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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.

3. Results

3.1. Effect of tranexamic acid treatment on the dorsal skin of NOA mice

The body weights of the mice in the control and tranexamic acid treatment groups did not differ to a statistically significant extent (Fig. 1(A)). The NOA mouse naturally develops dry skin, and elevated TEWL and decreased moisture retentions were observed during the 20-day experimental period. However, tranexamic acid treatment resulted in the amelioration of TEWL and moisture retention (Fig. 1(C)). The wrinkle formation of the dorsal skin of NOA mice at 20 days after tranexamic acid treatment is shown in Fig. 1(B). Tranexamic acid treatment suppressed the generation of wrinkles in the NOA mice, although wrinkle generation was observed following skin dryness. Furthermore, although a low expression of collagen type I was observed in the NOA mice, this expression increased following tranexamic acid administration. On the contrary, although the MMP-1 expression in the NOA mice was increased, it decreased following tranexamic acid administration (Fig. 1(D)).

3.2. Effects of tranexamic acid treatment on the plasma levels of ACTH, corticosterone and IgE in NOA mice

Increased plasma concentrations of ACTH, corticosterone, and IgE were observed in the NOA mice. However, these increases were inhibited by tranexamic acid treatment (Fig. 2).

3.3. Effects of tranexamic acid treatment on the proliferation of mast cells in the dorsal skin

Increased proliferation of mast cells in the dorsal skin was induced in NOA mice. However, this increase was suppressed by tranexamic acid treatment (Fig. 3).

3.4. Effects of tranexamic acid treatment on the plasma level of β-endorphin and on the expression of μ-opioid receptor in the dorsal skin

We next investigated the plasma level of β-endorphin, an opioid peptide, and the expression of μ-opioid receptor, a receptor of β-endorphin, in the dorsal skin. Both the β-endorphin level in the plasma and the expression of μ-opioid receptor in the dorsal skin increased following tranexamic acid treatment (Fig. 4).

3.5. Activation of μ-opioid receptor during skin disruption

The μ-opioid receptor expression increased in the dorsal skin following tranexamic acid treatment. We then identified cells with increased expression levels of μ-opioid receptor on their cell surfaces. We observed an increase in the μ-opioid receptor expression as well as co-localization of this receptor with mast cells and fibroblasts in tranexamic acid treated NOA mice (Fig. 5).

