Potential for tyndalized Lactobacillus acidophilus as an effective component in moisturizing skin and anti-wrinkle products

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Received March 26, 2015; Accepted April 25, 2016

DOI: 10.3892/etm.2016.3406

Abstract. It is widely accepted that ultraviolet (UV) irradiation induces skin damage. In the present study, a UVB-induced hairless mouse model of skin photoaging was developed to determine whether tyndalized Lactobacillus acidophilus was able to significantly enhance the repair of photodamaged skin. To evaluate the effects of tyndalized L. acidophilus on UVB-induced skin-wrinkle formation in vivo, HR-1 hairless male mice were exposed to UVB radiation and orally administered tyndalized L. acidophilus. Compared with the control group, the UVB irradiation mice displayed a significant increase in transepidermal water loss and a reduction in skin hydration. In mice with UVB-induced photodamage, the effacement of the fine wrinkles by tyndalized L. acidophilus was correlated with dermal collagen synthesis, accompanied by histological changes. Furthermore, western blotting was performed to investigate the protein expression levels of matrix metalloproteinases (MMPs) and mitogen-activated protein kinase. Notably, orally administered tyndalized L. acidophilus reduced the expression levels of MMP-1 and MMP-9. Based upon the aforementioned results, it was determined that tyndalized L. acidophilus effectively inhibited the wrinkle formation induced by UVB irradiation, and that this may be attributed to the downregulation of MMPs. Therefore, tyndalized L. acidophilus may be considered a potential agent for preventing skin photoaging and wrinkle formation.

Introduction

Skin is in continuous contact with the external environment, and protects the human body from potentially hazardous environmental threats, including physical, chemical and biochemical factors (1). Furthermore, skin prevents the entry of bacteria, fungi and viruses into the body, and provides a protective barrier that prevents moisture loss (2). Skin aging comprises several intrinsic processes, which are predominately genetically determined, and extrinsic aging that is typically associated with sun exposure; however, other factors may be involved in extrinsic skin aging, including ultraviolet (UV) irradiation, excessive alcohol consumption and environmental pollution (1,2).

Chronic exposure of human skin to the sun is characterized by epidermal hyperplasia and changes in the biomechanical properties of the dermis (3). These lead to wrinkle formation that may be observed histologically, and the development of deep wrinkles, nodules, irregular hyperpigmentation, telangiectasia and skin that has a leathery, rough texture, all of which are clinically evident (3).

Repeated exposure to UV radiation ultimately causes premature skin aging, or photoaging, which is characterized by the formation of fine and coarse wrinkles, an increase in the thickness of the skin, dryness, laxity and pigmentation (4). Exposure of the skin to UV light initiates the generation of active oxygen species in the skin (5), and exposure of the skin to UVB radiation is associated with elevated risks of erythema, edema, hyperplasia, sunburn-cell formation, photoaging, immune system suppression and skin cancer (6).

The role of probiotics in the regulation of intestinal health has been widely investigated for over 100 years. Probiotics are used with increasing frequency to treat medical conditions such as allergic diseases and atopic dermatitis, and to prevent dental caries and respiratory infections (7). Human clinical trials have demonstrated that probiotic supplementation may relieve atopic dermatitis and dry skin (8). Several studies involving Lactobacillus acidophilus have demonstrated...
that probiotics are effective against atopic dermatitis (9,10). However, the anti-wrinkle effects of tyndalized \textit{L. acidophilus} have not been investigated. Thus, the present study examined the effects of tyndalized \textit{L. acidophilus} on hairless mice that had developed skin damage following skin exposure to UVB radiation.

**Materials and methods**

**Materials.** Tyndalized \textit{L. acidophilus}, or ID-ACT3302, was obtained from Ildong Pharmaceutical Co., Ltd. (Seoul, South Korea). A total of 21 6-week-old HR-1 hairless male mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). UVB irradiation was administered using a UVM-225D Mineralight UV Display Lamp (UVP, Inc., Upland, CA, USA). Rabbit anti-phospho-stress-activated protein kinase/Jun-n-aminOO-terminal kinase (SAPK/JNK; cat. no. 9251), anti-SAPK/JNK (cat. no. 9252), anti-phospho-p44/42 mitogen-activated protein kinase (MAPK) extracellular signal-regulated protein kinases 1 and 2 (ERK1/2; cat. no. 9101), anti-p44/42 MAPK (ERK1/2; cat. no. 9102), anti-phospho-p38 MAPK (cat. no. 9211) and anti-p38 MAPK (cat. no. 9212) polyclonal antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Goat anti-matrix metalloproteinase (MMP)-1 polyclonal antibody (cat. no. sc12348), mouse anti-MMP-9 monoclonal antibody (cat. no. sc-21733), goat anti-β-actin polyclonal antibody (cat. no. sc-1616) and horseradish peroxidase (HRP)-conjugated anti-rabbit (cat. no. sc-2030) and anti-goat (cat. no. sc-2020) secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA).

