pISSN 1226-2587
J. Soc. Cosmet. Sci. Korea eISSN 2288-9507
Vol. 44, No. 2, June 2018, 111-116 http://dx.doi.org/10.15230/SCSK.2018.44.2.111

레드비트를 함유하는 화장품의 담배 연기에 의한 피부 지질 산화 방지 효과

서 초 롱 · 하 태 현 · 문 지 영* · 김 정 미* · 박 병 권* · 이 지 원* · 박 진 오 · 신 진 희†

피엔케이피부임상연구센타(주), *유씨엘(주)

(2018년 3월 14일 접수, 2018년 5월 3일 수정, 2018년 5월 23일 채택)

Protective Effect of Cosmetics Containing Red Beet against Cigarette Smoke-induced Oxidative Damage in Human Skin

Cho Rong Seo, Tae Hyun Ha, Ji Young Moon*, Jeong Mi Kim*, Byoung Kwon Park*, Ji Won Lee*, Jin Oh Park, and Jin Hee Shin†

P&K Skin Research Center., 4F, 25, Gukhoe-daero 62-gil, Yeongdeungpo-gu, Seoul, Korea
*UCL Co., Ltd., 119, Neungheodaero 649-gil, Namdong-gu, Incheon, Korea
(Received March 14, 2018; Revised May 3, 2018; Accepted May 23; 2018)

요 약: 최근 화장품 시장에서 안티폴루션 효능이 있는 화장품은 새로운 피부 건강을 위한 해결책으로 나타나고 있다. 외부(대기) 오염물질에 의해 피부의 산화 기전을 매개하는 주요 원인 인자들로 오존, UV, 미세먼지 및 담배연기 등을 꼽을 수 있다. 담배 연기의 노출은 직·간접적으로 피부 표피 내 지질의 산화를 야기한다. 피부의 지질 산화에 의해 squalene과 squalene monohydroperoxide의 비율 변화가 발생하고, malondialdehyde (MDA)가 지질산화 산물로 생성된다. 따라서, 본 연구에서는 담배연기 노출 시 발생하는 피부 산화에 대하여 레드비트를 함유하는 화장품이 방지할 수 있는 효능이 있는지에 대하여 MDA의 생성량으로 관찰하였다. 시험대상자 전박부를 세 영역(음성대조, 양성대조, 시험제품)으로 나누어 각 지름 3.3 cm의 원으로 구획하고, 15분간 담배연기에 노출 후 피부 표면으로부터 지질막을 걷어낸 후 TBARS assay를 통하여 MDA를 정량 하였다. 음성대조(무도포, 미노출)에 비해 양성대조(무도포, 담배연기 노출)의 MDA 생성량이 3.7배 증가된 결과로, 오염물질인 담배연기에 의한 피부의 산화적 손상을 확인하였다. 반면에 레드비트를 함유하는 화장품을 미리 도포한 영역은 양성대조에 비해 MDA 생성량이 25% 감소하는 결과를 나타내었다. 결론적으로, 담배연기 노출은 피부 표피 내 지질 산화를 야기하며, 레드비트를 함유하는 화장품이 이러한 대기환경오염으로부터 방어적 효과(안티폴루션 효과)를 보이는 것을 확인하였다.

Abstract: In cosmetics market, anti-pollution products recently come up with new solution for skin health. Environmental oxidation mechanisms are realized as bio-marker of atmospheric pollution upon skin by environmental pollutant such as ozone, UV rays, particulate matter (PM) as well as cigarette smoke. The exposure of cigarette smoke directly or indirectly causes the oxidation of the stratum corneum skin lipids, resulting in the conversion of squalene to squalene monohydroperoxide and/or generation of malondialdehyde (MDA) as a product of lipid peroxidation. The aim of this study is to see whether new cosmetics product containing red beet has anti-oxidation effect on skin exposed by cigarette smoke. So as to determine oxidative damage to human skin at biochemical level, each unit area of volar forearms was exposed to cigarette smoke through device (3.3 cm, diameter) for fifteen minutes, then measured MDA using standardized TBARS assay kit. Compared to negative control (untreated and unexposed area), the level of MDA was significantly

† 주 저자 (e-mail: jh.s@pnkskin.com)
call: 02)6925-1501

increased at positive control (untreated and exposed area) more than 3.7 times, indicating the pollutant induced-oxidative damage on the skin barrier. Whereas, the pre-applied area with the cosmetics products containing red beet revealed a decrease of 25% compared with positive control. As reports, these data demonstrated that cigarette smoke induce peroxidation of stratum corneum skin lipids. Conclusively, we suggest that anti-pollution effect of the cosmetics product containing red beet is beneficial to prevent the oxidation of skin lipids by atmospheric pollution.

