Original Article

The effect of H_1 and H_2 receptor antagonists on melanogenesis

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ABSTRACT

Background: Histamine was found to stimulate melanogenesis in cultured human melanocytes specifically mediated by histamine H_2 receptors via protein kinase A activation. Based on this finding, the effect of topically applied H_2 antagonist on UVB-irradiated Guinea pigs' skin was examined and found to be suppressive on the post-irradiation melanogenesis. **Aims:** In this study, we tried to explore the role of topically applied H_1 and H_2 receptor antagonists, in inhibition of UVB-induced melanization. **Methods:** The effect of topically applied H_1 and H_2 receptor antagonists in inhibition of melanization was done clinically and histochemically using Fontana Masson and DOPA reactions compared with placebo. **Results:** The post-irradiation pigmentation was found to be brownish/black instead of the original light brown color. This color change occurred below the shaved orange-red fur suggesting a switch of melanogenesis from pheomelanin to eumelanin. The induced pigmentation was suppressed by topically applied H_2 antagonist while both H_1 antagonist and vehicle had no effect. The microscopic examination showed that the keratinocytes in the H_2 antagonist-treated areas contained few melanosomes while the nearby dendrites are full of them. **Conclusion:** H_2 antagonists' inhibition of UVB-induced pigmentation is not only due to suppression of melanization but also due to a specific action on melanosomes' transfer.

Key words: H_1 blocker, H_2 blocker, histamine, melanocytes, melanosome transfer, melanogenesis

INTRODUCTION

In human skin, there is increased histamine release by dermal mast cells and keratinocytes after ultraviolet (UV) radiation.[1,2] These findings, together with the discovery of the existence of H_1 and H_2 receptors on the surface of human melanoma cells and melanocytes,[3] strongly suggested the involvement of histamine in UV-induced pigmentation.

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Histamine was found to stimulate melanogenesis in cultured human melanocytes specifically mediated by histamine H_2 receptors. Based on this finding, the effect of topically applied H_2 antagonist on ultraviolet B (UVB) irradiated Guinea pigs' skin was examined and found to be suppressive on the post-irradiation melanogenesis. Yoshida *et al.*[4] found that the melanogenic activity of histamine in cell cultures is specifically mediated by H_2 receptors through protein kinase A activation, while H_1 and H_3 antagonists had no role whatsoever. This melanogenic activity was blocked by H_2 antagonists in their cell cultures. Accordingly, the same group of authors confirmed this blocking effect in Guinea pigs' skin clinically and histologically.[5]

On the other hand, the inflammatory factors such as prostaglandins and nitric oxide which are mediated through H_1 receptors in the skin[6,7] were found to play

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an important part in triggering melanin synthesis in UV-induced pigmentation.[2,8] The degree of erythema induced by these inflammatory factors in the irradiated regions parallels the intensity of pigmentation and hence the suppression of erythema leads to a decrease of pigmentation.[9] Thus, despite that H_1 receptor was proven to have no role in inhibiting melanization in culture study, yet it still can have its effect *in vivo* through enhancing erythema formation as described above. Meanwhile, to the best of our knowledge, this theoretical role has not been explored yet.

In an animal model, we tried to confirm the role of H_2 receptor antagonism in inhibiting melanization and to examine if there is any effect of H_1 receptor antagonists on melanization clinically, histologically, and histochemically.

METHODS

The study was approved by Al-Minya University Committee concerned with the approval of the researches from the scientific and ethical points of views.

This study involved five female Guinea pigs with a patchy white and red/brown fur. Their hair was shaven from three areas on the dorsal skin of each animal in the red/brown patches using an electric shaver to avoid injuring the epidermis.

The animals were exposed to sessions of narrowband-UVB (NB-UVB) irradiation twice weekly for 2 weeks at a dose of 0.52 J/cm^2 per session using a NB-UVB unit consisting of 8 NB fluorescent tubes (Philips TL 100W/01 from Philips B.V) with a spectrum of 310 to 315 nm and a maximum wave length of 311 nm installed in a Waldman UV- 100 L unit (from Waldmann GmbH).

The three shaved areas in all the Guinea pigs were treated during the course of irradiation as follows:

Area 1: used as a control area and was exposed to NB-UVB and treated with cream base only twice daily.

Area 2: treated with topical H_1 antagonist (1.5% chlorphenoxamine hydrochloride in the same cream base, Allergex, Epico) twice daily.

