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Oral administration of collagen tripeptide improves dryness and pruritus in the acetone-induced dry skin model

Tomoko Okawa [a], Yukie Yamaguchi [a,*], Shinnosuke Takada [a], Yasuo Sakai [b], Noriaki Numata [b], Fumio Nakamura [c], Yoji Nagashima [d], Zenro Ikezawa [e], Michiko Aihara [a]

[a] Department of Environmental Immuno-Dermatology, Yokohama City University Graduate School of Medicine, Yokohama, Japan
[b] Central Research Institute, Jellice Co., Ltd., Japan
[c] Department of Molecular Pharmacology and Neurobiology, Yokohama City University Graduate School of Medicine, Yokohama, Japan
[d] Department of Molecular Pathology, Yokohama City University Graduate School of Medicine, Yokohama, Japan
[e] Department of Dermatology, International University of Health and Welfare Atami Hospital, Japan

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ABSTRACT

Background: Dry skin causes pruritus and discomfort in patients with xerosis and atopic dermatitis. General treatment for skin dryness involves the topical application of an emollient. However, more effective, simpler therapies are desired. Collagen tripeptide (CTP) is a highly purified, non-antigenic, low-allergenic collagen fraction that is known to have various biological effects.
Objective: To clarify the therapeutic effects of CTP for dry skin using acetone-induced dry skin model mice.
Methods: ICR mice were treated with acetone followed by oral administration of CTP (80 or 500 mg/kg/day) for 3 days. Hyaluronic acid production induced by CTP was assessed using human dermal fibroblasts in vitro and in an acetone-induced dry skin model mice in vivo. Transepidermal water loss (TEWL) and scratching behavior were evaluated. Furthermore, the effects of CTP on intraepidermal nerve fibers and expression of semaphorin 3A (Sema3A) and nerve growth factor (NGF) were examined by immunohistochemistry and quantitative RT-PCR.
Results: CTP enhanced hyaluronic acid production in human dermal fibroblasts in vitro and in murine skin in vivo. Oral administration of CTP in acetone-induced dry skin model mice significantly decreased TEWL and suppressed scratching behavior. Intraepidermal nerve growth was dramatically inhibited in CTP-treated mice. Quantitative PCR analysis and immunohistochemical study revealed that CTP abolished the increased NGF and decreased Sema3A levels induced by acetone treatment.
Conclusion: Oral administration of CTP improves dry skin and normalizes axon-guidance factors in the epidermis in addition to reducing pruritus. CTP may be used in a new therapeutic strategy against dry skin and pruritus.

1. Introduction

Dry skin causes pruritus in the aged and such common skin conditions as xerosis and atopic dermatitis (AD). Intolerable itching is frequently associated with abnormal innervations in the epidermis, and histamine H1 antagonists are often less effective against the hypersensation. A greater density of invasive nerve fibers in the epidermis has been observed in patients with AD, psoriasis, prurigo nodularis, and xerosis [1–4]. Recent studies suggest that epidermal innervations are regulated by the balance of nerve elongation factors—nerve growth factor (NGF), amphiregulin (AR), gelatinase—and nerve repulsion factors—semaphorin 3A (Sema3A), anosmin-1 [5,6]. NGF, which is released from keratinocytes and fibroblasts, is generally accepted as one of the most important growth factors causing abnormal skin innervations. Sema3A is known to induce retraction of NGF-sensitive neurons among dorsal root ganglion neurons, which prevent epidermal nerve invasions. In a dry skin model, cutaneous barrier disruption by acetone treatment has been shown to increase NGF and AR expression in the epidermis in addition to enhancing intraepidermal nerve growth [5]. The topical application of emollients ameliorated those reactions in an experimental animal

* Corresponding author at: Department of Environmental Immuno-Dermatology, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan. Tel.: +81 45 787 2675; fax: +81 45 786 0243.
E-mail address: yui1783@yokohama-cu.ac.jp (Y. Yamaguchi).

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model [7], which indicates that skin barrier disruption in dry skin is directly associated with skin innervations and pruritus.

Collagen is a major extracellular component in the skin. Collagen molecules comprise a highly repetitive sequence of glycine-X-Y (X, Y are arbitrary but are often occupied by proline, hydroxyproline, and alanine), and this formation and sequence makes them more resistant to degradation by general proteinases. Prepared using a collagenase digestion technique, collagen tripeptide (CTP) is a highly purified, non-antigenic, and low-allergic tripeptide fraction with a high percentage of basic units of glycine-X-Y [8]. Recently, collagen and collagen peptides, including CTP, have been implicated as functional foods with several biological effects. For example, CTP exerted beneficial effects on bone fracture healing in experimental animal models [9,10]. Compared with vehicle treatment, oral administration of collagen peptides produced faster wound healing and tissue regeneration, better angiogenesis, and helped to form thicker and better-organized collagen fiber deposition [10]. In in vitro studies, collagen-derived dipeptide (proline-hydroxyproline) stimulated hyaluronic acid (HA) production in synovial cells and skin fibroblasts [11,12]. Based on those findings, we hypothesized that CTP might display beneficial and therapeutic roles in common skin disorders. In this study, we therefore examined the effect of CTP oral administration on dry skin, epidermal innervations, and the levels of nerve guidance molecules, such as NGF and Sema3A, in acetone-induced dry skin model mice.

