pISSN 1226-2587
J. Soc. Cosmet. Sci. Korea eISSN 2288-9507
Vol. 44, No. 1, March 2018, 67-72 http://dx.doi.org/10.15230/SCSK.2018.44.1.67

녹차뿌리 특화 사포닌의 천연 계면 활성력을 이용한 새로운 안티폴루션 기작 연구

나 혜 원 · 이 영 란 · 박 준 성 · 이 태 룡 · 김 형 준†

(주)아모레퍼시픽 기술연구원

(2018년 3월 10일 접수, 2018년 3월 23일 수정, 2018년 3월 24일 채택)

Green Tea Root Is a Potential Natural Surfactant and Is Protective against the Detrimental Stimulant PM2.5 in Human Normal Epidermal Keratinocytes

Hye-Won Na, Yeongran Lee, Jun Seong Park, Tae Ryoung Lee, and Hyoung-June Kim†

AMOREPACIFIC R&D Center, Yongin-si, Gyeonggi-do 17074, Korea

(Received March 10, 2018; Revised March 23, 2018; Accepted March 24, 2018)

요 약: 녹차(*Camellia sinensis* L.)는 약용으로도 널리 사용되어 왔다. 하지만, 대부분의 연구는 녹차 잎을 연구의 대상으로 이루어졌으며, 지금까지도 잎 외의 다른 부분은 연구가 많이 이루어지지 않았다. 본 연구에서는 외부환경의 미세먼지(particulate matter 2.5; PM2.5)에 의한 normal human epidermal keratinocytes (NHEKs)의 손상을 뿌리 추출물이 효과적으로 보호할 수 있는 효과를 확인하였다. 30년 된 녹차뿌리 표본은 아모레퍼시픽의 Dolsongi tea field에서 채취되었으며 70%의 에탄올로 추출한 녹차 뿌리 추출물은 총 순수 사포닌 함량이 54%로, 인삼 추출물보다 더 많은 사포닌을 함유한 것을 확인하였다. 그리고 PM2.5에 의한 손상으로부터의 보호 효과를 평가하기 위해 건선환자의 특이적 바이오 마커인 IL-36G를 관찰한 결과 IL-36G mRNA 발현량은 PM2.5 처리시 녹차 뿌리 추출물 처리를 통해 IL-36G 식의 유의적인 감소를 확인할 수 있었다. 결론적으로, 30년 된 녹차뿌리 추출물은 사포닌 함량이 높은 자연 계면 활성제로 사용될 수 있으며, 또한 피부질환마커인 IL-36G 발현의 억제를 통해 PM2.5에 의해 유발되는 손상에 대한 보호 효과가 있음을 알 수 있었다.

Abstract: Green tea (*Camellia sinensis* L.) has been widely explored for its medicinal applications. However, most of the studies had targeted the green tea leaf, while other parts remained unexplored. In this study, protective effect of green tea root extract on Normal Human Epidermal Keratinocytes (NHEKs) against the damage induced by an external stimulant (PM2.5) was confirmed. Thirty-year-old green tea root samples were collected from Amorepacific's Dolsongi tea field and green tea root extract was prepared with 70% ethanol. Total crude saponin content in green tea root extract was 54%, which is much higher than that in ginseng extract. Our results suggest that green tea root extract can be used as a natural surfactant in cosmetics. For evaluating its protective effect against the damage induced by PM2.5, IL-36G was used as a biomarker. IL-36G mRNA expression level increased remarkable upon PM2.5 treatment in NHEKs. Moreover, IL-36G was recently reported to be expressed in psoriasis lesions. Results showed significant decrease of IL-36G expression by treatment of green tea root extract. In conclusion, thirty-year-old green tea root extract can be used as a natural surfactant with a high saponin content and may have protective effect against the damage induced by PM2.5.

Keywords: *green tea root, natural surfactant, PM2.5, skin care*

† 주 저자 (e-mail: leojune@amorepacific.com)
call: 031)280-5827

1. Introduction

Green tea (*Camellia sinensis* L.) is one of the most widely consumed beverages in the world, so its medicinal properties have been widely explored. But most of the studies were targeted on green tea leaf, while there lack studies regarding other parts of green tea tree, especially root. It is expected that tea root might also be a storehouse of many chemicals of medicinal and pharmacological interest[1]. However, when the tea plants are uprooted 30-100 years after plantation, the roots are used either for making ornamental furniture or as firewood. So, we became interested in the new effects of green tea root, and focused on the effects against the detrimental influences of fine particulate matter (PM2.5) among the various benefits expected from green tea root. Ambient air pollution is becoming more severe worldwide, posing a serious threat to human health. Fine airborne particles of PM2.5 show higher cytotoxicity than other coarse fractions. Indeed, PM2.5 induces cardiovascular or respiratory damage; however, few studies have evaluated the detrimental effect of PM2.5 to normal human skin.

This research is focused on evaluating the efficacies of roots of green tea trees on human skin and the analysis of its unique contents.

2. Materials and Methods

2.1. Preparation of Green Tea Root Extracts

30-year-old green tea root samples were collected in Amorepacific Dosun tea garden (Jeju island, South Korea). Green tea root was extracted with 70% ethanol (v/v) at room temperature for 12 h with constant stir. Suspension was filtered through filter paper to retain the clear solution. The solution was vacuum evaporated below 50 ℃. The dried extracts were stored at 4 ℃.