Area 3: treated with topical H_2 antagonist (2% famotidine in the same cream base) twice daily.

It is worthy to note that on days of irradiation, the topical applications were done following the UV exposure to exclude their barrier effect.

The three skin areas were photographed before and after the irradiation sessions to record the changes of skin color. Evaluation of the color changes in the three areas in each animal was done clinically by two independent blinded dermatologists.

Punch biopsies were taken from the three areas in each animal for histopathological examination using routine hematoxylin and eosin (H and E) and Masson-Fontana silver stains and histochemical examination using 3,4-dihydroxyphenylalanine (DOPA)-oxidase reaction to evaluate the melanogenic activity of melanocytes.

RESULTS

Pigmentation started to develop after two sessions, i.e., after one week, and reached the highest level of pigmentation after the fourth session of NB-UVB irradiation. In all the studied animals, the skin in areas 1 and 2 was markedly darkened to the same level while that in area 3 showed very little darkening [Figure 1].

The original color of the fur coat of the animals was red/brown and the skin underneath was light brown. After NB-UVB exposure, it was noticed that the skin color became brownish/black.

Microscopic examination of H and E-stained sections confirmed the integrity of the skin layers and cells and showed no marked difference between the three tested areas before irradiation. After irradiation, increased basal melanization was marked in areas 1 and 2 and mild or absent in area 3 [Figure 2].

Before NB-UVB exposure, the three areas showed no or mild basal pigmentation.

After NB-UVB irradiation, the melanin was heavily distributed in the basal layer of the epidermis, including melanocytes and keratinocytes, extending up to the horny layer, in areas 1 and 2 nearly to the same degree. In area 3, the melanocytes, with their characteristic dendrites, showed marked increase in melanin content while the nearby keratinocytes showed minimal increase [Figure 3].

Using the DOPA reaction, the three areas showed neither melanocyte nor basal pigmentation before NB-UVB exposure, whereas after NB-UVB exposure, DOPA-positive cells which were detected in the three areas were more marked in areas 1 and 2 [Figure 4].

DISCUSSION

In this work, NB-UVB irradiation induced hyperpigmentation in Guinea pigs. This effect was blocked by the application of H_2 antagonist but not with the vehicle or H_1 antagonist.

Melanin is classified into two subtypes; black-colored eumelanin and reddish-yellow pheomelanin.[10] We noticed that the induced pigmentation, underlying the shaved red fur coat, was brown/black which may suggest the shift of melanogenesis from pheomelanogenesis to eumelanogenesis on clinical basis. This goes in agreement with the findings of Lassalle *et al.*,[11] who examined the amounts of eumelanin and pheomelanin using high-performance liquid chromatography analysis after oxidation and hydrolysis of melanin produced after addition of histamine to cultured melanocytes and found that the ratio of eumelanin/pheomelanin increased significantly with nitric oxide

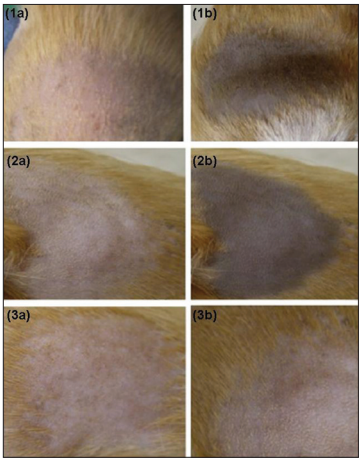

Figure 1: Guinea pigs' skin before (a) NB-UVB exposure in the three tested areas showing no pigmentation and after (b) exposure showing pigmentation in areas 1 and 2 and only mild pigmentation in area 3

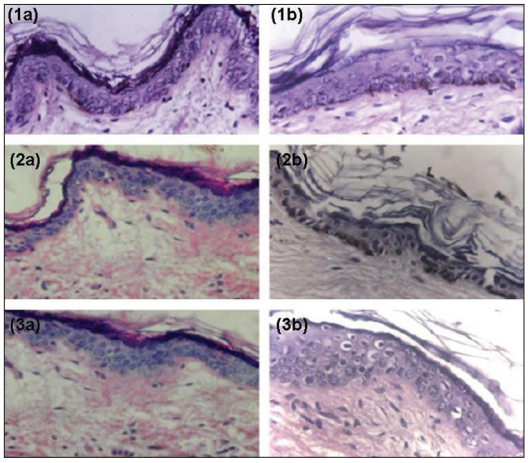

Figure 2: H and E-stained sections before (a) NB-UVB exposure in the three tested areas showing mild basal pigmentation in area 1 and no pigmentation in areas 2 and 3. After UVB exposure (b), areas 1 and 2 showed basal pigmentation, while area 3 showed no pigmentation