Keywords: *anti-pollution, cigarette smoke, red beet, malondialdehyde, cosmetics product*

1. Introduction

Although there have been the traditional pollutions associated with smoke and smog, current pollutions are more complex and dynamic due to accumulating data on their different triggers and various mode of actions. Nowadays, concerns regarding the harmful skin effect are growing as contamination of environment by any chemical, physical or biological agent that modifies the natural characteristics of the atmosphere.

It is well known that air pollution is caused by industry, vehicle fumes, and cigarette smoke. Particulate matter (PM) plays a major role in primary pollution. It comprises toxic organic compounds, heavy metals, smoke from driving automobile and burning plants, dust from industrial factories and roads.

The exposure to pollution may cause in cutaneous stress because pollutants can react with skin tissue. As consequence, the topical pollutants alter functioning of the skin barrier, penetrate the skin barrier and cause oxidative stress and inflammation damages by reacting with skin proteins, lipids and DNA.

There is growing evidence that various environmental factors influence on extrinsic skin ageing. In particular, exposure of skin to tobacco smoke was found to be an independent pathogenic factor[1]. A major mechanism by which environmental factors exerts its detrimental effects is through the generation of oxidative stress[2], an important contributor to extrinsic skin aging[1]. The higher mRNA level of matrix metalloproteinase (MMP-1) was observed in smoker's buttock dermal connective tissues[3]. The tobacco extracts induced the production of MMP-1 and MMP-3 in response of dose dependent manner in the fibroblast[4]. Additionally, the smoking-caused skin degeneration was prevented by alpha lipoic acid, an anti-oxidant, in animal[5], supporting the causative role of cigarette smoke in oxidative stress.

Cigarette smoking results in structural and compositional change of epidermal or dermal skin similar to those from long-term exposure of UV radiation. Several lines of evidence reported the association between wrinkle formation and cigarette smoking[6,7]. Smoker's premature skin aging was revealed to characterize melanosis and wrinkle formation. Interestingly, cigarette smoke directly induced the lipid oxidation on human skin, showing the conversion ratio of squalene (SQ) to squalene monohydroperoxide (SQHPO). Moreover, the increased lipid oxidation was blocked by anti-oxidants, such as glutathione, thiotaurine and hypotaurine[8]. Red beet root was evident at lowering the level of microsomal lipid peroxidation in the liver and enhancing the activity of superoxide dismutase[9]. As these evidences, we performed in this study whether a product containing red beet would be reduce the lipid peroxidation induced by cigarette smoke on human skin.

Recently, particulate matter 2.5 micrometer (PM2.5) has been issued because of penetration into skin in cosmetics fields. The tobacco extracts are mainly micro molar molecule as well. Therefore, we would suggest the methodological paradigm for evaluating anti-pollution of cosmetics through this study.

2. Materials and Methods

2.1. Materials

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical, L-ascor-

bic acid, xanthine, xanthine oxidase, nitroblue tetrazolium (NBT), EDTA, sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4) were purchased from Sigma Chemical Co. (USA).

2.2. DPPH Radical Scavenging Activity

The assay for free radical scavenging capacity was carried out according to the method that has been reported previously by Blois et al.[10]. For detection of DPPH radical, red beet extracts in response to final dose 1.25, 2.5, and 5% were added to a 0.2 mM solution of DPPH and the reaction mixture was shaken vigorously. For the amount of residual DPPH, the absorbance of each well was determined at 517 nm. These steps were three times repeated. The free radical scavenging activity of each sample was calculated according to the following formula :

DPPH radical scavenging activity (%) = [1-(ODS-ODB)/(ODC-ODB)] × 100

ODS is the absorbance of the experimental sample, ODB is the absorbance of the blank, ODC is the absorbance of the control at 517 nm.

The results are reported in terms of SC_{50} (the concentration needed to reduce 50% of DPPH). L-ascorbic acid, a representative antioxidant, was used as a control.

