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ORIGINAL ARTICLE

Topical tranexamic acid improves the permeability barrier in rosacea

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ABSTRACT

Objective: To evaluate the influence of tranexamic acid on epidermal permeability barrier function in rosacea and its potential mechanisms.
Methods: A randomized, vehicle controlled, split-face study was performed on 30 rosacea patients. This study involved 2 weeks of 3% tranexamic acid solution treatment and vehicle control treatment. Skin physiological parameters, including skin surface pH, stratum corneum hydration, and transepidermal water loss, were measured. The expression of protease-activated receptor 2 (PAR-2) in rosacea and normal skin samples was assessed with immunohistochemical staining. The expression of PAR-2 in HaCaT keratinocytes was determined using reverse transcription polymerase chain reaction after stimulation with tranexamic acid. Changes of intracellular calcium induced by PAR-2 activation were measured using Fluo-4 NW calcium assay.
Results: Individuals with rosacea expressed a higher baseline level of PAR-2 compared with normal skin. Tranexamic acid improved the permeability barrier function in rosacea patients and inhibited calcium mobilization in keratinocytes induced by PAR-2 activation. The PAR-2 expression was not altered by tranexamic acid stimulation.
Conclusion: Topical tranexamic acid could improve the epidermal permeability barrier function and clinical signs of rosacea, likely resulting from inhibition of PAR-2 activation and consequent calcium influx. Thus, tranexamic acid could serve as an adjuvant therapy for rosacea.

Introduction

Rosacea is a common inflammatory facial disorder with compromised epidermal permeability barrier function. The common manifestations of rosacea include recurrent erythema, telangiectasia, inflammatory papules and pustules on the mid face, or rhinophyma in severe cases. The current therapies for rosacea have certain limitations, and prevention of relapse requires a long-term maintenance therapy.[1] The treatments are often short of targets, largely due to its unknown pathogenesis.[2,3] Recent studies have suggested that protease-activated receptor 2 (PAR-2) could be involved in the pathogenesis of rosacea. PAR-2 is a G-protein-coupled 7-transmembrane domain receptor, which mediates inflammation in various tissues upon activation by serine proteases (SPs) such as kallikrein.[4,5] Consistently, the activities of SPs are increased in rosacea. SPs degrade the epidermal antimicrobial peptide to its active form, cathelicidin LL-37. The latter can mediate vascular action and inflammation, which are features of rosacea.[6–8]

Compromised epidermal permeability barrier function is another feature of rosacea.[9] While rosacea exhibits a higher level of transepidermal water loss, improvement of permeability barrier function alleviates rosacea.[10] Whether the defective permeability barrier is the cause or the resultant of rosacea is not clear, it is likely linked to the increased SP activity. Previous studies showed that the increased SP activity is associated with certain dermatoses, such as atopic dermatitis, accompanied by defective permeability barrier.[11] Conversely, inhibition of SP activity by either lowering stratum corneum pH or topical SP inhibitors, improves epidermal permeability barrier homeostasis in barrier-disrupted skin. As an SP inhibitor, tranexamic acid has been proved to accelerate the restoration of the damaged skin barrier. In this study, we chose tranexamic acid as a candidate treatment for rosacea, since it can act as an inhibitor of SP and accelerate the recovery of the damaged skin barrier resulting from tape stripping, acetone, *sodium dodecyl*

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sulfate, and other physical or chemical inducers.[12,13] Here, we hypothesize that in rosacea, activation of PAR-2 by SP results in inflammation and defective permeability barrier.

In the present study, we determined whether inhibition of SP activity by tranexamic acid improves rosacea *in vivo* and affects calcium mobilization induced by PAR-2 activation *in vitro*, which may further prove the importance of barrier abnormality in the etiology of rosacea, and indicate a new direction for rosacea treatment.

Methods

Participants

The study was conducted following the principles outlined in the Declaration of Helsinki. The protocol was read and approved by the Ethics Committee of Peking University First Hospital, Beijing, China. Written informed consent was obtained from all patients prior to the study. Thirty patients aged 18–65 years were recruited according to the diagnostic criteria of rosacea.[1] The exclusion criteria included people with facial acne, steroid-dependent dermatitis, or other skin or systemic diseases that might influence skin assessment; using antirosacea drugs (including antibiotics), steroids, or vasodilating agents topically during the past 2 weeks or orally during the past 1 month; allergic to the test ingredients; during gestation or lactation. A randomized, vehicle controlled, split-face study was performed on 30 rosacea patients. One side of each patient's face was topically treated with 5% tranexamic acid solution twice daily for 2 weeks while the vehicle-treated side served as a control. During the study, no other topical or systemic agents were allowed. This study was conducted in March–May 2011, in Beijing, China.

