Journal of Photoscience (2002), Vol. 9(2), pp. 205–208

Involvement of Nitric Oxide in UVB-induced pigmentation

Toshio Horikoshi [1]*, Minoru Sasaki [1,2], Michio Nakahara [1]
Hideyo Uchiwa [1] and Yoshiki Miyachi [2]

[1] Basic Research Laboratory, Kanebo Ltd., Odawara, 250-0002, Japan

[2] Department of Dermatology, Kyoto University, Graduate School of Medicine, Kyoto, 606-8507, Japan

Nitric oxide (NO) is a newly described transmitter involved with cell to cell communication that is generated in biologic tissues by specific types of nitric oxide synthase (NOS), which metabolize L-arginine and molecular oxygen to citrulline and nitric oxide. In the skin, NO has been reported to play an important role in such diseases as psoriasis, atopic dermatitis, and contact dermatitis, as well as act as an important modulator in UVB-induced erythema. Ultraviolet B irradiation to the skin evokes an increase in NO production in the epidermis through two pathways; induction of inducible NOS, mediated by inflammatory cytokines, and elevation of constitutive neuronal NOS activity. In a cell culture system, it has been demonstrated that NO functions as a melanogen after being produced in keratinocytes in response to UVB-irradiation. NO-stimulated melanogenesis in melanocytes is mediated by the cGMP/PKG pathway. In this study, up-regulation of tyrosinase gene expression by NO-stimulation and the involvement of NO in UVB-induced pigmentation were examined. In NO-induced melanogenesis, protein synthesis and tyrosinase activity increased along with an up-regulation of tyrosinase gene expression. In an animal model, UVB-induced pigmentation in skin was suppressed by sequential daily treatments with a specific inhibitor of NOS. Thus, NO plays an important role in UVB-induced pigmentation, where its function as a melanogen is considered to be one of the mechanisms. Together with its role in the development of erythema, NO contributes to the total protective response of skin against UVB-irradiation.

Key words : Nitric oxide, NOS, UV, pigmentation, melanogenesis, eumelanin

INTRODUCTION

Nitric oxide (NO) has been shown to have several physiological functions including vasodilation, as well as antitumor and antimicrobial effects, and is also known to have a role as a neurotransmitter. NO is generated by specific types of nitric oxide synthase (NOS), which metabolize L-arginine and molecular oxygen to citrulline and nitric oxide. Three different NOS isoforms, each encoded by different genes, have been characterized. Two enzyme isoforms are constitutively expressed [endothelial NOS (eNOS) and neuronal NOS (nNOS)], whereas the other is an inducible enzyme (iNOS).

*To whom correspondence should be addressed.
E-mail : PXB12765@nifty.com

Recently, several studies have identified a number of important roles for NO in such skin diseases as psoriasis, atopic dermatitis, itch-scratch response, and contact dermatitis, as well as in maintaining skin homeostasis such as wound healing and UVB-induced erythema [1]. NOS isoforms have been detected in skin cells as well as those in other tissues. Human keratinocytes express nNOS and iNOS, while all three are detected in melanocytes. These isoforms are activated or induced in skin cells in response to UVB irradiation. The activity of nNOS is elevated immediately after UVB-stimulation [2, 3], while a remarkable induction of iNOS has been detected in the basal cell layer of epidermis for long periods after UVB-irradiation [4]. This iNOS induction is thought to be mediated by inflammatory cytokines that are released in response to UV irradiation.

In the process of UVB-induced pigmentation, an increase in melanin production is an important event, which is catalyzed by tyrosinase expressed in melanocytes. Melanogenesis is regulated by melanogens, i.e. alpha-melanocyte-stimulating hormone (α-MSH), histamine, and endothelin-1, as well as several inflammatory factors, which are all released from cells surrounding melanocytes in response to UVB stimulation. On the other hand, stimulation of melanogenesis by NO was first reported by Romero-Graillet et al. [5]. **Figure 1** schematically illustrates melanogenesis stimulated by nitric oxide in the skin. In the present study, the up-regulation of tyrosinase gene expression caused by NO-stimulation and the involvement of NO in UVB-induced pigmentation were examined.