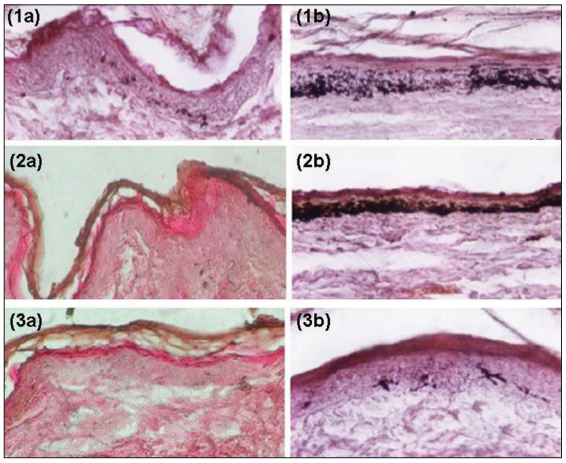

Figure 3: Masson-Fontana-stained sections before (a) NB-UVB exposure in the three tested areas showing mild basal pigmentation in area 1 and no pigmentation in areas 2 and 3. After UVB exposure (b), areas 1 and 2 showed pigmentation extending up to the horny layer, while area 3 showed only melanocytes with long dendrites

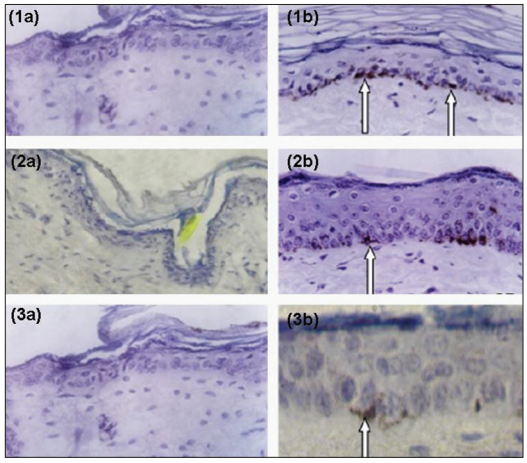

Figure 4: DOPA reaction in the three tested areas before (a) NB-UVB exposure showing neither melanocyte nor basal pigmentation. After (b) irradiation, areas 1 and 2 showed melanocytes (arrows) and basal pigmentation, while area 3 showed only melanocytes (arrow)

(NO) and histamine. The same results were obtained by examining this ratio in Guinea pigs' skin (To be published).

Histochemically, it was found that DOPA-positive cells that appeared in the three areas after irradiation stained more positively in areas 1 and 2 and to a lesser extent in area 3 [Figure 4]. This difference in melanocyte activity may be due to the inhibitory effect of H_2 antagonist on melanization reported by Yoshida *et al*.[5]

On examining the Masson-Fontana-stained sections, we noticed that despite that this stain does not detect the enzyme activity in the melanocytes, yet it was clear that active melanization and transfer to keratinocytes were not affected in areas 1 and 2, proved by the appearance of the pigment in all epidermal layers. On the other hand, in area 3, melanocyte melanization was less than the other two areas, but the keratinocytes showed no or little pigment [Figure 3].

Our work confirmed the previous reports on the role of H_2 antagonists on inhibition of melanization. However, we suggest that this inhibition is not only due to their effect on melanin formation as previously reported,[5] but also due to block of melanosomes' transfer from melanocytes to keratinocytes.

Based on these results,[12] two studies emerged; the first explained why vitiligo patients who were on antihistaminic therapy were not responding to conventional vitiligo therapy[13] and the second one showed that pigmentation is synchronized by a pH change during melanosome maturation and that the switch from pH 5.0 to 6.8 seems to depend on the proton pump p-protein in the melanosome membrane.[14]

CONCLUSION

This work gives a theoretical rationale for using topical famotidine 2% in the treatment of melasma, a useful application which is still waiting for clinical evaluation, and in case of success, it will be an addition to the treatment of this troublesome disease.

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