Physiological assessment

After facial cleansing at each visit, the patients were kept in a test room with a controlled temperature (20–22°C) and humidity (40–60%) for 30 minutes before physiological measurements. Corneometer CM825 (COURAGE+KHAZAKA electronic GmbH, Köln, Germany) was used to measure the water content in the stratum corneum. Tewameter TM300 and pH meter pH900 (both from COURAGE+KHAZAKA electronic GmbH, Köln, Germany) were used to measure transepidermal water loss and pH value, respectively. Chromameter CM2600d (Konica Minolta, Inc., Tokyo, Japan) was used for the measurement of Chroma, an indicator for the severity of erythema[14]; VISIA Image System (Canfield Scientific, Inc. Fairfield, NJ, USA) was used for image analysis.

Cell culture and reagents

Immortalized human keratinocyte cell line HaCaT were purchased from the China Center for Type Culture Collection (Wuhan, China) and cultured 1% penicillin-streptomycin at 37° in Dulbecco's Modified Eagle's medium (Gibco, Langley, OK, USA) supplemented with 10% fetal bovine serum and C in a humidified CO2 incubator (95% air, 5% CO_2). For stimulation, cells were cultured in 12-well plates (Corning, Inc., Corning, NY, USA). At 60–70% confluence, culture medium was replaced with serum-free medium, followed by incubation with tranexamic acid (200 μg/mL) for an additional 24 hours. Then the keratinocytes were harvested for analysis.

MTT assay

Three-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, MO, USA) assay was used to determine the viability of HaCaT cells and the appropriate concentration of tranexamic acid to treat cells.[15] Tranexamic acid of 400 μg/mL, 200 μg/mL, 100 μg/mL, and 50 μg/mL diluted in *phosphate-buffered saline* (PBS) were used to treat cells for 24 hours.[16] After incubation with MTT for 4 hours, the optical density of each well was read at 540 nm using a microplate reader.

Histology and immunohistochemistry

Four μm thin-sections of formalin-fixed, paraffin-embedded skin samples were stained using immunohistochemistry. Briefly, fixed sections were deparaffinized, rehydrated and washed in PBS (2 × 5 minutes). For antigen retrieval, the sections were put into 10mM citrate buffer (pH 6.0) and boiled for 15 minutes. After cooling at room temperature, sections were treated with 3% $H_2O_2$for 15 minutes, and incubated with primary antibodies (rabbit polyclonal antihuman, Santa Cruz Biotechnology, Santa Cruz, CA, USA, 1:200) overnight at 4°C. Secondary antibodies were polyperoxidase-antimouse/rabbit immunoglobulin G. Staining color was developed using 3,3′-diaminobenzidine for 1–3 minutes. The reaction was stopped with distilled water while being observed under a microscope. Harris hematoxylin was used for counterstaining. Slides were examined at 200 × and 400 × magnification. Images were evaluated using the Leica microscope with imaging software (Leica Application Suite, Leica Microsystems Limited, Switzerland).

RNA isolation, cDNA synthesis, and quantitative real-time polymerase chain reaction

After stimulation for 24 hours, keratinocytes were washed with PBS, and RNA was isolated using RNeasy 96 Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For cDNA synthesis RNA was reverse-transcribed with iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) and real-time polymerase chain reaction (PCR) was carried out with ViiA 7 Real-time PCR system (Applied Biosystem Life Technology, USA) according to the manufacturer's instructions. The relative expression of the target genes were calculated by comparing with the housekeeping gene GAPDH using a formula described previously.[17] The primers used are PAR-2 : forward 5′-ACATGGCAACAACTGGGTCT-3′ ; reverse, 5′-CGAT CACCCAGTACCTCTGC-3′. GAPDH: forward 5′-TGACGTGCCGCCTG GAGAAA-3′; R5′-AGTGTAGCCCAAGATGCCCTTCAG-3′. All real-time PCR experiments were performed at least in triplicate and the specificity of the reactions was confirmed by sequencing the PCR products.

Fluo-4 NW calcium assay to detect the in-cell calcium signaling of PAR-2 activation in HaCat

HaCaT cells were cultured on the chamber slice (Lab-Tek Nunc, Thermo Scientific, USA) and used at 50% confluent. After incubation with basal medium or tranexamic acid for 24 hours, the medium was removed and washed with Ca^{2+} free assay buffer. To each well 100 μL of the dye loading solution was added. The plates were incubated at 37°C for 30 minutes, then at room temperature for an additional 30 minutes. As a PAR-2 agonist 100nM trypsin was used; fluorescence images were measured and recorded using a laser confocal microscope (Leica TCS SP5 microsystem, Germany) for excitation at 488 nm.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5 (GraphPad Software Inc., San Diego, CA, USA). Calculations are based on the means of independent experiments (each individual