2.3. Superoxide Radical Scavenging Activity

The assay for superoxide radical scavenging capacity was carried out according to the method that has been reported previously by Parejo et al.[11].

The antioxidant activity of superoxide generated by the reaction of hypoxathine with uric acid by xanthine oxidase was investigated by the action of nitroblue tetrazolium (NBT) to eliminate superoxide. To the 50 μL of 200 mM sodium phosphate buffer (pH 7.5) containing 1 mM xanthine, 1 mM EDTA and 0.1 mM NBT, 50 μL of red beet extracts in response to final dose 1.25, 2.5, and 5% were mixed well. The reaction was started by adding 100 μL of xanthine oxidase (50 mU/mL). The reaction mixture was incubated at 37 ℃ for 30 min. These steps were three times repeated. Absorbance was measured at 550 nm. The superoxide scavenging activity of each sample with red beet extracts was calculated according to the following formula :

superoxide radical scavenging activity (%) = [1-(ODS-ODB)/(ODC-ODB)] × 100

ODS is the absorbance of the experimental sample, ODB is the absorbance of the blank, ODC is the absorbance of the control at 550 nm.

2.4. Subjects

This study was performed with subjects who were aged 20 to 60. Subjects were total of 13 people, 12 males and 1 female, and the average age was 40.6 ± 8.4 years old. Selected subjects did not show any adverse effects nor any medical or drug history that might affect the study.

All subjects were given a detailed description of the study and signed a consent form prior to participation in the study. This study was approved by the Institutional Review Board of P&K Skin Research Center. The reception number for the IRB review is P1705-96.

2.5. Skin Treatment and Cigarette Smoke Exposure

The forearm was divided 3 areas by 3.3 cm diameter circle, which are test area (product-applied and cigarette smoke-exposed site), positive control (only cigarette smoke-exposed site), and negative control (nothing treated). On test area, cosmetics product containing red beet (4 $\mu L/cm^2$) was applied and absorbed for 15 min and subsequent exposed to cigarette smoke for 15 minutes. Either positive control was exposed by cigarette smoke or negative control was unexposed by it. Cigarette smoke was applied simultaneously on each site using the air compressor chamber. Six cigarettes were used for one application.

2.6. Skin Sample Collection and Thiobarbituric Acid Reactive Substances (TBARS) Assay

The swap sampling was performed to obtain the stratum corneum lipids on each test area using the sterilized cotton swabs soaked with the absolute ethanol (Merck, Germany). The stratum corneum lipids were extracted in 1 mL absolute ethanol by vortexing for 1 min. The lipid

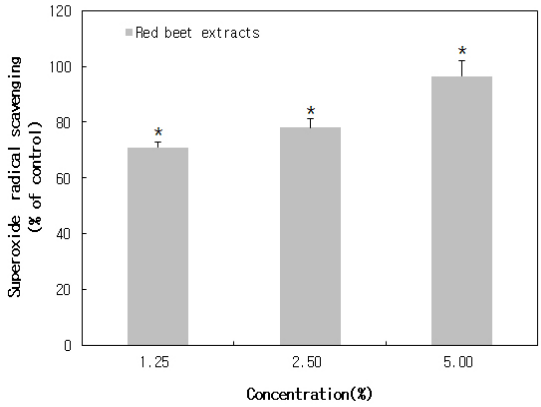

Figure 1. Scavenging effect on DPPH free radical of red beet extracts. The rate about DPPH radical scavenging comparison to control was increased by red beet extracts in a dose-dependent manner. The DPPH radical was fully blocked at 5% red beet extract. Data represent the mean ± S.D. with three separate experiments. *, $p < 0.05$ vs. control.

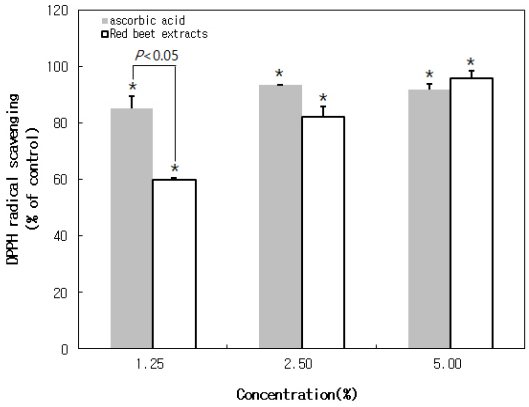

Figure 2. Scavenging effect on superoxide radical of the red beet extracts. The rate about superoxide radical scavenging comparison to control was increased by red beet extracts at indicated concentration. Data represent the mean ± S.D. with three separate experiments. *, $p < 0.05$ vs. control.

extracts were centrifuged at 1,600 g for 1 minute, and then supernatant was transferred into the new tube.