2. Materials and methods

2.1. Animals

Five-week-old male ICR mice under specific pathogen-free conditions were purchased from SLC (Shizuoka, Japan). They were kept under conventional conditions in cages at 23 ± 3 °C and humidity of 40–60% with a 12-h light and dark cycle. The animals were given standard feed and water. This study was conducted according to the guidelines for animal experiments at Yokohama City University Graduate School of Medicine.

2.2. Reagents

CTP was supplied by Jellice (Sendai, Japan).

2.3. Enzyme-linked immunosorbent assay (ELISA) assay for detection of hyaluronic acid produced by human dermal fibroblasts

Twenty-four-well plates (Corning, NY, USA) were inoculated with normal human dermal fibroblasts (Kurabo Industries, KF-4009, Osaka, Japan) at a concentration of 5×10^4 (cells/mL) per well. The plates were incubated in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal calf serum (FCS; Wako Pure Chemical Industries, Osaka, Japan) at 37 °C and 5% CO_2 for 3 days. The medium was replaced with DMEM containing 0.5% FCS, and then 10 μg/mL CTP was added. After further incubation for 24 h, the cultured supernatant was used to measure the amount of HA produced by means of the hyaluronan assay kit (Seikagaku Biobusiness, Tokyo, Japan). Purified water was used for the negative control and N-acetyl glucosamine (N-Ace.Glu) for the positive control. N-Ace.Glu was added to achieve a final concentration of 0.1 mM.

2.4. Acetone-induced dry skin model mice

For skin barrier disruption, acetone treatment was used as described previously with some modifications [13]. Briefly, the dorsal area of 6-week-old ICR mice was carefully clipped under isoflurane anesthesia (Intervet, Tokyo, Japan) at least 1 day before the acetone treatment. The mice were treated with acetone-soaked cotton balls for 5 min under anesthesia on 3 days (days 1–3). For the control groups, saline was used instead of acetone.

2.5. Oral administration of CTP

CTP was dissolved in saline and administered to the mice at a dose of 80 mg/kg, or 500 mg/kg orally, at 2 h after acetone treatment on days 1–3. As a vehicle control, saline was given to the mice in the same way. Dorsal skin samples were collected on day 4 by 5-mm punch biopsies (Dermapunch®, Maruho, Osaka, Japan) under anesthesia and were subjected to immunohistochemistry or RNA preparation.

2.6. Real-time polymerase chain reaction

Harvested skin samples were immediately kept in RNAlater (Invitrogen, Carlsbad, CA, USA) at 4 °C. Within 1 week, total RNA was extracted from the samples using the Illustra RNAspin Mini RNA Isolation Kit (GE Healthcare, Buckinghamshire, UK) according to the manufacturer's protocol. The RNA concentration was measured using the NanoDrop® ND-100 spectrophotometer (LMS, Tokyo, Japan). cDNA was synthesized from the same amount used for the RNA samples by means of TaqMan® Reverse Transcription Reagents (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. To detect the expression of hyaluronan synthase 2 (HAS2), Sema3A, and NGF, quantitative polymerase chain reaction (PCR) analysis was performed using the ABI PRISM® 7500 Sequence Detection System (Applied Biosystems) with the TaqMan® Gene Expression Assays probe and primer mix (Applied Biosystems) and TaqMan® Universal PCR Master Mix (Applied Biosystems) according to the manufacturers' instructions. The assay identification numbers of HAS2, Sema3A, NGF, and β-actin were Mm00515089m1, Mm00436469m1, Mm00443039m1, and Mm01205647g1, respectively. In the ABI Sequence Detection 7500 System, PCR reactions were carried out with the following thermal cycler conditions: stage 1, hold for 5 min at 50 °C, stage 2, hold for 10 min at 95 °C; stage 3, 40 cycles of 95 °C for 15 s, followed by 60 °C for 1 min. The results were normalized to the gene expression level of β-actin. All amplification reactions were performed in triplicate. An analysis of relative gene expression data was performed using the $2^{-\Delta\Delta CT}$ method with ABI PRISM® 7500 Sequence Detection System Software (Applied Biosystems). The fold change in studied gene expression, normalized to endogenous controls, was calculated using $RQ = 2^{-\Delta\Delta CT}$. RNA quality was further assessed by agarose gel electrophoresis.

2.7. Measuring of transepidermal water loss (TEWL) and corneum hydration

At 2 h after acetone treatment on days 1, 2, and 3, TEWL was measured on the back using a Tewameter® (TM300, Courage & Khazaka, Cologne, Germany) following the manufacturer's protocol. Measurements were taken at a stable level 20 s after the probe was placed on the skin. TEWL was automatically calculated and expressed in $g/m^2/h$. The room conditions were kept at 24 ± 2 °C and relative humidity of $45 \pm 5\%$. In some experiments, level of stratum corneum hydration was also measured using Corneometer® (CM825, Courage & Khazaka, Cologne, Germany).

2.8. Measurement of scratching behavior

The scratching behavior of mice was recorded using a digital video camera (Hitachi, Tokyo, Japan) for 30 min at 1 h after the