the drug of choice for the treatment of Wilson's disease due to its copper-chelating effect. This drug is a cysteine, doubly substituted with methyl groups (Figure 1). A free sulphydryl group acts as the copper-chelator. Penicillamine also induces the production of metallothionein, an intracellular cysteine-rich protein that is an endogenous chelator of metals (2). Tyrosinase, one of the essential enzymes in the synthesis of melanin, needs copper for its proper function. D-penicillamine, by chelating copper ions, can inhibit the activity of tyrosinase (3).

Moreover, penicillamine is a one-electron donor and prevents the production of dopachrome by reducing an oxidation product of dopa, probably a free radical. During the reaction the -SH group of penicillamine is oxidized (4). This can further contribute to its melanogenesis-inhibitory effect. Because nonpigmented melanoma cells are much more sensitive to gamma radiation and, given the melanogenesis inhibitory effect of D-penicillamine, this agent has recently been employed in vitro as a radiation sensitizer for killing melanoma cells (5). Penicillamine has also

been shown to inhibit melanoid pigment production by trichophyton species (6).

This study was conducted to evaluate the skin-lightening effect of topical D-penicillamine. Ten black guinea pigs were randomly divided into two groups of five pigs, groups A and B. Five-percent D- Penicillamine ointment with Eucerin as the base was made and applied on the right ears of group A pigs and left ears of group B pigs twice daily for three months. The other ears received the vehicle (Eucerin) as a placebo. The pigs' ears were evaluated for any color change weekly by two observers blinded to the treatment identification. Contrary to expectations and the strong theoretical grounds, no color change was detected by observers at any point in the study. Microscopic examination of drug- and placebo-treated sites using H & E and Masson-Fontana's stains also revealed no noticeable change.

Although D-penicillamine is a melanogenesis inhibitor, our study, which is the first in vivo study on the subject, did not reveal any lightening effect of this agent. The reason for the negative result of our study despite the strong theoretical background could be insufficient absorption of D-penicillamine through the stratum corneum. Nonpolar molecules lighter than 500 Dalton are believed to penetrate well through the stratum corneum, which is the major skin barrier (7), and D-penicillamine is a small molecule of 149.21 Dalton molecular weight. However, because D-penicillamine possesses both acidic and basic groups and can produce different ionic species depending on the pH, we surmise that in the acidic pH of mammalian skin it is highly polar and is thus unable to penetrate the skin well.

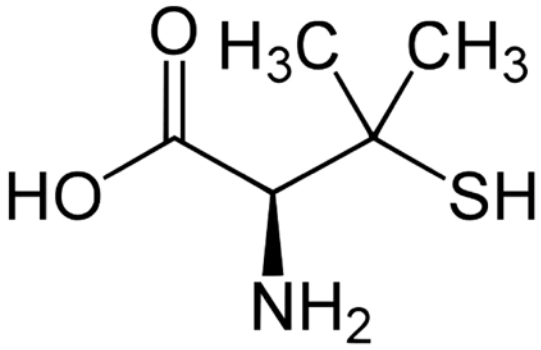

Figure 1. Chemical structure of D-penicillamine.

Given the results of our study, we discourage future in vivo and human studies on this agent for skin lightening and suggest, for the first time, that even small molecules, if having an amino group, do not penetrate well into the skin due to polarization by absorbing a proton in the acidic pH of the skin surface.

Acknowledgement

We thank Iran's National Elite Organization for its support.

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AUTHORS' ADDRESSES

Mohammad R. Namazi, Shiraz Skin Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, corresponding author, E-mail: namazi_mr@yahoo.com

Maryam Sharifian, Shiraz Skin Research Center, same address

Fatemeh Sari-Aslani, Pathology Department, same address

Bahram Hemmatinejad, Pharmacy School, same address

Mohammad K. Fallahzadeh, Shiraz Health Policy Research Center, same address

Mohammad J. Khoshandish, Dermatology Department, same address

Ramin Miri, Pharmacy School, same address

Soleyman Mohammadi-Samani, Pharmacy School, same address

Farideh Jowkar, Dermatology Department, same address

Behrooz Kasraee, Department of Dermatology, Geneva University Hospital, Geneva, Switzerland