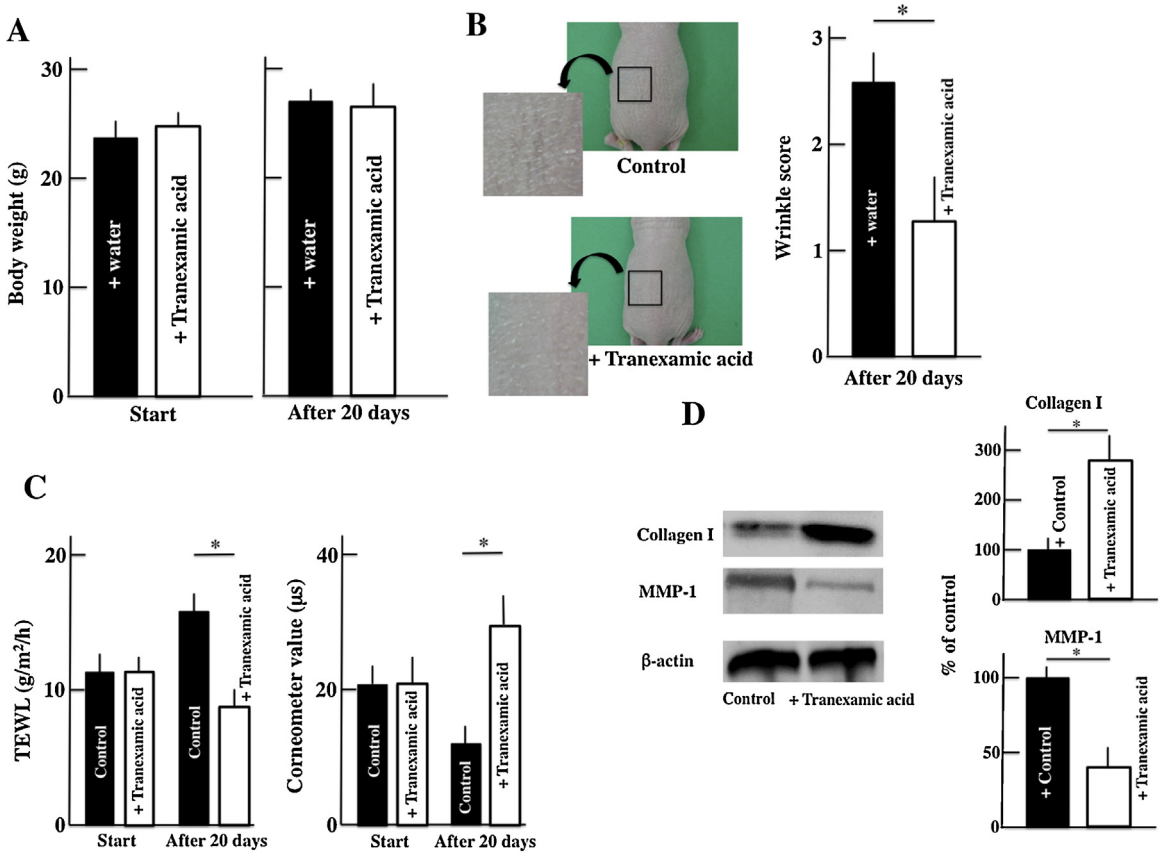

Fig. 1. Effects of tranexamic acid on body weight (A), wrinkle formation (B), TEWL and moisture retention (C), and the expression of MMP-1 and collagen type I (D) in the dorsal skin of NOA mice. The values are expressed as the mean ± SD derived from 10 animals. *$p < 0.05$.

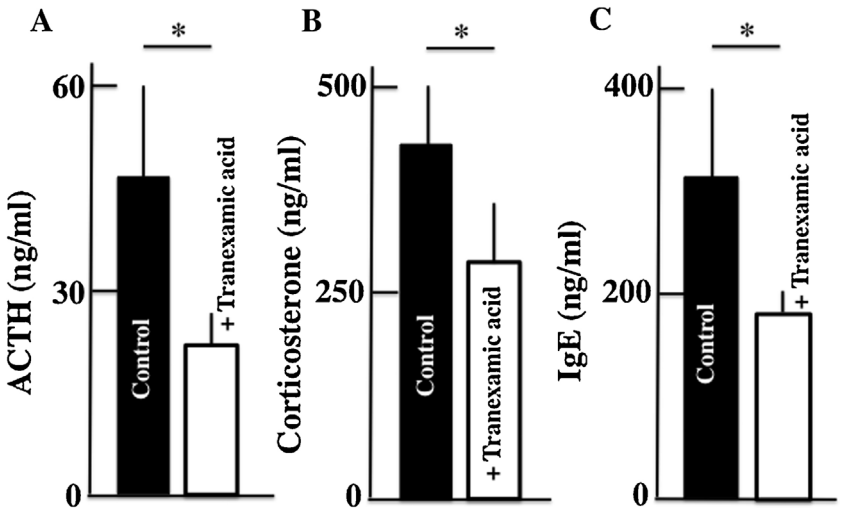

Fig. 2. Effects of tranexamic acid on the plasma levels of ACTH (A), corticosterone (B), and IgE (C) in NOA mice. The values are expressed as the mean ± SD derived from 10 animals. *$p < 0.05$.