To assess the malondialdehyde (MDA) production in the stratum corneum lipids, MDA was measured using TBARS assay kit (Cayman Chemical, Ann Arbor, MI) according to manufacturer's methods.

2.7. Statistics

The statistical analysis package SPSS 19.0 was used to evaluate the efficacy of test product for skin changes. The parametric-test, Independent t-test was used according to the statistical treatment for analysis.

2.8. The Extraction of Red Beet

The red beet originated from Jeju was freeze-dried, and then 10g freeze-died red beet and 1L purified water were leached and filtered. The red beet-extracted liquid was used in this study.

2.9. All Ingredients in the Face Serum

Water, butylene glycol, niacinamide, cyclopentasiloxane, 1,2-hexanediol, cyclohexasiloxane, betaine, silica, glycerin, cetearyl olivate, sorbitan olivate, dimethicone, pentaerythrityl tetraethylhexanoate, sodium polyacrylate, *Beta vulgaris* (beet) root extract, sodium acrylates/sodium acryloyldimethyl taurate copolymer, polyisobutene, tocopheryl aceta, *Camellia sinensis* seed oil, *Citrus limon* (lemon) peel, glyceryl polyacrylate, *Helianthus annuus* (sunflower) seed oil, stearyl glycyrrhetinate, *Opuntia coccinellifera* fruit extract, *Aloe barbadensis* leaf extract, *Camellia sinensis* leaf extract, *Citrus junos* fruit extract, *Citrus paradisi* (grapefruit) fruit extract, *Eucalyptus globulus* leaf extract, *Eugenia caryophyllus* (clove) flower extract, *Sophora flavescens* extract, adenosine, caprylyl/capryl glucoside, disodium EDTA, phenoxyethanol, *Alkanna tinctoria* root extract, *Anthemis nobilis* flower oil, *Citrus aurantifolia* (lime) oil, *Eucalyptus globulus* leaf oil, *Juniperus mexicana* oil, *Lavandula angustifolia* (lavender) oil, *Pelargonium graveolens* flower oil, *Rosmarinus officinalis* (rosemary) leaf oil, tocopherol.

3. Results

3.1. The Free Radical Scavenging Effect of Red Beet Extracts

The red beet extract increased scavenging activity against DPPH free radical. The effects were observed in dose dependent manner. The anti-oxidative effect of red beet was similar to ascorbic acid, a positive control, at the

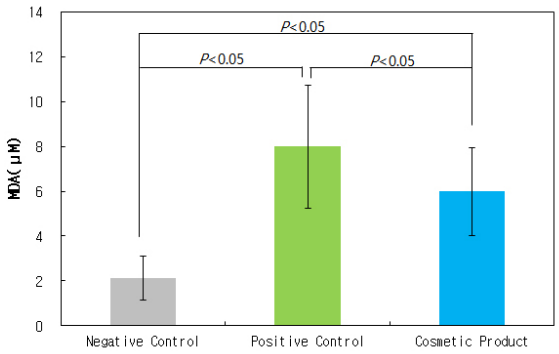

Figure 3. The anti-oxidative effect of the cosmetics product containing red beet in human skin. The level of MDA was significantly increased on the site exposed by only cigarette smoke(positive control), compared with on the site without exposure of cigarette smoke (negative control). The increased level of MDA was decreased by application of cosmetics product containing red beet on the site exposed by cigarette smoke. Means ± S.D. are shown (n = 13 subjects), significant difference among negative control, positive control, and cosmetic product, $p < 0.05$.

final dose 5% (Figure 1). Also, the anti-oxidative potency of red beet was confirmed through superoxide radical scavenging activity (Figure 2). The lowering effect of superoxide production was dose dependent about red beet. These results revealed that red beet is potent at scavenging free radical.

