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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Methods.

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Letter to the Editor

N-Nicotinoyl dopamine, a novel niacinamide derivative, retains high antioxidant activity and inhibits skin pigmentation

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Abstract: We synthesized a novel derivative of a well-known skin-lightening compound niacinamide, N-nicotinoyl dopamine (NND). NND did not show inhibitory effects of tyrosinase and melanin synthesis in B16F10 mouse melanoma cells. However, NND retains high antioxidant activity without affecting viability of cells. In a reconstructed skin model, topical applications of 0.05% and 0.1% NND induced skin lightening and decreased melanin production without affecting the viability and morphology of melanocytes and overall tissue histology. Moreover, no evidence for skin irritation or sensitization was observed when 0.1% NND emulsion was applied onto the skin of 52 volunteers. The effect of NND on skin lightening was further revealed by pigmented spot analyses of human clinical trial. Overall, NND treatment may be a useful trial for skin lightening and treating pigmentary disorders.

Key words: antioxidant effect – niacinamide-N-nicotinoyl dopamine – skin pigmentation

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Background

Hyperpigmentation disorders, such as melasma, postinflammatory hyperpigmentation and lentigo senilis, are associated with the abnormal accumulation of melanin pigments (1). The regulation of human skin pigmentation has been a long-standing goal for cosmetics and pharmaceutical applications (2). Skin pigmentation is determined by the amount and the type of melanin synthesized by the melanocytes and its transfer to the neighbouring keratinocytes (3). Niacinamide (NA, vitamin B3, nicotinamide and 3-pyridinecarboxamide) is a well-known skin-lightening compound, which functions by inhibiting melanosome transfer (4,5). However, the associated mechanism for its action remains to be elucidated. In this study, we synthesized a novel NA derivative, N-nicotinoyl dopamine (NND), by reaction of nicotinic acid and dopamine hydrochloride (Figure S1a); skin lightening related with the pigmentation process was investigated.

Questions addressed

In this study, we synthesized a novel NA derivative, NND, which contains antioxidant activity, and observed the effects of this on skin lightening.

Experimental design

The effect of NND on the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was estimated as previously described (6) with a minor modification. HaCaT cells (ATCC, Manassas,

VA, USA) were cultured in RPMI medium (Gibco, Grand Island, NY, USA) containing 10% (v/v) fetal bovine serum (FBS) (Gibco). After 24-h incubation, HaCaT cells were treated with NND for 2 h. To check intracellular reactive oxygen species (ROS) generation induced by UVB, cells were exposed to UVB (20 mJ/cm^2) at 30 min after the treatment of dichlorofluorescein diacetate (DCF-DA) (Molecular Probes, Eugene, OR, USA). The intracellular ROS was determined using a fluorescence microscope Axiovert 200-HBO 100 (Zeiss, Jena, Germany) and fluorescence microplate reader Infinite F500 (Tecan, Mannedorf, Switzerland). Reconstructed human epidermis MEL-300-B (MatTek, Ashland, MA, USA) consisted of normal human epidermal keratinocytes and normal human epidermal melanocytes. Human repeated insult patch test was conducted by modified Shelanski method (7). For the human clinical study, for depigmentating effect, 23 volunteers were included in the study to observe the depigmentation effect. After the pigmentation induction on the test area in the arm, the samples were applied for 8 weeks (for details, see online Supporting information).

