DCP-LA REVIVES UV IRRADIATION-INDUCED ELASTIC FIBER DEGRADATION AND EPITHELIAL HYPERPLASIA

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ABSTRACT

Ultraviolet (UV) exposure causes skin damage, which includes skin thickness, formation of wrinkles, and loss of skin elasticity. UVB irradiation to the dorsal skin of HR-1 hairless mice reduced the density of elastic fibers in the extracellular matrix and thickened the epidermis. Application of the linoleic acid derivative 8-[2-(2-pentyl-cyclopropylmethyl)-cyclopropyl]-octanoic acid (DCP-LA) to the dorsal skin of mice apparently suppressed UVB irradiation-induced reduction of elastic fibers and epithelial hyperplasia, while linoleic acid and ceramide exhibited no significant beneficial effect. This indicates that DCP-LA has the potential to restore UV exposure-induced skin damage including formation of wrinkle.

Key Words: Ultraviolet ray, Elastic fiber degradation, Epidermal thickness, DCP-LA, Restoration
INTRODUCTION

Aged skin is characterized by skin thickness, wrinkle formation, and loss of skin elasticity. Elastin in the skin is indispensable for keeping skin elasticity, and ultraviolet (UV) exposure disrupts elastin, resulting in wrinkled or stretched-out skin. UV irradiation causes photo-oxidative damage to the skin by producing reactive oxygen species (ROS) (Herrling et al., 2006; Watson et al., 2014; Rinnerthaler et al., 2015). Superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), which reduce oxidant levels, are capable of protecting the skin from ROS-induced damage by reducing oxidant levels (Knaggs, 2009; Oresajo et al., 2012; Wölfl et al., 2014).

The linoleic acid derivative 8-[2-(2-pentyl-cyclopropyl)methyl]-cyclopropyl]-octanoic acid (DCP-LA) with cyclopropane rings instead of cis-double bonds on linoleic acid, that we have devised, serves as a selective and direct activator of the novel protein kinase C (PKC) isozyme PKCe (Kanno et al., 2006; Shimizu et al., 2011). Moreover, DCP-LA exhibits an anti-oxidant effect, i.e., DCP-LA protects neurons from oxidative stress-induced apoptosis by inhibiting caspase-3/-9 activation (Yaguchi et al., 2010). Then, we postulated that DCP-LA might ameliorate UV exposure-induced skin damage. We show here that DCP-LA suppresses UVB irradiation-induced elastic fiber degradation and epithelial hyperplasia in the HR-1 hairless mouse skin.

MATERIALS AND METHODS

Animal care
All procedures were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**UVB irradiation**

Male HR-1 hairless mice (7-8 weeks of age) were purchased from Japan SLC Inc. (Shizuoka, Japan). Mice were separated and housed in the special cage equipped with a Toshiba FL-20 SE fluorescent lamp (Toshiba Electric, Tokyo, Japan) with the range of wavelengths from 280 to 340 nm, with the maximum wavelength of 305 nm. The intensity of irradiation was set at 0.3 mW/cm² by using a UV radiometer. The time of irradiation was changed to control the UVB energy applied to the dorsal skin of mice. The value of the minimal erythema dose (MED) was about 54 mJ/cm². Long-term repeated UVB irradiation was carried out by the method as previously described (Inomata et al., 2003). Briefly, the dose was raised from 36 to 216 mJ/cm² in a step-by-step manner at 1-week intervals. UVB was irradiated at three times per week for 9 weeks, and the total exposure dose was approximately 4.6 J/cm².

DCP-LA (1 mg/mL), linoleic acid (1 mg/mL), or ceramide (1 mg/mL), which is diluted with polyethylene glycol (PEG), or PEG alone was applied once a day to the back skin of mice for a week prior to the end of last UVB irradiation.

**Immunohistochemical and histological analysis**

After UVB irradiation, mice were sacrificed and the skin was removed. Then, the skin was mounted in an optimal cutting temperature compound and cut at an
approximately 10-µm in thickness using a cryostat. Sections were blocked with 5% (v/v) goat serum in PBS-Triton X-100 for 1 h and incubated with an anti-elastin antibody (1:2000)(Millipore) for overnight at 4 °C followed by a goat anti-mouse IgG (1:500)(Invitrogen, Carlsbad, CA, USA) for 1 h at room temperature. After DAPI staining, sections were visualized on a confocal scanning laser microscope (Axiovert/LSM510; Carl Zeiss).