3.2. The Protective Effect of the Cosmetics Product Containing Red Beet on Oxidation of Stratum Corneum Lipid

Next, we performed TBARS assay at stratum corneum extracts after exposure to cigarette smoke in human whether the product containing red beet would effectively inhibit lipid peroxidation production. The volar forearms of 13 subjects was exposed to cigarette smoke for 15 min after product application or nothing applied. The collected corneum lipids by swabbing were analyzed for MDA production, a lipid peroxidation marker, using TBARS assay. The level of MDA was significantly increased approximately 3.7 times in positive control which was exposed only cigarette smoke, comparison to negative control. However, the increased MDA was dramatically inhibited by treatment with the cosmetics product containing red beet (Figure 3). This result showed that the anti-oxidative effect of red beet is reproducible in human as well as *in vitro*. Additionally, cigarette smoke, one of PMs, induced stratum corneum contamination showing elevated lipid peroxidation and the product containing red beet fundamentally revealed anti-pollution effect.

4. Discussion and Conclusion

From now, the method for anti-pollution effect of cosmetics was not represented in Korea. Several reports supported that cigarette smoke may play role as environmental pollutant on human skin[6,7]. As previous reported[8], the exposure of cigarette smoke caused lipid oxidation on human skin in our experiment. In this study, the red beet was potent active ingredient for anti-oxidative activation, showing DPPH and superoxide radicals scavenging *in vitro*. The effectiveness on anti-oxidation of the red beet was repeatable in human, lowering MDA production. In our previous test, the product without anti-oxidative action could not inhibit the increased MDA on skin (data not shown). Taken together, the exposure of cigarette smoke, as a pollutant, thoroughly induced the lipid peroxidation products in stratum corneum. The product containing red beet meaningfully blocked the increased MDA amounts. Therefore, we suggest methodological paradigm for analyzing anti-pollution effect of cosmetics product through this study.

Reference

1. P. Schroeder, S. M. Schieke, and A. Morita, Premature skin aging by infrared radiation, tobacco smoke and ozone, *Skin Aging*, 45 (2006).
2. K. Donaldson, L. Tran, L. A. Jimenez, R. Duffin, D. E. Newby, N. Mills, W. MacNee, and V. Stone, Combustion-derived nanoparticles: a review of their toxicology following inhalation exposure, *Part Fibre Toxicol.*, **21**, 2 (2005).
3. C. Lahmann, J. Bergemann, G. Harrison, and A. R.

Young, Matrix metalloproteinase-1 and skin ageing in smokers, *Lancet*., **357**, 935 (2001).
4. L. Yin, A. Morita, and T. Tsuji. Alterations of extracellular matrix induced by tobacco smoke extract, *Arch. Dermatol. Res*., **292,** 188 (2000).
5. F. Yıldırım Baş, D. Bayram, B. Arslan, I. Armağan, Ş. Yeşilot, E. Yeşilot, and E. Yorgancıgil, Effect of alpha lipoic acid on smoking-induced skin damage, *Cutan Ocul Toxicol*., **36**, 67 (2017).
6. H. W. Daniell, Smoker's wrinkles, A study in the epidemiology of "crow's feet", *Ann. Intern. Med.*, **75**, 873 (1971).
7. L. Yin, A. Morita and, T. Tsuji, Skin aging induced by ultraviolet exposure and tobacco smoking: evidence from epidemiological and molecular studies, *Photodermatol. Photoimmunol. Photomed.*, **17**, 178 (2001).
8. M. Egawa, Y. Kohno, and Y. Kumano, Oxidative effects of cigarette smoke on the human skin, *Int. J. Cosmet. Sci*., **21**, 83(1999).
9. M. Kujawska, E. Ignatowicz, M. Murias, M. Ewertowska, K. Mikołajczyk, and J. Jodynis-Liebert, Protective effect of red beetroot against carbon tetrachloride- and N-nitrosodiethylamine-induced oxidative stress in rats, *J. Agric. Food Chem.,* **25**, 2570 (2009).
10. M. S. Blois, Antioxidant determinations by the use of a stable free radical, *Nature*, **29**, 1199 (1958).
11. I. Parejo, F. Viladomat, J. Bastida, A. Rosas-Romero, N. Flerlage, J. Burillo, and C. Codina, Comparison between the radical scavenging activity and antioxidant activity of six distilled and nondistilled mediterranean herbs and aromatic plants, *J. Agric. Food Chem.*, **50**, 6882 (2002).