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Original Article

Tranexamic acid inhibits the plasma and non-irradiated skin markers of photoaging induced by long-term UVA eye irradiation in female mice

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ABSTRACT

Photoaging can be induced by long-term ultraviolet (UV)A eye irradiation, but an ameliorating method for such photoaging is not known. In this study, we examined the effects of tranexamic acid (trans-4-aminomethylcyclohexanecarboxylic acid) on photoaging of the skin induced by UVA eye irradiation. We used the C57BL/6 j female mice and locally exposed their eyes to UVA at a dose of 110 kJ/m^2 using an FL20SBLB-A lamp multiple times a week for one year. The plasma urocortin 2, β-endorphin, methionine enkephalin (OGF), and histamine content, as well as the expression of the corticotropin-releasing hormone receptor (CRHR) type 2, μ-opioid receptor, opioid growth factor receptor (OGFR), T-bet, and GATA3 increased in the mice subjected to UVA eye irradiation. However, the increased levels of urocortin 2, methionine enkephalin, histamine, OGFR, T-bet, and GATA3 were suppressed by tranexamic acid treatment. Conversely, the levels of β-endorphin and μ-opioid receptor increased with tranexamic acid treatment. In addition, the expression of the estrogen receptor-β on the surface of mast cells was increased by tranexamic acid. These results indicate that photoaging induced by UVA eye irradiation can be ameliorated by tranexamic acid through the regulation of hypothalamo-pituitary hormones. Furthermore, this ameliorative effect on photoaging may be due to an increase in estrogen receptor-β after tranexamic acid treatment.

1. Introduction

Humans are exposed to ultraviolet rays (UV) on a daily basis, which can cause sunburn, suntan, and photoimmunosuppression of the skin with short-term exposure [1], and photoaging and skin cancer with long-term exposure [2]. Unlike natural aging, photoaging can induce various symptoms, such as skin dryness, pigmentation maculation (spot), tylosis, and elasticity depression (wrinkles) [2]. Active oxygen species induction is one of the causes of deep wrinkles that occur due to photoaging. If keratinocytes and fibroblasts are irradiated with UV, reactive oxygen species (ROS) are generated, which can damage the DNA. Furthermore, the activity of enzymes, such as tyrosine phosphatase, which can be oxidized and inactivated by UV irradiation, causes changes in the metabolic and cellular functions [3]. In addition, matrix metalloproteinase (MMP) is activated [4], resulting in the secretion of MMP-1, MMP-3, and MMP-9 and degradation of collagen and elastin in the dermis. It is known that the secretion of the transcription factor AP-1 is induced by tyrosine phosphatase, inhibiting the expression of the procollagen gene in fibroblasts [5,6]. Furthermore, UV causes DNA damage directly and indirectly through the genesis of active oxygen species; thus, the cells that have damaged DNA produce vascular endothelial growth factors (VEGFs) and inflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α, that can accumulate and degrade collagen in cells [5].

Another unique characteristic of photoaging is the induction of skin spots. Skin spots that occur due to photoaging are also called as senile pigment spots, which usually start off brown and gradually turn black over time. The skin spots can develop when the skin is exposed to UV because it induces a signal to keratinocytes, which then promotes melanocytes to generate melanin. However, if the spot is continued to be exposed to UV for a long period, excessive melanin will be synthesized and the spot turns darker in color [6,7]. Furthermore, UV irradiation can induce the metabolic failure of the *stratum corneum*; in other words, the outer layer of the skin cannot slough off and melanin accumulates in these skin layers [8].

Although long-term UV skin exposure can cause photoaging, it can also be caused by long-term local UV exposure to the eye [9,10]. We have reported the use of tranexamic acid as an anti-inflammatory agent

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against wrinkles of dry skin [11] and for the suppression of pigmentation caused by UV irradiation [12]. Tranexamic acid functions as a regulator of the α-melanocyte-stimulating hormone (α-MSH), adrenocorticotropic hormone (ACTH), and β-endorphin, which are secreted from pro-opiomelanocortin (POMC) [13]. Generally, UVA irradiation increases the occurrence of wrinkles, spots, and skin cancer through the POMC system, thus, causing photoaging [14]. Our previous study on photoaging induced by UVA eye irradiation indicated that the brain hormones ACTH, α-MSH, corticotropin-releasing hormone (CRH), and urocortin 2 are affected by UVA eye irradiation [15]. Therefore, tranexamic acid may regulate the expression of these additional peptide hormones that are induced during photoaging. In this milieu, we examined the effect of tranexamic acid on photoaging induced by long-term UVA eye irradiation in mice and analyzed the mechanism.

