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Original article

The amelioration effect of tranexamic acid in wrinkles induced by skin dryness

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ABSTRACT

Tranexamic acid (trans-4-aminomethylcyclohexanecarboxylic acid) is a medical amino acid widely used as an anti-inflammatory and a whitening agent. This study examined the effect of tranexamic acid administration in wrinkle formation following skin dryness. We administered tranexamic acid (750 mg/kg/day) orally for 20 consecutive days to Naruto Research Institute Otsuka Atrichia (NOA) mice, which naturally develop skin dryness. In these NOA mice, deterioration of transepidermal water loss (TEWL), generation of wrinkles, decrease of collagen type I, and increases in mast cell proliferation and tryptase and matrix metalloproteinase (MMP-1) release were observed. However, these symptoms were improved by tranexamic acid treatment. Moreover, the increase in the β-endorphin level in the blood and the expression of μ-opioid receptor on the surface of fibroblasts increased by tranexamic acid treatment. In addition, when the fibroblasts induced by tranexamic acid treatment were removed, the amelioration effect by tranexamic acid treatment was halved. On the other hand, tranexamic acid treated NOA mice and mast cell removal in tranexamic acid treated NOA mice did not result in changes in the wrinkle amelioration effect. Additionally, the amelioration effect of mast cell deficient NOA mice was half that of tranexamic acid treated NOA mice. These results indicate that tranexamic acid decreased the proliferation of mast cells and increases the proliferation of fibroblasts, subsequently improving wrinkles caused by skin dryness.

1. Introduction

Wrinkles in the skin occur according to various causes. The predominant cause of wrinkles is a depression of the skin function by aging. Other causes of wrinkles include skin dryness, ultraviolet-ray exposure, and allergy (for example, atopic dermatitis) [1–6]. These responses decrease collagen and elastin fibers and induce wrinkles. Furthermore, oxidative stress in the skin plays a major role in the aging process. Oxidative stress is a source of reactive oxygen species and plays a significant role in wrinkle formation [7].

Dry wrinkles often observed in human include epidermal wrinkles due to decreased moisture in the stratum corneum of the epidermidis. Decreased moisture in the stratum corneum of the epidermidis induces chapping and dry, rough skin. As a result, the skin surface contracts and the width of the sulcus cutis and crista cutis spread. The sulcus cutis becomes deep and wrinkles appear. Furthermore, dry wrinkles include dermis wrinkles. Collagen and the natural moisturizing factors in the dermis decrease, elasticity is lost, and as a result, deep wrinkles are formed.

The Naruto Research Institute Otsuka Atrichia (NOA) mouse is a hair-deficient mutant that has been established as an inbred strain [8]. The development of dry skin was observed under individual housing conditions in NOA mice [9,10]. Furthermore, the proliferation of mast cells and eosinophils are increased, along with elevated immunoglobulin E (IgE) levels in these mice; thus, the NOA mouse is useful as an animal model of allergic dermatopathy.

In the study, we examined the improvement effect of tranexamic acid on the genesis of wrinkles using NOA mice, which naturally develop wrinkles due to skin dryness.

2. Materials and methods

2.1. Animal experiments

Specific pathogen-free, 4-week-old male NOA mice (CLEA, Suita, Osaka, Japan) were used in the experiments. The mice were

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kept individually in cages in an air-conditioned room at 23 ± 1 °C under SPF conditions. There were 10 mice per group. Skin samples and blood samples were obtained 20 days after the start of the experiment. This study was carried out in strict accordance with the recommendations of the guide for the care and use of laboratory animals of Suzuka University of Medical Science (approval number: 34). All surgeries were performed under pentobarbital anesthesia, and all efforts were made to minimize suffering.

2.2. Tranexamic acid treatment [11]

Approximately 750 mg/kg of tranexamic acid (Daiichi Sankyo Healthcare Co., Ltd., Tokyo, Japan) in saline was administered orally for 20 consecutive days, while saline was administered to the control animals.

2.3. Chemical treatment

2.3.1. N-(4-amino-2-methylquinolin-6-yl)-2-(4-ethylphenoxymethyl) benzamide monohydrochloride (JTC-801) treatment [12]

JTC-801 is an antagonist of opioid receptor. JTC-801 (1 mg/kg/day; Selleck Chemicals, Houston, TX) was suspended in a 1:175 solution of DMSO:PBS, which was administered orally every other day during the 20 days. Control animals were treated with vehicle only.

2.3.2. Chloroquine phosphate treatment [13]

Approximately 10 mg/kg of chloroquine phosphate (fibroblast inhibitor; LKT Laboratories, Inc., St. Paul, MN) in saline was injected intraperitoneally into the mice every other day during the 20 days. Saline alone was injected into the control mice.

2.3.3. SCF-Rabbit anti-Mouse polyclonal antibody (kit) treatment [14]

Approximately 21 μg/kg of the kit (Lifespan Biosciences Inc., Seattle, WA) in saline was injected intraperitoneally into the mice every other day during the 20 days. Saline was injected into the control mice.

