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Topical application of glycolic acid suppresses the UVB induced IL-6, IL-8, MCP-1 and COX-2 inflammation by modulating NF-κB signaling pathway in keratinocytes and mice skin

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ABSTRACT

Background: Glycolic acid (GA), commonly present in fruits, has been used to treat dermatological diseases. Extensive exposure to solar ultraviolet B (UVB) irradiation plays a crucial role in the induction of skin inflammation. The development of photo prevention from natural materials represents an effective strategy for skin keratinocytes.
Objective: The aim of this study was to investigate the molecular mechanisms underlying the glycolic acid (GA)-induced reduction of UVB-mediated inflammatory responses.
Methods: We determined the effects of different concentrations of GA on the inflammatory response of human keratinocytes HaCaT cells and C57BL/6J mice dorsal skin. After GA was topically applied, HaCaT and mice skin were exposed to UVB irradiation.
Results: GA reduced the production of UVB-induced nuclear factor kappa B (NF-κB)-dependent inflammatory mediators [interleukin (IL)-1β, IL-6, IL-8, cyclooxygenase (COX)-2, tumor necrosis factor-α, and monocyte chemoattractant protein (MCP-1)] at both mRNA and protein levels. GA inhibited the UVB-induced promoter activity of NF-κB in HaCaT cells. GA attenuated the elevation of senescence associated with β-galactosidase activity but did not affect the wound migration ability. The topical application of GA inhibited the genes expression of IL-1β, IL-6, IL-8, COX-2, and MCP-1 in UVB-exposed mouse skin. The mice to UVB irradiation after GA was topically applied for 9 consecutive days and reported that 1–1.5% of GA exerted anti-inflammatory effects on mouse skin.
Conclusion: We clarified the molecular mechanism of GA protection against UVB-induced inflammation by modulating NF-κB signaling pathways and determined the optimal concentration of GA in mice skin exposed to UVB irradiation.

1. Introduction

Alpha hydroxy acids (AHAs), including glycolic acid (GA) and citric acid, are naturally occurring organic acids commonly present in foods. GA has been established as a safe, nontoxic substance in fruits and participates in multiple bioactivities such as inducing antioxidant activity, increasing the effects of melasma treatment, and biosynthesizing ceramide [1,2]. GA was initially used to treat disorders of keratinization [3], and was used for increasing the production of dermal and epidermal glycosaminoglycans to

Abbreviations: AHAs, alpha hydroxy acids; COX-2, cyclooxygenase-2; GA, glycolic acid; GM-CSF, granulocyte-macrophage colony-stimulating factor; HRP, horseradish peroxidase; IL-1β, interleukin-1β; MCP-1, monocyte chemoattractant protein; NHEKs, normal human epidermal keratinocytes; NF-κB, nuclear factor kappa B; ROS, reactive oxygen species; SA-β-gal, senescence-associated-β-gal; TNF-α, tumor necrosis factor-α; UVB, ultraviolet B.

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prevent both dermal and epidermal atrophy resulting from long-term topical corticosteroid use [4]. Studies have reported that UV irradiation induces phototoxic responses involving peeling of drug-treated skin. However, GA has also been used in multifunctional cosmetic products containing skin photo-protective agents [5–7]. In our previous study, we clarified that the photo-protective or phototoxic effects of GA in UVB-treated human keratinocytes in vitro and in animal skin are dependent on its concentrations [8]. We demonstrated that GA either alone or with UVB irradiation reduces the expression of inflammasome genes, such as NLRC4 and ASC, through epigenetic modification by increasing total DNA methyltransferase activity [8]. Recently, we demonstrated that GA significantly prevented the UVB-induced loss of skin cell viability, reactive oxygen species (ROS) formation, and DNA damage in normal human epidermal keratinocytes (NHEKs) (data not show). Currently, knowledge regarding the anti-inflammatory and photo-protective effects of GA and its mechanism of action in keratinocytes is limited.

UV irradiation typically induces acute phase responses and stimulates inflammatory factors in the skin, such as interleukin (IL)-1, IL-6, IL-7, IL-8, IL-12, IL-15, monocyte chemoattractant protein (MCP)-1, tumor necrosis factor (TNF)-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF) from keratinocytes, leading to inflammation of skin [9–12]. These cytokines play crucial roles in various inflammatory skin diseases such as psoriasis and atopic dermatitis [13–15]. Moreover, IL-1, IL-6, IL-8, TNF-α, and MCP-1 are closely related to the wound healing process and are used for wound age determination [16,17]. Nuclear factor kappa B (NF-κB) is a major factor mediating UVB-induced inflammatory responses through the expression of various proinflammatory proteins such as inducible nitric oxide synthase, TNF-α, and IL-6 [18–20]. UVB induces the constitutive expression of cyclooxygenase (COX)-2, which is the primary source of elevated PGE2 in the skin [21]. Repeated exposure to UV irradiation leads to ROS accumulation, photo-aging, and photocarcinogenesis [22]. Numerous studies reported that topical application of antioxidants engenders photo-protective effects and reduces UVB-induced inflammatory responses or ROS accumulation in hairless mouse skin [19,22,23]. ROS contributes to the induction of cellular senescence, as evidenced by premature ROS accumulation on treatment with antioxidants or inhibitors of cellular oxidant scavengers. GA has various biological activities such as antioxidant anti-inflammatory effects. Furthermore, GA not only exists in all whole fruits but also acts as an antioxidant, which protects keratinocytes from the ROS-mediated damage induced by UVB irradiation [24].