**Experimental animals and oral administration.** After purchase, the HR-1 hairless male mice were stabilized for 1 week prior to the commencement of the study. The animals were housed in a climate-controlled facility at a temperature of 24°C, at 50% humidity with a 12-h dark:light cycle and \textit{ad libitum} access to food and water. All experimental protocols were approved by the Korea Institute of Oriental Medicine Institutional Animal Care and Use Committee (Daejeon, South Korea). The mice were divided into 3 groups as follows: Control (n=5), UVB-treated vehicle (n=5), and UVB-treated tyndalized \textit{L. acidophilus} (n=5) groups. Mice from the UVB-treated tyndalized \textit{L. acidophilus} group were orally administered 0.1 ml water containing 100 mg of tyndalized \textit{L. acidophilus}/kg body weight/day. The control group did not receive irradiation with UVB, or any other form of treatment. The vehicle group mice were orally administered 0.1 ml water and underwent irradiation with UVB.

**UVB irradiation.** UVB irradiation was administered using a UVM-225D Mineralight UV Display Lamp (UVP) that emitted at a wavelength of 302 nm. The strength of the UV radiation was measured using a HD2102-2 UV meter (Delta OHM Srl, Padova, Italy). UVB radiation was applied to the backs of the mice 3 times/week for 12 weeks. The amount of irradiation was progressively increased from 60 mJ/cm²/exposure during week 1 (1 minimal erythematous dose = 60 mJ/cm²) to 90 mJ/cm²/exposure during week 7.

**Skin hydration and transepidermal water loss (TEWL).** A corneometer (Courage + Khazaka electronic GmbH, Cologne, Germany) was used to determine the hydration levels of the skin, and a Tewameter (Courage + Khazaka electronic GmbH) was used to measure TEWL, which is an indicator of the barrier function of the skin's epidermis.

**Histological investigation.** The dorsal skin was removed from each hairless mouse and fixed in 10% neutral-buffered formalin. Using a conventional method, the fixed tissue samples were washed with distilled water, dehydrated using an ethanol gradient, cleared with xylene and embedded in paraffin wax, after which 5 µm sections were cut using a microtome. The tissue sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome stain for collagen fiber analysis. The thickness of the epidermis was measured under light microscopy using an eyepiece micrometer (Olympus Corporation, Tokyo, Japan).

**Western blotting.** Protein was extracted from the skin tissue samples using radioimmunoprecipitation assay lysis buffer.
The hydration of the stratum corneum was significantly reduced in the UVB-treated vehicle group, as compared with the control group ($P<0.0001$; Figure 2B). The protective effect of tyndalized L. acidophilus against changes in the number of collagen fibers (Figure 2C) was also observed.

**Results**

**Evaluation of TEWL and skin hydration.** The hydration of the stratum corneum was significantly reduced in the UVB-treated vehicle group, as compared with the control group ($P<0.0001$; Figure 2B). The protective effect of tyndalized L. acidophilus against changes in the number of collagen fibers (Figure 2C) was also observed.

**Statistical analysis.** All measurements were undertaken in triplicate, and all values are presented as the mean ± standard error. An analysis of variance and Tukey’s test was used to determine differences in the results among the study groups. Statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). $P<0.05$ was considered to indicate a statistically significant difference.