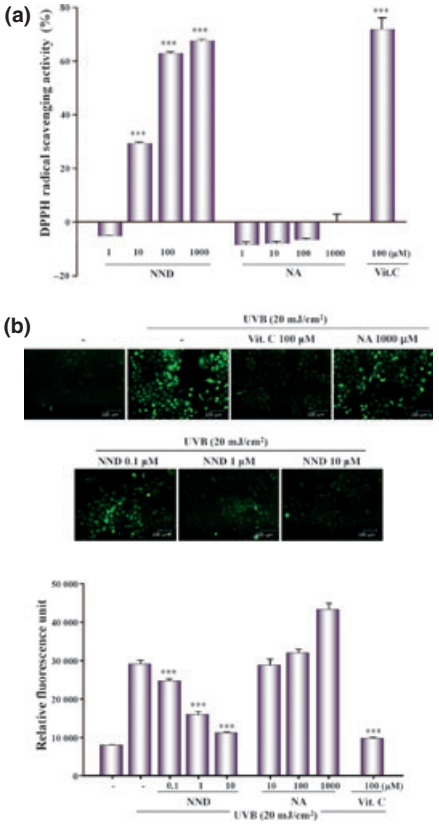
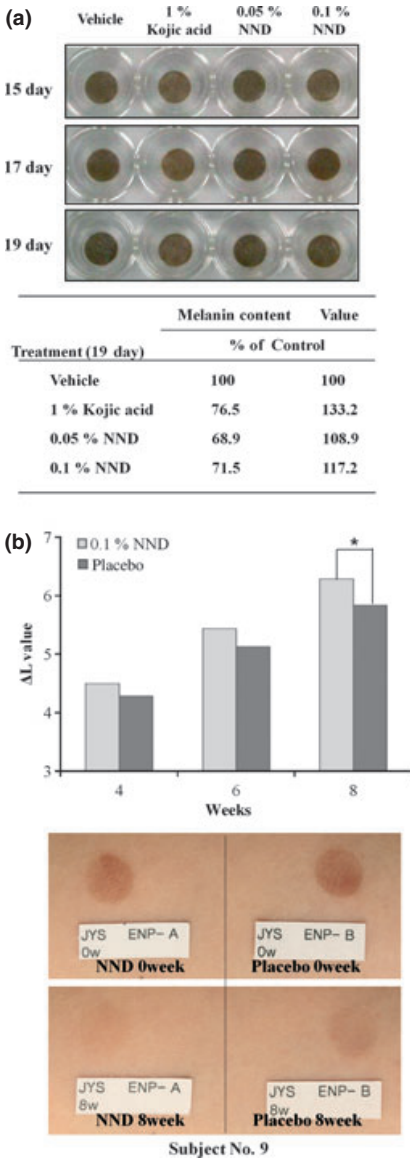


Figure 1. Effect of N-nicotinoyl dopamine (NND) or Niacinamide (NA) on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and intracellular reactive oxygen species (ROS) accumulation induced by UVB irradiation in HaCat cells. (a) HaCat cells were treated with NND, NA or vitamin C, and DPPH scavenging activities were estimated as described in the Materials and methods. (b) HaCat cells were grown, as Fig. 1a, and treated with dichlorofluorescein diacetate with or without NND, NA and vitamin C for 2 h followed by exposed to 20 mJ/cm^2. Fluorescent microscope images of intracellular ROS accumulation were measured. Lower panel, Relative fluorescence unit was quantified by dichlorofluorescein, and the error bars indicate the SD of three independent experiments. Asterisks indicate a significant difference compared with the UVB group, ***$P < 0.001$.

Results

N-nicotinoyl dopamine retains high antioxidant activity as shown by the measurement of the DPPH radical scavenging reaction (Fig. 1). The intracellular ROS scavenging activity, which was monitored by [dichlorofluorescein (DCF)] fluorescence intensity (8), was increased by UVB irradiation (Fig. 1b). NND did not change the mushroom tyrosinase activity and melanin synthesis in B16F10 cells as NA (Figure S1b,c). In addition, NND reduced



Treatment (19 day)	Melanin content % of Control	Value
Vehicle	100	100
1 % Kojic acid	76.5	133.2
0.05 % NND	68.9	108.9
0.1 % NND	71.5	117.2