3.6. Effects of tranexamic acid treatment on the dorsal skin after JTC-801, chloroquine phosphate and SCF antibody injection

In the tranexamic acid treated NOA mice, the amelioration of TEWL and wrinkles and the suppression of decreased collagen type I levels were observed. On the other hand, the amelioration effect of tranexamic acid treatment was inhibited by both chloroquine phosphate and JTC-801, which were administered with half-dose tranexamic acid administration. Furthermore, the NOA mice treated by both SCF antibody and tranexamic acid exhibited similar results as NOA mice treated with tranexamic acid alone. In addition, the amelioration effect of the NOA mice treated only by SCF antibody was similar to the tranexamic acid treated NOA mice administered chloroquine phosphate or JTC-801 (Table 1A and 1B and Fig. 6).

4. Discussion

This study examined the amelioration effect of tranexamic acid in wrinkle formation of the skin using NOA mice. An increase in collagen type I and decrease in MMP-1 in the skin were observed following tranexamic acid administration. Additionally, tranexamic acid induced the expression of β-endorphin and the proliferation of fibroblasts, and decreased the proliferation of mast cells and the release of mast cell tryptase. Furthermore, the expression of μ-opioid receptor, which is a receptor of β-endorphin, increased on the cell surface of fibroblasts.

The NOA mouse naturally develops dry skin and induces the formation of wrinkles, which is accompanied by skin xerosis. A reduction in skin tightness due to decreased collagen type I levels is one of the cause of wrinkle generation of a skin [18]. Tryptase mainly released from mast cells activates MMP-1, which is the degradative enzyme of collagen type I, leading to subsequent decreased collagen type I levels [19]. In this study, the proliferation of mast cells, and the expression of tryptase and MMP-1 increased in NOA mice; as a result, collagen type I levels decreased and wrinkles formed. On the other hand, when the NOA mice were treated with tranexamic acid, decreased proliferation of mast cells and decreased tryptase and MMP-1 levels were observed, with the subsequent increased expression of collagen type I (Figs. 1 and 3). These results demonstrate that tranexamic acid affects mast cells. Tranexamic acid is a protease inhibitor with antiplasmin activity

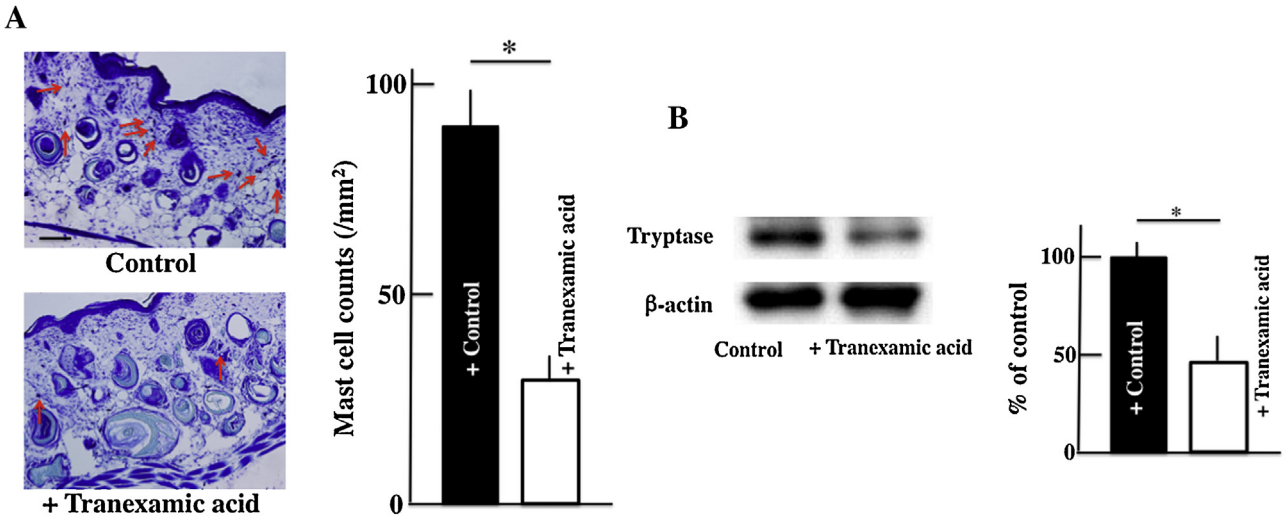

Fig. 3. Effects of tranexamic acid on the mast cells (A) and mast cell tryptase (B) in the dorsal skin of NOA mice. Skin specimens were stained with toluidine blue. Scale bar = 100 μm. The values are expressed as the mean ± SD derived from 10 animals. *$p<0.05$.