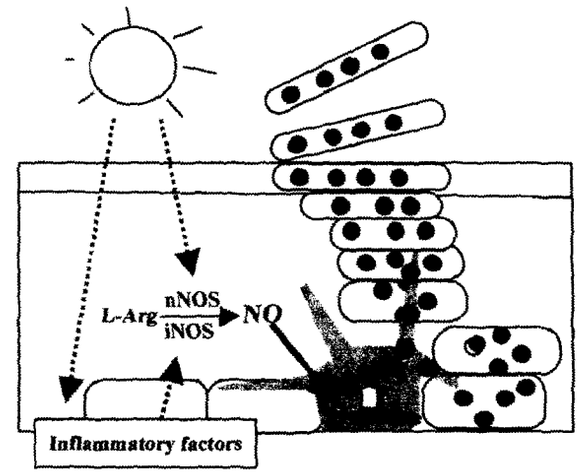

Fig. 1. Schema of melanogenesis caused by nitric oxide in the skin, as proposed by Romero-Graillet et al. [4], with some modifications. NO; Nitric oxide, NOS; NO synthase.

MATERIALS AND METHODS

Cell culture

Human melanocytes were incubated in the presence of SNAP (S-nitroso-N-acetyl-L-arginine), then RNA was extracted and subjected to Northern blotting analysis using tyrosinase cDNA.

Animal model for UVB-induced pigmentation

An L-NAME (N-nitro- L-arginine methyl ester hydrochloride) or D-NAME (N-nitro-D-arginine methyl ester hydrochloride) solution at a final concentration of 40 mM was topically applied to one of two separated areas on back skin of brownish guinea pigs for 2 days (100 μl/application, twice/day) prior to UVB irradiation. These areas were then irradiated with 350 mJ/cm^2 of UVB from an SE lamp, which was repeated 6 days later. Sequential topical applications of the solutions were performed twice per day for 13 days after the initial irradiation. The degree of pigmentation was evaluated by measuring the L^* value,

using a colorimeter (CR-300, Minolta, Tokyo, Japan), which indicated the lightness of the skin color.

RESULTS AND DISCUSSION

NO-induced melanogenesis in human melanocytes

The function of NO as a melanogen was first demonstrated by Romero-Graillet et al. [5] in a cell culture system. They extended their experiment to a transwell culture system using keratinocytes and melanocytes, and showed that NO was initially produced in UVB-irradiated keratinocytes and then stimulated melanocytes [6]. In their study, increases in both tyrosinase activity and tyrosinase protein levels were observed after daily stimulation with a NO donor for 4 days. We investigated the details of tyrosinase gene expression within the first 24 hours of NO-induced melanogenesis, and found that tyrosinase mRNA expression was induced 12 hours after a single treatment with SNAP (a NO donor) (**Fig. 2A**). An increase of tyrosinase activity was also detected time-dependently within the first 24-hour period (**Fig. 2C**), which was accompanied by an increase of tyrosinase protein levels (**Fig. 2B**).

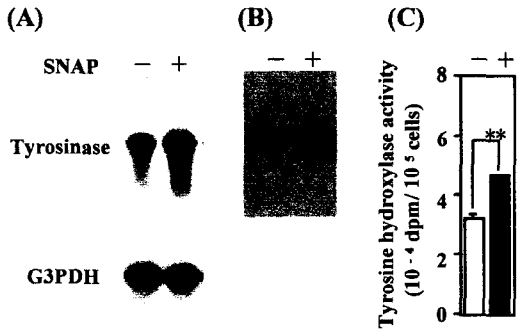

Fig. 2. Effect of nitric oxide on tyrosinase expression in melanocytes. Increases in (A) tyrosinase mRNA, (B) tyrosinase protein, and (C) tyrosinase activity in NO-stimulated melanogenesis are demonstrated. SNAP; the NO donor.

It has been reported that an increase in tyrosinase activity results in an increase in the ratio of eumelanin and pheomelanin [7]. A significant increase in the ratio of eumelanin/pheomelanin has also been observed with the stimulation of melanocytes by NO (Lassalle et al., personal communication).

Involvement of nitric oxide in UV-induced pigmentation

Although these *in vitro* results imply the involvement of NO in UVB-induced pigmentation, there are no known *in vivo* studies that have attempted to determine whether NO is an important factor in pigmentation. We examined the contributions of NO towards UV-induced pigmentation in an animal model, using a NOS inhibitor. UVB-induced erythema in guinea pig skin was reduced when the NOS inhibitor L-NAME was topically applied to skin daily, beginning 3 days before UVB-irradiation (data not shown). Further, delayed pigmentation in the skin was markedly suppressed by sequential daily treatments with L-NAME (**Fig. 3**).