Figure 2. Effect of N-nicotinoyl dopamine (NND) emulsion on depigmentation in three-dimensional human reconstructed epidermis model (a) and in human clinical study (b). (a) Reconstructed human epidermis MEL-300-B was incubated at 37°C in a humidified atmosphere of 5% CO_2. The following 25 mg of test emulsions was applied to the apical surface of the tissues: 0.05%, 0.1% NND and 1% kojic acid for 19 days. Table indicated quantification of melanin amount which was monitored by OD 450 nm measurements of the lysates of reconstructed epidermis. Values are data for skin lightness using semiquantitative image analysis programme. (b) 0.1% NND and vehicle-treated sides of skin during treatment period of 8 weeks as assessed by Chromameter. *L*-value changes from baseline for 0.1% NND and vehicle-treated side. Lower panel, Representative photographs of 0.1% NND treatment at 0 and 8 week. *$P < 0.05$ when compared with placebo group.

pigmentation in the reconstructed epidermis. Topical application of 0.05% and 0.1% NND emulsions resulted in significant skin lightening, and melanin production was decreased by 31.1% and 28.5%, respectively (Fig. 2a). We did not observe any evidence for irritation or sensitization when the 52 subjects were tested by a human repeated insult patch test for 0.1% NND. To identify the effect of NND on pigmentation and lightening, we evaluated the efficacy after topical application of 0.1% NND for 8 weeks to the forearm skin of 23 Korean women. The *L*-value representing efficacy of skin whitening and visual assessment by dermatologists was significantly higher by 0.1% NND application for 8 weeks compared to the placebo control ($P < 0.05$) (Fig. 2b).

Conclusions

N-nicotinoyl dopamine, a newly synthesized NA derivate, which retains high antioxidant activity, was characterized as a relatively safe and effective skin-whitening reagent. NND significantly reduced the degree of cutaneous pigmentation when analysed by the reconstructed epidermis model. The depigmentation and whitening of skin by NND was further clinically confirmed by human clinical studies by applying non-irritating 0.1% NND on the forearm skin. Previously, most studies involving melanogenesis and skin whitening are mostly focussed on the inhibition of tyrosinase within the melanosomes of melanocytes (9). However, the inhibition of melanin synthesis is not a cause of the depigmentation by NND as revealed by the absence of effects of NND in the tyrosinase activity in B16F10 cells. In addition, NND was not found to inhibit the expression of melanogenesis-related factors, including tyrosinase, TRP-1 and TRP-2, and MITF (data not shown). Recently, the inhibition of melanin transfer from melanocytes to keratinocytes has become an attractive target for the research of melanogenesis and skin whitening (9,10). The effects of NA on skin lightening by inhibiting melanosome transfer are well known (4,11). Therefore, the skin-lightening effect of NND may also be attributed to the inhibition of the melanocyte melanosome transfer into keratinocytes. The inhibition of melanosome transfer can be attributed to several factors such as phagocytosis involving proteinase-activated receptor-2 (PAR-2), melanocyte dendricity involving E-cadherin and Rho, and cell–cell recognition involving lectins and neoglycoproteins (12–15). The controlling factors for the distribution patterns of melanosomes within keratinocytes are complex and should be further investigated to clarify the antimelanosome transfer mechanism of NND. The strong antioxidant effect of NND is potentially beneficial for the prevention of oxidative damage to the skin (16). NND has proven to be well tolerated to skin by the human repeated insult patch test. Overall, NND may potentially be used for cosmetics or drugs for improvement of skin whitening and therapies related to skin pigmentation disorders.

Acknowledgements

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Conflict of interest

The authors state no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of the article:

Data S1. Materials and methods.

Figure S1. (a) NND synthesis. NND was synthesized by addition of Dicyclohexylcarbodiimide to pyridine solution containing dopamine hydrochloride and Nicotinic acid. (b) Effect of NND on mushroom tyrosinase activity and cellular melanin synthesis. L-tyrosine solution with or without a sample chemical and mushroom tyrosinase were incubated at 37°C for 10 min followed by measurement of the light absorbance at 475 nm. (c) Effect of NND on cellular melanin synthesis. B16F10 cells treated α-MSH was added with NND (0.1, 1, or 10 μM), NA (1 mM), or Kojic (500 μM) acid for 3 days. Melanin productions were expressed as the relative percentages for untreated controls. The error bars indicate the SD of three independent experiments.

Figure S2. Effect of NA emulsion on de-pigmentation in three dimensional human reconstructed epidermis model.

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