**Figure 2.** Tyndalized Lactobacillus acidophilus treatment reduced UVB-induced skin thickening in hairless mice. (A) Tyndalized L. acidophilus suppressed the UVB-induced increase in epidermal thickness. Hematoxylin and eosin staining of UVB-irradiated hairless mice skin. Magnification, x200. (B) Epidermal thickness of the dorsal skin. Data are presented as the mean ± standard error. ***$P<0.001$ vs. the control group. ##$P<0.01$ vs. the vehicle group. (C) Protective effect of tyndalized L. acidophilus against changes in the number of collagen fibers. Histological staining of hairless mouse skin stained with Masson’s trichrome stain. Collagen fibers are stained blue. Magnification, x200. Lac, tyndalized L. acidophilus; UVB, ultraviolet B.
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Fig. 1 A). The hydration of the skin from the UVB-treated tyndalized L. acidophilus group mice was not reduced to the same extent as that observed in the UVB-treated vehicle group and it was significantly different, as compared with the UVB-treated vehicle group (P<0.001; Fig. 1B).

Histological evaluation of the anti-wrinkle effect of L. acidophilus in UVB-irradiated hairless mice. To determine the wrinkle-reducing effects of tyndalized L. acidophilus, skin was removed from the hairless mice and skin tissue sections were stained. H&E staining of the skin confirmed that the thickness of the stratum corneum and the epidermis had increased in the UVB-treated vehicle group compared with the skin from the control group (Fig. 2A and B; P<0.001). Masson's trichrome staining revealed that the collagen was uniformly distributed in the dermal layer (Fig. 2C). Collagen fibers were increased in the UVB-treated tyndalized L. acidophilus group compared with the UVB-treated vehicle group (Fig. 2C). These results suggest that tyndalized L. acidophilus reduced the level of skin-wrinkling resulting from UV irradiation.

Evaluation of the anti-wrinkle effect of tyndalized L. acidophilus based on changes in epidermal thickness. To evaluate the anti-wrinkle effect of tyndalized L. acidophilus, changes in the thickness of the epidermis were investigated by measuring the distance from the keratin layer to the epidermal basement membrane in skin sections stained with H&E using a microscope. The skin epidermal thickness was significantly increased in the UVB-treated vehicle group, as compared with the control group (P<0.001; Fig. 2B). The skin epidermal thickness of the the UVB-treated tyndalized L. acidophilus group was decreased by 54.1%, as compared with the UVB-treated vehicle group, and this difference was statistically significant (P<0.01). These results suggest that tyndalized L. acidophilus is able to reduce the thickness of the epidermis and may be an effective anti-wrinkle agent.

Western blotting. UVB irradiation increased the expression of the MMPs, MMP-1 and MMP-9. Orally administered 100 mg tyndalized L. acidophilus reduced the expression levels of MMP-1 and MMP-9 (Fig. 3A). Furthermore, tyndalized L. acidophilus treatment suppressed the UVB-induced upregulation of MMP-1 and MMP-9. The effect of tyndalized L. acidophilus on UVB-induced MAPK phosphorylation was examined in mouse skin, as MMP-9 is primarily regulated by MAPK activation. As revealed in Fig. 3B, UVB irradiation induced the phosphorylation of p38, ERK 1/2, and JNK. Pretreatment with tyndalized L. acidophilus attenuated the phosphorylation of JNK 1/2, and p38. (C) Tyndalized L. acidophilus inhibited the phosphorylation of ERK or MEK, and ERK 1/2. Lac, tyndalized L. acidophilus; con, control; UV, ultraviolet; MMP, matrix metalloproteinase; JNK, Jun-amino-terminal kinase; MEK, MAPK/ERK kinase; ERK1/2, extracellular signal-regulated protein kinase 1/2. **P<0.01, ***P<0.001 vs. the vehicle group. "P<0.01, ""P<0.001 vs. the control group.

Discussion

Photoaging refers to premature skin aging caused by chronic exposure to UV radiation, particularly the UVB component, which is regarded as the primary cause of skin damage (11). Acute or chronic exposure to UVB radiation is a major cause of dermatologic disorders. UVB irradiation of the skin induces...
with the downregulation of the MAPK family of protein kinases and MEK. It was determined that the expression levels of JNK, p38, and ERK increased in UVB-irradiated skin, and that tyndalized *L. acidophilus* attenuated the elevations in the expression levels of the aforementioned proteins.

In conclusion, tyndalized *L. acidophilus* effectively inhibited wrinkle formation induced by UVB irradiation, and this inhibition was attributed to the downregulation of MMPs. Therefore, tyndalized *L. acidophilus* may serve as a potential agent for preventing skin photoaging and wrinkle formation.

Acknowledgements

The present study was supported by a grant from the Korea Institute of Oriental Medicine (grant no. K14101).

References