For a different set of examinations, sections of the dorsal skin were subjected to hematoxylin and eosin staining. Then, five fields per stained section under a light microscope were randomly chosen and epidermal thickness was measured as the distance from the basement membrane in the interfollicular epidermis to the bottom of the stratum corneum.

Statistical analysis

Statistical analysis was carried out using Dunnett’s test.

RESULTS AND DISCUSSION

UVB irradiation decreased the density of elastic fibers in the extracellular matrix of the dermis in the dorsal skin of HR-1 hairless mice, as compared with that for control mice without UVB irradiation and application of DCP-LA (Figure 1). UVB irradiation markedly reduced the density of elastic fibers, and application of DCP-LA to the dorsal skin of mice suppressed UVB irradiation-induced elastic fiber degradation (Figure 1).
UVB irradiation, alternatively, thickened the epidermis in the mouse skin to an extent much greater than that for control mice (Figure 2A-C). Application of DCP-LA significantly diminished UVB-induced epidermal hyperplasia (Figure 2A). In contrast, application of linoleic acid and ceramide had no significant effect on UVB irradiation-induced epithelial hyperplasia (Figure 2B,C).

We have earlier found that DCP-LA has an anti-oxidant effect (Yaguchi et al., 2010). The protective effect of DCP-LA against UVB irradiation-induced skin damage obtained here might be attributed to the anti-oxidant effect of DCP-LA. Fibulin-5/DANCE is an elastin-binding protein and forms elastic fibers (Yanagisawa et al., 2002; Hirai et al., 2007). In the preliminary study, DCP-LA increased extracellular elastin and elastic fibers in cultured human fibroblasts, and the effect was abolished by the PKC inhibitor GF109203X or knocking-down PKCe (unpublished data). DCP-LA indeed revived UVB irradiation-induced elastic fiber degradation in the HR-1 hairless mouse skin. This raises the possibility that DCP-LA promotes formation of elastic fibers in the extracellular matrix by activating PKCe, to restore UVB irradiation-induced elastic fiber degradation, by the mechanism distinct from the anti-oxidant effect.

Epidermal hyperplasia causes formation of wrinkle as well as reduced elastic fibers (Mizushima, 2013). DCP-LA significantly inhibited UVB irradiation-induced epidermal thickness, while linoleic acid and ceramide exhibited no beneficial effect. This indicates that the effect of DCP-LA on the epidermal thickness is not due to the non-specific action, e.g., the sun-block action. Collectively, these results suggest that DCP-LA enables recovery from UV irradiation-induced wrinkle formation in the skin.
We have obtained the data that the concentrations of DCP-LA in the epidermis and dermis increased after 3-h application on the hairless mouse skin (unpublished data). Moreover, the concentration of cytosolic DCP-LA, when extracellularly applied to PC-12 cells, increased in a concentration-dependent manner in parallel with increased concentrations in the plasma membrane (Kanno et al., 2015). These results provide evidence that DCP-LA actually permeates the skin, arriving in the epidermis and dermis.

CONCLUSION

The results of the present study demonstrate that the linoleic acid derivative DCP-LA neutralizes UVB irradiation-induced elastic fiber reduction in the extracellular matrix of the dermis and epidermal hyperplasia in the HR-1 hairless mouse skin. This indicates that DCP-LA could become an anti-wrinkle compound.
REFERENCES


Yaguchi T, Fujikawa H, Nishizaki T. Linoleic acid derivative DCP-LA protects


Figure 1. The effect of DCP-LA on UVB irradiation-induced elastic fiber degradation. DCP-LA (1 mg/mL) or PEG [DCP-LA (-)] was applied once a day to the back skin of hairless mice for a week prior to the end of last UVB irradiation. After sacrifice, the dorsal skin was removed and sections were subjected to immunohistochemistry using an anti-elastin antibody. Bars, 50 µm.
Figure 2. The effect of DCP-LA on UVB irradiation-induced epithelial hyperplasia.

DCP-LA (1 mg/mL)(A), linoleic acid (LA)(1 mg/mL)(B), or ceramide (1 mg/mL)(C) was applied once a day to the back skin of hairless mice for a week prior to the end of last UVB irradiation. Sections of the dorsal skin were subjected to hematoxylin and eosin staining and epidermal thickness was measured. In the graphs, each column represents the mean (± SEM) epidermal thickness (n=3-6 independent mice). *P* values, Dunnett's test. Bars, 50 µm.