2. Materials and methods

2.1. Animals

Specific-pathogen-free 8-week-old female C57BL/6 j mice (SLC, Hamamatsu, Shizuoka, Japan) were used in all the experiments. The mice were maintained individually in cages in an air-conditioned room at 23 ± 1°C. There were six mice per treatment group. UVA irradiation was performed as previously described [16]. Briefly, under light pentobarbital anesthesia, UVA (wavelength of 320–400 nm) was applied to the eye at a dose of 110 kJ/m^2 (irradiation time: 30 min. d) using an FL20SBLB-A lamp (wavelength: 300–400 nm, peak emission: 352 nm; Toshiba Co., Tokyo, Japan). The UVA light was passed through a glass filter to block the UVB rays. During irradiation, we covered the mice with aluminum foil, except the eye. The control group received visible light irradiation (wavelength: 400–700 nm) to the eye. The eyes of mice were exposed three times each week over a one-year period. No signs of injury to the eye were observed with the doses of UVA radiation administered. In addition, we covered the control group mice also with aluminum foil during visible light irradiation, and it was carried out under the same conditions as UVA irradiation. Furthermore, with respect to the physical stress caused by covering with aluminum foil, the mice were acclimated against physical stress by prolonged maintenance under stress condition (data not shown). This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals at the Suzuka University of Medical Science (approval number: 34). All surgeries were performed under pentobarbital anesthesia and all efforts were made to minimize animal suffering.

2.2. Tranexamic acid treatment

Approximately 750 mg/kg of tranexamic acid (Daiichi Sankyo Healthcare Co., Ltd., Tokyo, Japan) in distilled water was administered orally three times a week for one-year; the control animals were administered distilled water [9]. The dosis of tranexamic acid used this experiment was proportionate to that administered to human.

2.3. Preparation and staining of epidermal sheets and dorsal skin

For histological studies, the mice were sacrificed one year after the start of the experiment. Skin samples (0.5 cm × 0.5 cm) were obtained from the ears and incubated for 2 h at 37°C in 2 M sodium bromide. The epidermis was then separated from the dermis to obtain epidermal sheets. Dopa-positive melanocytes in the epidermal sheets were stained as previously described [17]. The dorsal skin specimens were fixed in 4% phosphate-buffered paraformaldehyde, embedded in frozen Tissue Tek OCT compound, and cut into 5-μm thick sections. The sections were then stained with hematoxylin-eosin (HE) in accordance with the established procedures to enable the histological analysis of the skin. In addition, the skin specimens were stained using antibodies for the immunohistological analysis according to a previously published method [18]. Briefly, the specimens were incubated with either rabbit polyclonal anti-estrogen receptor-β (1:100; Affinity BioReagents, Golden, CO, USA) or goat polyclonal anti-mast cell tryptase (1:50; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) primary antibodies. The samples were then washed and incubated with fluorescein isothiocyanate-conjugated anti-goat and tetramethylrhodamine isothiocyanate-conjugated anti-rabbit (1:30; Dako Cytomation, Glostrup, Denmark) secondary antibodies, respectively. The expression of mast cell tryptase and estrogen receptor-β was evaluated immunohistochemically by fluorescence microscopy.

2.4. Quantification of adrenocorticotropic hormone, β-endorphin, histamine, and methionine-enkephalin using an enzyme-linked immunesorbent assay

Blood samples were collected from the heart of test mice one-year after the start of the experiments. The plasma levels of adrenocorticotropic hormone (ACTH), β-endorphin, histamine, and methionine-enkephalin were determined using a commercial enzyme-linked immunesorbent assay (ELISA) kit (ACTH and β-endorphin: Phenix Pharmaceuticals Inc., Burlingame, CA, USA; histamine: Bertin Pharma, Montigny-le-Bretonneux, France; methionine-enkephalin: Peninsula Laboratories International Inc., San Carlos, CA, USA) according to the instructions of the respective manufacturers.

2.5. Western blot analysis of the dorsal skin

The skin samples were homogenized in lysis buffer (Kurabo, Osaka, Japan) and centrifuged at 8000 × g for 10 min. The supernatant from each sample was then separated and stored at −80 °C until further analysis. We performed the western blot analysis as previously described [19]. Briefly, 10 μg of protein was separated by electrophoresis and transferred on to nitrocellulose membranes, which were incubated at 25 °C for 1 h with primary antibodies against collagen type I (1:1000; EMD Chemicals Inc., Darmstadt, Germany), melanocortin receptor 2 (MC2R) (1:1000; Chemicon, Temecula, CA, USA), μ-opioid receptor (1:1000; Abcam, Tokyo, Japan), T-bet (marker of T helper 1 (Th1) cell, 1:1000; Abcam), GATA3 (marker of Th2 cell, 1:1000; Cell Signaling Technology, Denvers, MA, USA), opioid growth factor receptor (OGFR) (1:1000; MyBioSource, San Diego, CA, USA), or β-actin as a loading control (1:5000; Sigma-Aldrich, St. Louis, MO, USA). The membranes were then treated with horseradish peroxidase-conjugated secondary antibody (1:1000; Novex, Frederick, MD, USA), and the immune complex was detected using the Immunostar Zata reagent (Wako, Osaka, Japan). The images of the membranes were acquired with the Multi-Grade software program (Fuji-film, Greenwood, SC, USA).

2.6. Statistical analyses

All data are presented as mean ± standard deviation (SD). The statistical significance of these data was analyzed using Microsoft Excel 2010 and by the one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test using the SPSS software, version 20 (IBM, Armonk, NY, USA). The results with $p < 0.05$ were considered significant.

3. Results

3.1. Effect of tranexamic acid treatment on photoaging induced by long-term UVA eye irradiation

The eyes of test mice were topically irradiated with UVA three times a week for 1 h per day over a period of one year, and then ear skin specimens were collected after the last UVA irradiation session. The chronic UVA exposure resulted in an increase in the dopa-positive cells

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