DCP-LA Exerts an Antiaging Action on the Skin

Tomoyuki Nishizaki

Innovative Bioinformation Research Organization, Kobe, Japan

Key Words

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.

© 2016 S. Karger AG, Basel

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- κ B 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

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.

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 sup-

plemented 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.

Fig. 2. DCP-LA prevents SNP-induced death of keratinocytes. Cultured mouse keratinocytes were treated with DCP-LA (100 nM) in the presence and absence of SNP at the concentrations indicated for 24 h, followed by MTT assay. Data represent the mean ($\pm$ SEM) percentage of basal MTT intensities for cells without treatment with DCP-LA in the absence of SNP ($n = 4$ independent experiments). p values: unpaired t test.

Results and Discussion

The nitric oxide (NO) donor sodium nitroprusside (SNP) produces $\text{NO}_3^-/\text{NO}_2^-$ to induce NO stress (oxidative stress). One would think that H_2O_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 for the skin has been provided. Some hormones and com-

pounds such as 17 β -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 C ϵ [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 3 β , which is induced by protein kinase C ϵ [30]. In this preliminary study, DCP-LA ameliorated SNP-induced degradation of extracellular elastin/elastic fibers in cultured human fibroblasts in a protein kinase C ϵ -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.

Acknowledgments

I thank Prof. Akito Tanaka (Hyogo University of Health Sciences, Kobe, Japan) for synthesizing and providing DCP-LA.

Statement of Ethics

The author has no ethical conflict to disclose.

Disclosure Statement

The author has no conflict of interest to declare.

