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Hypothesis Letter

Are tyrosinase inhibitors in sunscreens and cosmetics enhancing UV carcinogenicity?

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Background

Melanin is the pigment synthesized in the skin responsible for the adaptive pigmentation in humans which protects from the dangerous and possible carcinogenic effects of UV rays present in the sunlight (1). The main enzyme synthesizing melanin in the skin melanocytes is tyrosinase whose natural substrate is tyrosine, but can accept as substrates a large number of aromatic compounds (S1) which may interfere with the production of melanin.

Premises

A larger number of aromatic and non-aromatic compounds inhibit competitively or not competitively melanin activity reducing or abolishing the melanin formation, reducing or preventing the sun tanning of the skin (2). The vast majority of the organic UV absorbers present in the sunscreens are aromatic compounds (AC), and about one-third are phenols present at high concentration between 4 and 15% (www.tga.health.gov.au/docs/html/argom.htm). AC are frequently present also in other cosmetics, in drugs and, in general, in products with which humans are in contact daily. Most of these compounds were not properly tested for their effects on tyrosinase, and particularly intriguing is their possible presence in sunscreen products. Salicylic acid (and presumably salicylates), para-aminobenzoic acid (PABA) and benzoic acid, largely present in sunscreens or other skin products, are in fact established tyrosinase inhibitors (S2–S4).

Hypothesis

In the last years, many researchers have epidemiologically studied whether sunscreen use influences the malignant melanoma (MM) incidence upon sun exposure. Surprisingly, most of the researchers (3–5; S5–S11) observed that sunscreen users have a higher incidence of MM than non-users although at present a considerable controversy exists. Huncharek and Kupelnick (6), in a meta-analysis of these papers, have concluded that sunscreen use does not increase nor diminish the MM incidence, a rather unexpected conclusion given that sunscreens are supposed to filter UV light which is claimed as the main cause of MM incidence.

In a previous paper (7), we have suggested that these hard to explain epidemiological data may be explained by the presence in sunscreens and in other cosmetic products of tyrosinase inhibitors impairing the melanin synthesis which shields the skin from the dangerous action of UV rays (Fig. 1). We have therefore decided to test ten aromatic organic UV absorbers present in sunscreen formulations and cosmetics. It may be seen in Fig. S1 that five of the ten organic UV absorbers tested are almost insoluble in water, and therefore, their activity on tyrosinase cannot be appreciated in our experiments and their activity *in vivo*, in which the conditions are very different, remains unknown. Three of the five remaining compounds, Uvinul® D50, Uvinul® M40 and Uvinul® MS40 (BASF, Ludwigshafen, Germany), are tyrosinase inhibitors. It is noteworthy that Uvinul® D50 is one of the most potent tyrosinase inhibitors ever tested with a potency comparable to that of 4-n-butylresorcinol (Fig. 2a,b). The other two are less potent and inhibit tyrosinase at micromolar and millimolar concentrations (Fig. 2c,d). Uvinul® MS40 suppressed tyrosinase activity of approximately 33% at 1 and 3 mM.

Our data demonstrate that other compounds, beside those already reported, that are PABA, salicylic acid and benzoic acid, may inhibit tyrosinase presumably hampering tanning. Two of the organic UV absorbers, Uvinul® M40 and MS40, are used prevalently in the sunscreen products at high concentration. The third product, Uvinul® D50, is not present in the sunscreens, at least in USA and Europe as it was not authorized by the Food and Drug Administration and the European authorities because it has potent oestrogenic effects (S12), but incredibly it is present in an enormous number of cosmetics, soaps, shampoos, etc. and therefore may be used daily for whole life. Uvinul® D50 is incredibly potent, few micrograms inhibiting totally the tyrosinase.

Even if they are effective in filtering the UV rays, the likely inhibition of the melanin synthesis in the skin is probably harmful

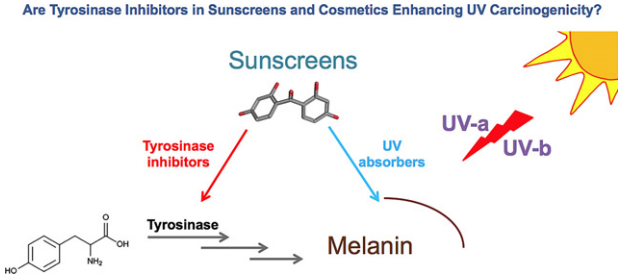

Figure 1. Cartoon showing the hypothesized double role of organic UV absorbers.