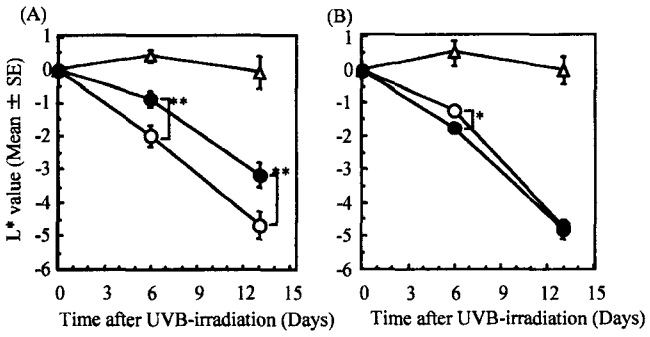

Fig. 3. Inhibition of UVB-induced hyperpigmentation by topical application of a specific inhibitor of NOS in brownish guinea pigs. (A) Effect of L-NAME (●) as compared with control area (○). (B) Effect of D-NAME (●) as compared with control area (○). (△), non-irradiated area. *P<0.05, **P<0.01.

Moreover, suppression of increased numbers of DOPA-positive melanocytes in the skin was also observed with sequential daily L-NAME treatment (**Fig. 5**). In contrast, D-NAME, an ineffective isomer of L-NAME, had no effect on these UV-induced skin responses. These results suggest that NO production may contribute to the regulation of UVB-induced pigmentation.

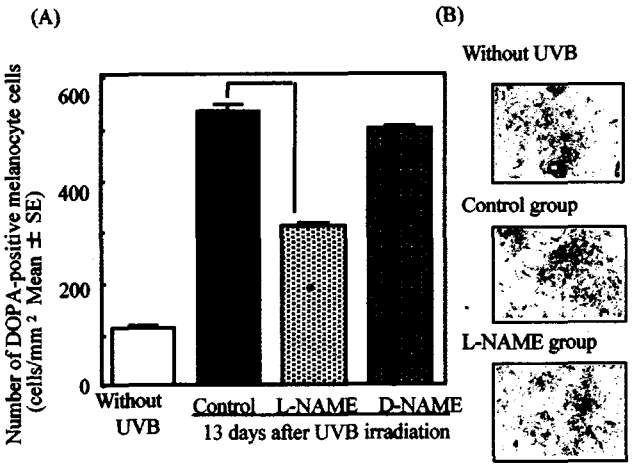

Fig. 4. Suppressive effect of L-NAME on the increase in DOPA-positive melanocyte cells in back skin of guinea pigs after UVB-irradiation. (A) Numbers of DOPA-positive melanocyte cells 13 days after UVB- irradiation, counted under an optical microscope after staining. (B) Pictures showing DOPA-positive melanocyte cells 13 days after UVB-irradiation.

CONCLUSION

As we have shown, NO plays an important role in UVB-induced pigmentation, where its function as a melanogen is considered to be one of the mechanisms. Together with its role in the development of erythema, NO contributes to the total protective response of skin against UVB-irradiation.

REFERENCES

1. Bruch-Gerharz D., T. Ruzicka and V. Kolb-Bachofen (1998) Nitric oxide and its implications in skin homeostasis and disease – a review. *Arch. Dermatol. Res.* 290, 643-651.
2. Kang-Rotondo C. H., S. Major, T. M. Chiang, L. K. Myers and E. S. Kang (1996) Upregulation of nitric oxide synthase in cultured human keratinocytes after ultraviolet B and bradykinin. *Photodermatol. Photoimmunol. Photomed.* 12, 57-65.
3. Sasaki M., J. Yamaoka and Y. Miyachi (2000) The effect of ultraviolet B irradiation on nitric oxide synthase expression in murine keratinocytes. *Exp. Dermatol.* 9, 417-422.
4. Kuhn A., K. Fehsel, P. Lehmann, J. Krutmann, T. Ruzicka and V. Kolb-Bachofen (1998) Aberrant timing in epidermal expression of inducible nitric oxide synthase after UV irradiation in cutaneous lupus erythematosus. *J. Invest. Dermatol.* 111, 149-153.
5. Romero-Graillet C., E. Aberdam, N. Biagoli, W. Massabni, J. P. Ortonne and R. Ballotti (1996) Ultraviolet B radiation acts through the nitric oxide and cGMP signal transduction pathway to stimulate melanogenesis in human melanocytes. *J. Biol. Chem.* 271, 28052-28056.
6. Romero-Graillet C, E. Aberdam, M. Clement, J. P. Ortonne and R. Ballotti (1997) Nitric oxide produced by ultraviolet-irradiated keratinocytes stimulates melanogenesis. *J. Clin. Invest.* 99, 635-642.
7. Hunt G, S. Kyne, K. Wakamatsu, S. Ito and A. J. Thody (1995) Nle^4Phe^7 α-Melanocyte-stimulating hormone increases the eumelanin:pheomelanin ratio in cultured human melanocytes. *J. Invest. Dermatol.* 104, 83-85.