In this study, we hypothesized that GA has anti-inflammatory effects on UVB-stimulated human keratinocytes, and we tested this hypothesis in an animal model. Specifically, the objectives of this study were to (I) determine the GA-induced inhibition of UVB-induced cytokine and chemokine expressions in HaCaT and NHEK cells, (II) elucidate the mechanisms underlying GA-regulated anti-inflammatory cytokine expression, (III) determine the photo-protective effects of GA in the animal model, and (IV) clarify the effectiveness of GA in fostering ant senescence and in regulating the wound healing process of skin cells.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice (8–9 weeks old) were purchased from the National Laboratory Animal Center (R.O.C.) and housed in the Laboratory Animal Center, Tzu-Chi University. The mice were maintained in the animal resource facility for at least 1 week before experimental use and under standard conditions of 12-h light/dark cycles at a temperature of 24 ± 2 °C and relative humidity of 50% ± 10%. The mice were anesthetized with an intraperitoneal injection of ketamine:xylazine (80:10 mg/kg body weight) before being subjected to experimental procedures. The mice were categorized into five groups ($n = 6$). The hair on the back of the mice was removed using commercially available hair removal creams containing thioglycolate trihydrate (approximately 250 μL/mouse) 2 to 3 days before the experiments. We determined the effects of the topical application of aqueous solutions of GA (1%, 1.5%, 2%, 2.5%, and 3%; Sigma Chemical Co., USA). These solutions were prepared, and the back of each animal was marked with India ink, dividing it into five squares ($0.6 \times 0.6\ cm^2$ each). Four of these squares were treated with 10 μL of each concentration of peeling agents, whereas one square was left untreated. The mice were pretreated with GA once daily before they were exposed to UVB irradiation ($50\ mJ/cm^2$). For long-term UVB irradiation, GA was topically applied on mice in group 2 for 9 consecutive days. On day 10, the mice in group 2 were subjected to UVB irradiation ($50\ mJ/cm^2$), and they were sacrificed on day 11. We removed their dorsal skin and analyzed the proinflammatory cytokines. The mice were irradiated using KLBiotech STS-1 sunlamps (KLBiotech, Taiwan). The energy output of UVB (290–390 nm) was $1.5\ mW/cm^2$, as determined using a Dermaray UV meter and detector (Gigahertz-Optik, Pochheim, Germany).

Our animal protocols were approved by the Animal Care Committee, and they comply with the commonly accepted three Rs of Tzu-Chi University (approval ID 103-29-1). Mouse skin samples were obtained, formalin fixed, paraffin embedded, and sectioned for hematoxylin and eosin staining, according to previously reported methods [25,26]. For immunohistochemistry staining, 4-μm-thick serial sections were mounted on glass slides and stained with anti-IL-6 and -IL-8 (Biobyt, California, USA) and MCP-1 (Santa Cruz Biotechnology Inc.), followed by a horseradish peroxidase (HRP)-labeled secondary antibody, and visualized (Dako Cytomation). Immunohistochemistry was performed according to the manufacturer's instructions.

2.2. Cell culture and drug treatments

HaCaT cells and primary NHEKs were obtained from CLS (Cell Lines Service GmbH, Germany). The HaCaT cells were grown in Dulbecco's modified Eagle's medium (Gibco–Invitrogen, Carlsbad, CA, USA), with 10% fetal bovine serum (Gibco–Invitrogen); the NHEKs were used up to passage four and maintained in the undifferentiated state by maintaining calcium concentrations below 0.06 mM. The NHEKs were grown in KGM2 medium containing epidermal growth factor (Lonza Co., Ltd.). All the cells were cultured in a humidified atmosphere containing 5% CO_2 at 37 °C. Cells at 70–80% confluence were stimulated with GA at 0.1 (pH 7.4) or 5 mM (pH 7.1) for 24 h. Subsequently, the cells were harvested at the indicated time for RNA or protein isolation.

2.3. UVB irradiation

UVB was supplied by a closely spaced array of KLBiotech STS-1 sunlamps. The energy output of UVR (290–320 nm), as measured using a UVB photometer (LT Lutron, UV-340A photometer, International Light, Taiwan), was determined to be $1.5\ mW/cm^2$. Immediately before UVB irradiation, the medium was replaced with phosphate-buffered saline (PBS, pH 7.3).

2.4. Cytokine assay, quantitative determination of cytokine, and viability assay

HaCaT cell-conditioned media were centrifuged for 5 min at $1200 \times g$, and the supernatants were then diluted for use in the

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