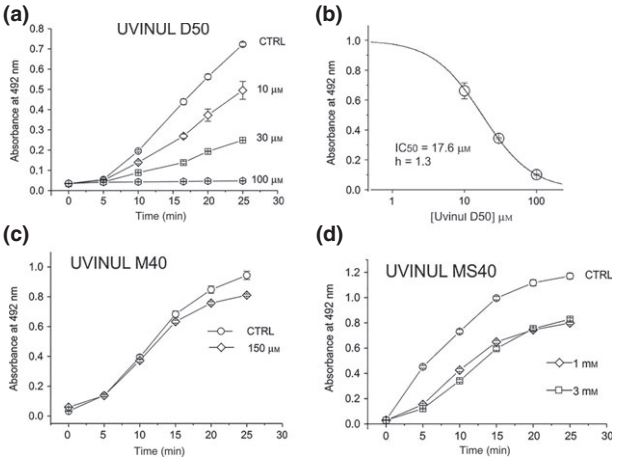

Figure 2. (a) Inhibition of tyrosinase by different concentrations of Uvinul® D50. (b) Dose-inhibition relationship of tyrosinase activity estimated at 20 min. The line represent the best fit of the data with Hill equation Abs norm = 1/(1 + ([Uvinul® D50]/IC_{50})^h), where IC_{50} and h represent the concentration the block half of tyrosinase activity and the Hill coefficient, respectively. (c) Inhibition of tyrosinase by Uvinul® M40. (d) Inhibition of tyrosinase by Uvinul® MS40 at different concentrations. L-Tyrosine oxidation by tyrosinase was spectrophotometrically determined as previously described with minor modifications [S13]. 150 µl of 5 mM L-tyrosine in 67 mM sodium phosphate buffer (pH 6.8) was mixed with 50 µl of the same buffer with or without the compound to test in a 96-well plate. The reaction was started by further added 50 µl (150 U/ml) of mushroom tyrosinase (Sigma-Aldrich, St. Louis, MO, USA). Dopachrome formation from the reaction mixture was determined as the increase of absorbance at wavelength 492 nm per min (Δ*A* 492/min) by using a Molecular Devices microplate reader every five minutes for 120 min. All compounds used in the assay (Fig. S1) were dissolved in DMSO and diluted to the finals concentrations of 50 and100 µM with exception UVINUL MS40 that was dissolved in water. The final DMSO concentration in the assay was always <3%.

increasing the genetic damage produced by UV rays and contributing to the continuous raising trend of MM. This can be counterintuitive if considering a possible compensatory effect due to the sunscreen, but it is realistic to hypothesize that the compensatory effect is not complete. Due to the long human evolution pathway (8), skin melanin should have, at least in theory, a much higher efficacy (UV absorbing and protecting properties) than anthropogenic sunscreens.

How to test the hypothesis

The relationship between melanoma and sunscreen use is difficult to make. This hypothesis would be far less speculative if *in vivo* data would be available. First of all, the real concentration of tyrosinase inhibitor sunscreens at the level of the basal lamina should be measured using microdialysis, and then, the effect of these compounds on melanoma induced by carcinogenesis protocols should be tested in mice.

Relevance and perspectives

Sunscreens are devised to protect us from the harmful effects of UV rays: it seems incredible and paradoxical that these products are not tested to evaluate their activity on tyrosinase, the enzyme that provides us with the natural protection afforded by melanin. It is our opinion that its use should be banned in any product that may enter in contact with human skin. Alternatively, advanced formulation strategies, which contemplate the organic UV absorbers encapsulation or inclusion with no or minimal release, should be strongly promoted. These novel formulation approaches permit to reduce or completely eliminate the contact between these molecules and the skin avoiding absorption. Encapsulated sunscreens remain efficient UV absorbers on the skin surface (9,10).

Authors' contributions

Giorgio Morpurgo (Y, B), Luigi Catacuzzeno (Y), Sara Peruzzi (X, A), Paolo Blasi (Z, B) and Bernard Fioretti (X, Y, B).

Conflict of interests

The authors have declared no conflicting interests.

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Supporting Information

Additional supporting data may be found in the supplementary information of this article.

Appendix S1. References.

Figure S1. Names, molecular formula, molecular weight, solubility and chemical structure of the 10 compounds mentioned in the study.

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Letter to the Editor

Xenobiotic metabolizing enzymes in human skin and SkinEthic reconstructed human skin models

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Abstract: Skin metabolism is becoming a major consideration in the development of new cosmetic ingredients, skin being the first organ exposed to them. In order to replace limited samples of Excised human skin (EHS), *in vitro* engineered human skins