References

- Yaar M, Eller MS, Gilchrist BA: Fifty years of skin aging. *J Invest Dermatol Symp Proc* 2002;7:51–58.
- Chung JH: Photoaging in Asians. *Photodermatol Photoimmunol Photomed* 2003;19:109–121.
- Tiwari AK: Antioxidants: new-generation therapeutic base for treatment of polygenic disorders. *Curr Sci* 2004;86:1092–1102.
- Herrling TH, Jung K, Fuchs J: Measurements of UV-generated free radicals/reactive oxygen species (ROS) in skin. *Spectrochim Acta A Mol Biomol Spectrosc* 2006;63:840–845.
- Watson RE, Gibbs NK, Griffiths CE, Sherratt MJ: Damage to skin extracellular matrix induced by UV exposure. *Antioxid Redox Signal* 2014;21:1063–1077.
- Rinnerthaler M, Bischof J, Streubel MK, Trost A, Richter K: Oxidative stress in aging human skin. *Biomolecules* 2015;5:545–589.
- Sivanandham V: Free radicals in health and diseases. *Pharmacol Online* 2011;1:1062–1077.
- Akasaka E, Takekoshi S, Horikoshi Y, Toriumi K, Ikoma N, Mabuchi T, Tamiya S, Matsuyama T, Ozawa A: Protein oxidative damage and heme oxygenase in sunlight-exposed human skin: roles of MAPK responses to oxidative stress. *Tokai J Exp Clin Med* 2010;35:152–164.
- Costa C, Catania S, De Pasquale R, Stancaneli R, Scribano GM, Melchini A: Exposure of human skin to benzo[a]pyrene: role of CYP1A1 and aryl hydrocarbon receptor in oxidative stress generation. *Toxicology* 2010;271:83–86.
- Katiyar SK, Meeran SM: Obesity increases the risk of UV radiation-induced oxidative stress and activation of MAPK and NF- κ B signaling. *Free Radic Biol Med* 2007;42:299–310.
- Hwang IS, Kim JE, Choi SI, Lee HR, Lee YJ, Jang MJ, Son HJ, Lee HS, Oh CH, Kim BH, Lee SH, Hwang DY: UV radiation-induced skin aging in hairless mice is effectively prevented by oral intake of sea buckthorn (*Hippophae rhamnoides* L.) fruit blend for 6 weeks through MMP suppression and increase of SOD activity. *Int J Mol Med* 2012;30:392–400.
- Lee JK, Ko SH, Ye SK, Chung MH: 8-Oxo-2'-deoxyguanosine ameliorates UVB-induced skin damage in hairless mice by scavenging reactive oxygen species and inhibiting MMP expression. *J Dermatol Sci* 2013;70:49–57.
- Lee YJ, Kwon SB, Kim CH, Cho HD, Nam HS, Lee SH, Lee MW, Nam DH, Choi CY, Cho MK: Oxidative damage and nuclear factor erythroid 2-related factor 2 protein expression in normal skin and keloid tissue. *Ann Dermatol* 2015;27:507–516.
- Ryu J, Kwon MJ, Nam TJ: Nrf2 and NF- κ B signaling pathways contribute to porphyria-334-mediated inhibition of UVA-induced inflammation in skin fibroblasts. *Mar Drugs* 2015;13:4721–4732.
- Zhu X, Jiang M, Song E, Jiang X, Song Y: Selenium deficiency sensitizes the skin for UVB-induced oxidative damage and inflammation which involved the activation of p38 MAPK signaling. *Food Chem Toxicol* 2015;75:139–145.
- Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O, Peacocke M, Campisi J: A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci USA* 1995;92:9363–9367.
- Lee BY, Han JA, Im JS, Morrone A, Johung K, Goodwin EC, Kleijer WJ, DiMaio D, Hwang ES: Senescence-associated beta-galactosidase is lysosomal beta-galactosidase. *Aging Cell* 2006;5:187–195.
- Bassaneze V, Miyakawa AA, Krieger JE: A quantitative chemiluminescent method for studying replicative and stress-induced premature senescence in cell cultures. *Anal Biochem* 2008;372:198–203.
- Gary RK, Kindell SM: Quantitative assay of senescence-associated β -galactosidase activity in mammalian cell extracts. *Anal Biochem* 2005;343:329–334.
- Itahana K, Campisi J, Dimri GP: Methods to detect biomarkers of cellular senescence: the senescence-associated β -galactosidase assay. *Methods Mol Biol* 2007;371:21–31.
- Yaguchi T, Fujikawa H, Nishizaki T: Linoleic acid derivative DCP-LA protects neurons from oxidative stress-induced apoptosis by inhibiting caspase-3/-9 activation. *Neurochem Res* 2010;35:712–717.
- Kanno T, Nishimoto T, Fujita Y, Gotoh A, Nakano T, Nishizaki T: Sphingosine induces apoptosis in MKN-28 human gastric cancer cells in an SDK-dependent manner. *Cell Physiol Biochem* 2012;30:987–994.
- Son ED, Lee JY, Lee S, Kim MS, Lee BG, Chang IS, Chung JH: Topical application of 17 β -estradiol increases extracellular matrix protein synthesis by stimulating TGF- β signaling in aged human skin in vivo. *J Invest Dermatol* 2005;124:1149–1161.
- Mitts TF, Bunda S, Wang Y, Hinek A: Aldosterone and mineralocorticoid receptor antagonists modulate elastin and collagen deposition in human skin. *J Invest Dermatol* 2010;130:2396–2406.
- Kanno T, Yamamoto H, Yaguchi T, Hi R, Mukasa T, Fujikawa H, Nagata T, Yamamoto S, Tanaka A, Nishizaki T: The linoleic acid derivative DCP-LA selectively activates PKC- ϵ , possibly binding to the phosphatidylserine binding site. *J Lipid Res* 2006;47:1146–1156.
- Shimizu T, Kanno T, Tanaka A, Nishizaki T: α , β -DCP-LA selectively activates PKC- ϵ and stimulates neurotransmitter release with the highest potency among 4 diastereomers. *Cell Physiol Biochem* 2011;27:149–158.
- Kanno T, Tsuchiya A, Shimizu T, Mabuchi M, Tanaka A, Nishizaki T: DCP-LA activates cytosolic PKC ϵ by interacting with the phosphatidylserine binding/associating sites Arg50 and Ile89 in the C2-like domain. *Cell Physiol Biochem* 2015;37:193–200.
- Kanno T, Yaguchi T, Nagata T, Tanaka A, Nishizaki T: DCP-LA stimulates AMPA receptor exocytosis through CaMKII activation due to PP-1 inhibition. *J Cell Physiol* 2009;221:183–188.
- Tsuchiya A, Kanno T, Nagaya H, Shimizu T, Tanaka A, Nishizaki T: PTP1B inhibition causes Rac1 activation by enhancing receptor tyrosine kinase signaling. *Cell Physiol Biochem* 2014;33:1097–1105.
- Kanno T, Tsuchiya A, Tanaka A, Nishizaki T: Combination of PKC ϵ activation and PTP1B inhibition effectively suppresses A β -induced GSK-3 β activation and tau phosphorylation. *Mol Neurobiol* 2015, Epub ahead of print.