2.4. Measurement of wrinkles

In accordance with the method of Bissett et al. [15], we scored the wrinkles of the NOA mice on the 20th day after tranexamic acid treatment as follows: 0: no wrinkles, 1: light wrinkles, 2: slightly deep wrinkles, and 3: deep wrinkles.

2.5. Measurement of transepidermal water loss (TEWL) and capacitance of the dorsal skin

TEWL and capacitance of the dorsal skin were measured. TEWL measurements were obtained to determine skin permeability (reflecting the barrier function of the skin), using a Tewameter TM300 probe (Courage + Khazaka Electronic GmbH, Cologne, Germany), as described previously [16]. Values were recorded after the responses stabilized, approximately 10 s after the probe was placed on the skin. The average of three independent measurements is reported.

The capacitance level of the stratum corneum (reflecting skin hydration in the outermost layer of the skin) was measured using a Corneometer CM825 probe (Courage + Khazaka Electronic GmbH, Cologne, Germany), as described previously [17]. The Corneometer probe was applied to the dorsal skin surface of each mouse, and the degree of skin hydration was determined by obtaining electrical capacitance measurements, expressed in arbitrary unit (a.u.). The average of three independent measurements per test area is reported.

2.6. Preparation and staining of the dorsal skin

For the histological studies, the mice were sacrificed 20 days after experiment onset. The dorsal skin specimens were fixed in phosphate-buffered paraformaldehyde (4%), embedded in frozen Tissue Tek, OCT compound, and cut into 5 μm thick sections. The skin sections were stained with standard toluidine blue stain to evaluate their mast cell content.

For the analysis of the expression of mast cell tryptase, μ-opioid receptor, and the number of fibroblasts, dorsal skin sections were washed in PBS and subsequently incubated overnight at 4 °C with a goat anti-mast cell tryptase (1:50) polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA), mouse anti-μ-opioid receptor (1:100) monoclonal antibody (Abcam, Tokyo, Japan) or rabbit-anti-S100A4 (anti-fibroblast; 1:50) polyclonal antibody (Thermo Scientific, Fremont, CA), respectively. After staining, the specimens were washed in PBS and incubated at room temperature for 2 h with tetramethylrhodamine isothiocyanate-conjugated (TRITC) anti-mouse immunoglobulin or fluorescein isothiocyanate-conjugated (FITC) anti-goat and anti-rabbit immunoglobulin (1:30; Dako Cytomation, Glostrup, Denmark). The expression levels of tryptase, μ-opioid receptor, and S100A4 were evaluated immunohistochemically with fluorescence microscopy.

2.7. Western blot analysis

The dorsal skin samples were homogenized in cold NR-10025 suspension reagent (Kurabo, Osaka, Japan). After centrifugation at 8000g for 10 min, the supernatant fractions were isolated and stored at −80 °C until further analysis. After thawing, equal amounts of protein (12.5 μg/line) were loaded onto a 4–12% BIS-TRIS Blot Gel (Life Technologies, Carlsbad, CA) and electrophoresed at 200 V for 20 min. Following separation, the proteins were transferred to a nitrocellulose membrane using the iBlot Western Blotting System (Life Technologies, Carlsbad, CA), which was subsequently blocked with 5% skim milk at 4 °C overnight. The next day, the membranes were incubated at 25 °C for 1 h with a primary antibody against collagen type I (1:1000; Millipore, Billerica, MA), matrix metalloproteinase (MMP-1) (1:1000; Santa Cruz Biotechnology Inc., Santa Cruz, CA), mast cell tryptase (1:500; Santa Cruz Biotechnology Inc., Santa Cruz, CA), μ-opioid receptor (1:1000; Abcam, Tokyo, Japan) or β-actin (1:5000; Sigma–Aldrich, St. Louis, MO). Membranes were then treated with a horseradish peroxidase-conjugated secondary antibody (Dako Cytomation, Glostrap, Denmark). Immune complexes were detected with ImmunoStar Zeta reagent (Wako, Osaka, Japan). Images were acquired using the Multi-Gauge Software program (Fujifilm, Greenwood, SC).

2.8. Quantification of adrenocorticotropic hormone (ACTH), corticosterone, immunoglobulin e (IgE), and β-endorphin using enzyme-linked immunosorbent assays (ELISA)

Blood samples were taken from the heart at 20 days after experiment onset, and then the plasma was fractionated. The plasma levels of ACTH, corticosterone, IgE, and β-endorphin were determined using commercial ELISA kits (ACTH and β-endorphin: Phoenix Pharmaceuticals Inc., Burlingame, CA; corticosterone: Assaypro, St. Charles, MO; and IgE: Yamasa, Chiba, Japan) according to the manufacturer's instructions.

2.9. Statistical analysis

All data are presented as the means ± standard deviation. For comparisons among groups, Student's *t*-test was applied, with $p < 0.05$ considered to be statistically significant.