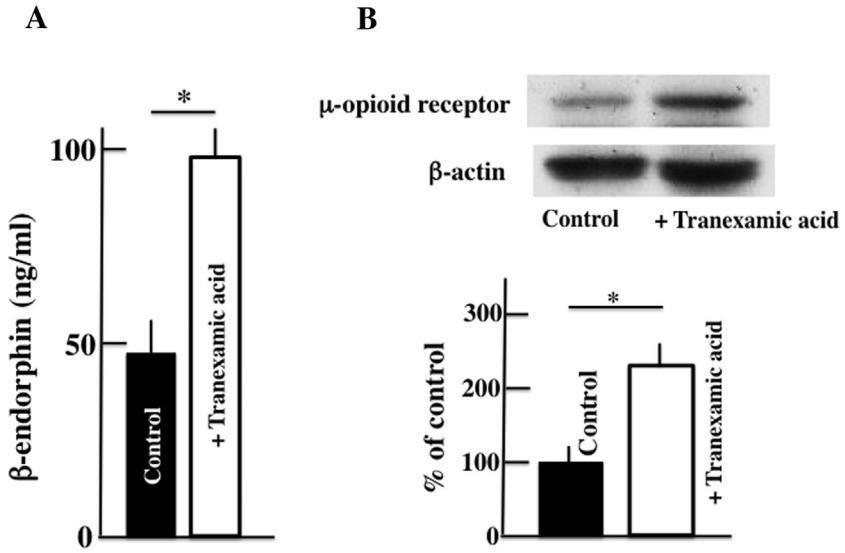

Fig. 4. Effects of tranexamic acid on the plasma levels of β-endorphin (A) and on the expression of μ-opioid receptor in the dorsal skin of NOA mice. The values are expressed as the mean ± SD derived from 10 animals. $^{*}p < 0.05$.

