DCP-LA Exerts an Antiaging Action on the Skin

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Aged skin · Antiaging · DCP-LA · NO stress · Senescence-associated β-galactosidase

Abstract
The present study assessed the possibility for the linoleic acid derivative 8-[2-(2-pentyl-cyclopropylmethyl)-cyclopropyl]-octanoic acid (DCP-LA) as an antiaging compound for the skin by assaying senescence-associated β-galactosidase (SA-β-Gal), a biomarker of senescence and cell viability. The nitric oxide (NO) donor sodium nitroprusside (SNP) increased in SA-β-Gal-positive cells in cultured human fibroblasts and mouse keratinocytes, and DCP-LA significantly inhibited the effect of SNP. Moreover, SNP induced cell death in cultured mouse keratinocytes, and DCP-LA significantly prevented NO stress-induced death of keratinocytes. Taken together, these results indicate that DCP-LA exerts an antiaging action on the skin.

Introduction
Skin aging is classified into two types: natural aging, which results from the passage of time, and photoaging, which results from habitual ultraviolet (UV) exposure [1, 2]. The latter is superimposed on intrinsic aging, and both natural aging and photoaging are cumulative. Of the factors responsible for facilitating skin aging, oxidative stress plays a critical role. Oxidative stress is induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are produced in the normal cellular redox process [3]. UV exposure enhances ROS/RNS production [4–6], and the free radicals cause skin damage including apoptosis by interacting with DNAs, RNAs, and proteins [7]. Several lines of evidence have pointed to the implication of mitogen-activated protein kinase, aryl hydrocarbon receptor, transcription factors, such as nuclear factor-κB and nuclear factor erythroid 2-related factor 2, or matrix metalloproteinase in the degradation of dermal connective tissue following oxidative stress-induced skin damage [8–15].

Senescence-associated β-galactosidase (SA-β-Gal) has been proposed as a biomarker of senescence [16]. Overexpression and accumulation of endogenous lysosomal β-Gal is found specifically in senescent cells [17]. β-Gal hydrolyzes β-galactosides into monosaccharides. The quantitative β-Gal assay using X-Gal, a substrate, was devised, where senescent cells contain blue-dyed precipitate [18–20]. The assay is widely used to assess cell senescence, although expression of β-Gal is not required for senescence.

A variety of skin care products and food supplements which exhibit an antioxidant effect have been produced. We have earlier synthesized the linoleic acid derivative
8-[2-(2-pentyl-cyclopropylmethyl)-cyclopropyl]-octanoic acid (DCP-LA), which contains cyclopropane rings instead of cis-double bonds on linoleic acid. We have found that DCP-LA inhibits oxidative stress-induced apoptosis of neurons by inhibiting caspase-3/9 activation [21]. Then, we were prompted to assess the effect of DCP-LA on oxidative stress-induced skin damage. The results of the present study demonstrate that DCP-LA exhibits an antiaging effect on the skin.

**Materials and Methods**

**Cell Culture**

Normal human dermal fibroblasts were purchased from Lonza (Verviers, Belgium). Cells were grown in FGM™-2 BulletKit™ (Lonza) in a humidified atmosphere of 5% CO₂ and 95% air at 37°C. Fibroblasts with 3–5 passages after initial culture were used for experiments.

Epidermal keratinocytes were prepared from HR-1 mouse skin using 1.5% dispase overnight at 4°C. After mechanical dissociation, cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal bovine serum, penicillin (final concentration, 100 U/ml), and streptomycin (final concentration, 0.1 mg/ml) in a humidified atmosphere of 5% CO₂ and 95% air at 37°C. Keratinocytes (passage 3) were used for experiments.

**β-Gal Staining**

Cells were fixed with 2% (w/v) paraformaldehyde for 5 min and incubated with fresh SA-β-Gal staining solution (1 mg/ml 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 150 mM NaCl, 2 mM MgCl₂, and 40 mM trisodium citrate, pH 6.0 with NaH₂PO₄) for 4 h at 37°C. SA-β-Gal-reactive cells were visualized with an inverted microscope (Ti-E; Nikon, Tokyo, Japan).

**Assay of Cell Viability**

Cell viability was assayed using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) using a method described previously [22]. MTT-reactive cells were quantified at an absorbance of 570 nm using a microplate reader (SpectraMax Plus 384; Molecular Devices, Sunnyvale, Calif., USA).

**Statistical Analysis**

Statistical analysis was carried out using Dunnett’s test and unpaired t test.

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**Fig. 1.** DCP-LA inhibits SNP-induced increase in SA-β-Gal-positive cells. Cultured human fibroblasts (a) or mouse keratinocytes (b) were treated with DCP-LA (100 nM) in the presence and absence of SNP (1 mM) for 24 h, followed by SA-β-Gal staining. In the graphs, each column represents the mean (± SEM) percentage of SA-β-Gal-positive cells against the number of total cells (n = 4 independent experiments). p values: Dunnett’s test.
Results and Discussion

The nitric oxide (NO) donor sodium nitroprusside (SNP) produces NO\(^3\)/NO\(^2\) to induce NO stress (oxidative stress). One would think that H\(_2\)O\(_2\) treatment is more physiological to induce oxidative stress than SNP treatment. We have confirmed that SNP induces oxidative stress, thereby activating caspase-3 and -9 responsible for neuronal cell death, and that DCP-LA neutralizes the effect of SNP \([21]\). To induce oxidative stress, therefore, SNP was used in the present experiments. SNP markedly increased the numbers of SA-β-Gal-positive cells in cultured human fibroblasts and mouse keratinocytes compared to non-SNP-treated control cells (fig. 1a, b). DCP-LA significantly neutralized the effect of SNP in both cell types (fig. 1a, b), which indicates that DCP-LA restores oxidative stress-induced skin aging.

SNP apparently reduced cell viability in cultured mouse keratinocytes in a concentration (0.3–1 mM)-dependent manner, and DCP-LA significantly prevented SNP-induced death of keratinocytes (fig. 2). This indicates that DCP-LA protects keratinocytes from oxidative stress-induced death. Overall, these results interpret that DCP-LA has the potential to suppress oxidative stress-induced skin aging.

So far, a number of antiaging drugs and compounds such as 17β-estradiol, aldosterone, and mineralocorticoid receptor antagonists, which are implicated in the synthesis and modulation of elastin, may exhibit an antiaging effect on the skin \([23, 24]\). DCP-LA serves as a direct and selective activator of protein kinase Cε \([25–27]\). DCP-LA also activates calcium/calmodulin-dependent protein kinase II by inhibiting protein phosphatase 1 \([28]\). DCP-LA, alternatively, inhibits protein tyrosine phosphatase 1B, thereby enhancing the receptor tyrosine kinase signaling pathway relevant to Rac1 activation \([29]\). Moreover, DCP-LA activates Akt and glycogen synthase kinase 3β, which is induced by protein kinase Cε \([30]\). In this preliminary study, DCP-LA ameliorated SNP-induced degradation of extracellular elastin/elastic fibers in cultured human fibroblasts in a protein kinase Cε-dependent manner. This effect of DCP-LA appears to be caused by a mechanism distinct from that of any other compounds previously shown. Taken together, DCP-LA may be developed as a novel antiaging compound for the skin.

Conclusions

DCP-LA inhibited the NO stress-induced increase in SA-β-Gal-positive cells and prevented NO stress-induced cell death in cultured fibroblasts and/or keratinocytes. This raises the possibility that DCP-LA may serve as an antiaging compound for the skin.

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Statement of Ethics

The author has no ethical conflict to disclose.

Disclosure Statement

The author has no conflict of interest to declare.
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