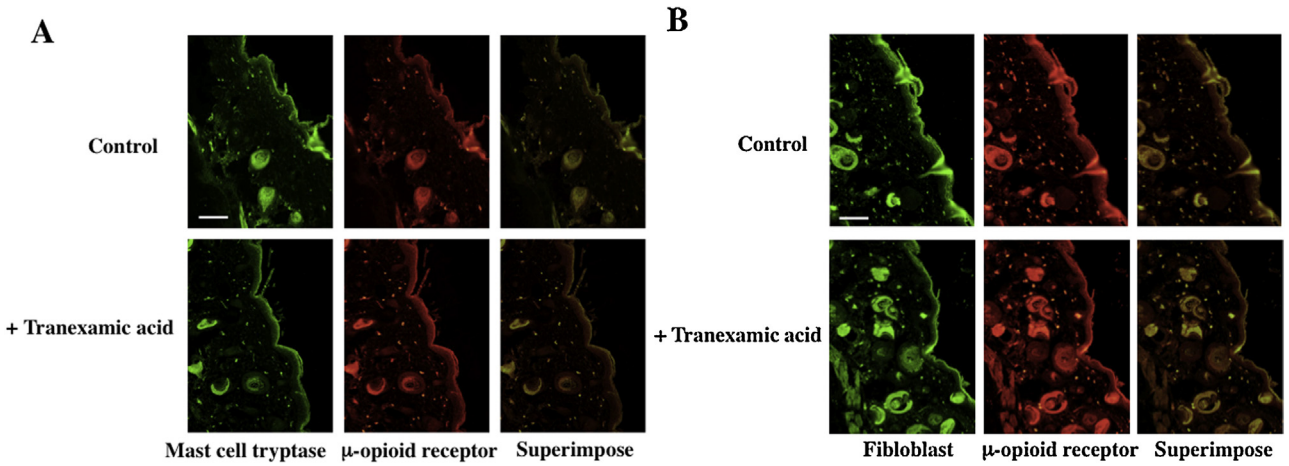

Fig. 5. Effects of tranexamic acid on the expression of mast cell tryptase and μ-opioid receptor (A) and proliferation of fibroblasts and μ-opioid receptor (B) in the dorsal skin of NOA mice. The data show one representative experiment performed on 10 animals. Scale bar = 100 μm.

and anti-inflammatory effects [20]. Briefly, plasmin increases the

Table 1A
Effects of JTC-801, chloroquine phosphate, and SCF antibody on TEWL, capacitance, and wrinkle in the dorsal skin of tranexamic acid treated NOA mice. The value at the time of the start of experiment.

	TEWL ($g/m^2/h$)	Corneometer (μs)	Wrinkle score
Non-treatment groups			
Control	11.03 ± 0.58	20.65 ± 1.77	1.18 ± 0.11
+Kit (SCF antibody)	11.05 ± 1.82	20.06 ± 1.81	1.05 ± 0.17
+Chloroquine	11.85 ± 1.43	20.05 ± 1.65	1.25 ± 0.13
+JTC-801	11.27 ± 0.80	20.87 ± 1.76	1.21 ± 0.16
Tranexamic acid treatment groups			
Control	12.05 ± 0.77	20.08 ± 2.01	1.16 ± 0.21
+Kit (SCF antibody)	12.38 ± 2.37	21.90 ± 2.70	1.04 ± 0.17
+Chloroquine	11.92 ± 1.59	20.83 ± 1.79	1.21 ± 0.15
+JTC-801	11.02 ± 1.69	21.43 ± 2.95	1.16 ± 0.10

The values are expressed as the mean ± SD derived from 10 animals. There was no significant difference in all parameters.

Table 1B
Effects of JTC-801, chloroquine phosphate, and SCF antibody on TEWL, capacitance, and wrinkle in the dorsal skin of tranexamic acid treated NOA mice. The value 20 days after treatment.

	TEWL ($g/m^2/h$)	Corneometer (μs)	Wrinkle score
Non-treatment groups			
Control	18.00 ± 1.75	15.90 ± 0.64	2.73 ± 0.18
+ Kit (SCF antibody)	14.63 ± 1.77*	24.15 ± 4.30*	1.81 ± 0.18*
+ Chloroquine	19.62 ± 1.44	14.90 ± 0.52	2.59 ± 0.16
+ JTC-801	19.17 ± 1.82	14.85 ± 2.15	2.72 ± 0.21
Tranexamic acid treatment groups			
Control	9.60 ± 0.59	34.62 ± 2.61	0.95 ± 0.17
+ Kit (SCF antibody)	8.73 ± 0.87	37.45 ± 3.15	0.88 ± 0.15
+ Chloroquine	14.58 ± 1.96*	24.00 ± 4.30*	2.00 ± 0.16*
+ JTC-801	13.93 ± 1.08*	23.72 ± 3.48*	2.09 ± 0.17*

The values are expressed as the mean ± SD derived from 10 animals.
* $p < 0.05$ indicates a significant difference between the control and each treatment groups.

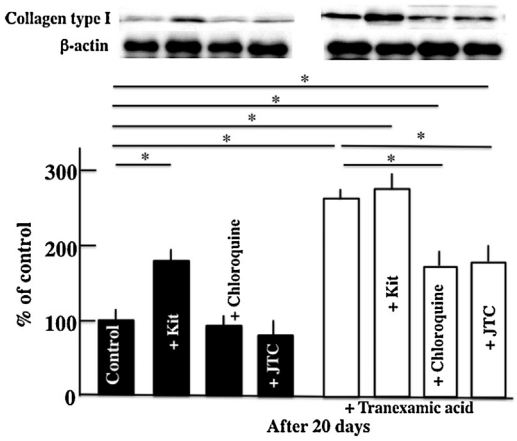

Fig. 6. The effects of JTC-801, chloroquine phosphate, and SCF antibody on the expression of collagen type I in the dorsal skin of tranexamic acid-treated NOA mice. The values are expressed as the mean ± SD derived from 10 animals. $^{*}p < 0.05$.

production of kinin, leading to pain and inflammation. Tranexamic acid reduces the production of kinin by suppressing the activity of plasmin. Similarly, tryptase is a serine protease and tranexamic acid may have inhibited the action of tryptase. Furthermore, the proliferation of mast cells may be decreased, and tranexamic acid may have directly affected the mast cells. However, the effect of tranexamic acid on mast cells is not known and further examination is necessary.

In this study, we additionally investigated the intervention of β-endorphin and fibroblasts as another component of the wrinkle amelioration effect of tranexamic acid. We observed an increased β-endorphin level in the plasma and increased expression of μ-opioid receptor in the skin following the administration of tranexamic acid (Figs. 4 and 5). Furthermore, the proliferation of fibroblasts in the skin and the expression of μ-opioid receptor on the surface of the fibroblasts increased (Fig. 5). It is known that β-endorphin can induce the proliferation of fibroblasts [21,22]. Therefore, the increased β-endorphin expression following tranexamic acid treatment induced the proliferation of fibroblasts. The fibroblasts increased the production of collagen type I and, as a result, led to the amelioration of wrinkles. In addition, tranexamic acid increases the cleavage of β-endorphin from proopiomelanocortin [11]. β-endorphin combines with μ-opioid receptor on the fibroblast surface, increasing the secretion of β-endorphin from the fibroblasts [23]. Thus, we considered the potential increase in collagen type I production through the loop we refer to as the tranexamic acid-β-endorphin-fibroblasts-β-endorphin loop.

In order to examine the wrinkle amelioration effect of mast cells and fibroblasts, we created mice deficient in mast cells and the fibroblasts and examined the effect of tranexamic acid. The tranexamic acid treated NOA mice deficient in mast cells or fibroblasts had a significantly greater amelioration effect of wrinkles compared with the tranexamic acid non-treated group. However, the wrinkle amelioration effect of tranexamic acid treated fibroblast deficient or μ-opioid receptor deficient NOA mice decreased by 50% compared with tranexamic acid only treatment (Figs. 5, 6 and Table 1A and 1B). On the other hand, the wrinkle amelioration effect of the tranexamic acid treated mast cell deficient NOA mice did not change as compared with the only tranexamic acid treated NOA mice. Moreover, the wrinkle amelioration effect of the mast cell deficient NOA mice was 50% of the only tranexamic acid treated NOA mice. From these findings, we speculate that the amelioration effect of the wrinkles by tranexamic acid administration involves both mast cells and fibroblasts. On the other hand, β-endorphin induces the disengagement of histamine from mast cells [24]. However, the number of mast cells decreased and the expression μ-opioid receptor on mast cells decreased by tranexamic acid administration, suggesting that tranexamic acid reduces the influence of β-endorphin on mast cells.

From the above results, we speculated that tranexamic acid acted on both mast cells and fibroblasts and improved wrinkles by modulating the production of collagen type I with suppressed tryptase release from mast cells and increased fibroblast proliferation. Tranexamic acid may be useful as a cosmetics additive, as this treatment has anti-inflammatory, whitening, and anti-wrinkle action. However, further study is necessary to elucidate the detailed mechanisms of tranexamic acid on mast cells and fibroblasts.

Conflict of interest

The authors declare that they have no conflicts of interest concerning this article.

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