2.2. Ultra Performance Liquid Chromatography (UPLC) Analysis of Amino Acids

The UPLC analysis of 21 kinds of amino acids and γ-aminobutyric acid was based on the Waters AccQ-Tag™ method (http://www.waters.com). Amino acid derivation with AccQ-Tag™ reagents was conducted according to the manufacturer's protocol. Briefly, 10 μL of either a standard amino acid mix solution, or a root extract were mixed with 70 μL of AccQ-Tag™ Ultra borate buffer, and 20 μL of AccQ-Tag™ reagent previously dissolved in 1.0 mL of AccQ-Tag™ Ultra reagent diluent were added. The reaction was allowed to proceed for 10 min at 55 ℃. Liquid chromatographic analysis was performed on a Waters Acquity UPLC system, equipped with a binary solvent manager, an autosampler, a column heater and a photodiode array (PDA) detector. The separation column was a Waters AccQ-Tag™ Ultra column (2.1 mm i.d. × 100 mm, 1.7 μm particles). The column heater was set at 55 ℃ and the mobile phase flow rate was maintained at 0.7 mL/min. Eluent A was 10% AccQ-Tag™ solvent A, and eluent B was 100% AccQ-Tag™ solvent B. The non-linear separation gradient was 0-0.54 min (99.9% A), 5.74 min (90.0% A), 7.74 min (78.8% A), 8.04-8.64 min (40.4% A), 8.73-10 min (99.9% A). One microliter of sample was injected for analysis. The PDA detector was set at 260 nm, with a sampling rate of 20 points/sec.

2.3. Total Crude Saponin Contents

Total crude saponin contents in green tea root extract were estimated by vanillin-sulfuric acid colorimetric methods[2]. To 100 μL of methanol solution of the sample, 0.3 mL vanillin solution of 8% (w/v) and then 4.0 mL of sulfuric acid of 75% (v/v) were added and thoroughly mixed in an ice water bath. The mixture was then warmed in a bath at 60 ℃ for 10 min then cooled in ice-cold water. Absorbance at 535 nm was recorded against the blank with the reagents using a Cary 60 UV/VIS spectrophotometer (Agilent Technologies).

2.4. Preparations of PM2.5

PM2.5 was collected on a Teflon filter (Zefluor, Pall Life Science, Ann Arbor, MI, USA) with a low-volume air sampler consisting of a cyclone (2.5-μm size cut, URG-2000-30EH), two upstream denuders (annular, URG-2000-30x242-3CSS), Teflon filter, back up filter,

and back up denuder in series. The collection started at around 10:00 a.m., replacing the filters every 24 h, at a flow rate of 16.7 L/min. The sampling region was located 35 km southeast of downtown Seoul, Korea (37.34 °N, 127.27 °E, 167 m above sea level).

2.5. Culturing of Normal Human Keratinocytes

Normal human epidermal keratinocytes (NHEKs) purchased from Lonza, Inc. (Walkersville, MD, USA) were cultured in a CO_2 incubator under the condition of 37 ℃ and 5% CO_2. The cells were cultured according to the instructions of Lonza, Inc. 500 mL of KBM-2 (CC-3103) and KGM-2 Bullet kit CC-3107 (BPE (bovine pituitary extract), human epidermal growth factor (hEGF), insulin, hydrocortisone, transferrin, epinephrine, gentamycin sulfate + amphotericin-B (GA-1000) were used.

2.6. Measurement of Cytotoxicity

In order to investigate the cytotoxicity of green tea root, Cell Counting Kit-8 (CCK-8) assay (Dojindo, MD, USA) was conducted. HaCat cells were seeded in triplicate in 96-well plate and green tea root extract was treated for 24 h on the next day. Then CCK-8 solution was added and incubated for 2 h. The absorbance was determined by measuring absorbance at 540 nm.

2.7. Treatment with PM2.5 and Quantitative Real-time PCR

NHEKs were seeded in triplicate and PM2.5 or PM2.5 with green tea root extract was treated for 24 h on the next day. Total RNA was isolated by using TRIzol™ reagent according to the manufacturer's instructions (Invitrogen, CA, USA). 4 μg of the RNA was reverse-transcribed into cDNA. The cDNA samples were determined by analyzing the expression level with the following probes: interleukin-36 gamma (IL-36G, Hs00219742_m1). All relative mRNA expression normalized to ribosomal protein L13a (RPL13A, Hs04194366_g1) (7500Fast, Applied Biosystems, CA, USA).

2.8. Analysis of Change in Genes in Cells by Microdust by Next-generation Sequencing (NGS)

For mRNA sequencing (RNA-seq) data processing and analysis, the general analysis method developed by Trapnell et al.[3] was used. Fast QC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used for quality control of the RNA-seq data and FASTX (http://hannonlab.cshl.edu/fastx_toolkit/) was used to remove base and adaptor sequences of low accuracy. Then, alignment was performed using Tophat[4] and human genome (hg19) and the data quantity for each sample was confirmed using EVER-seq renamed as quality control of RNA-seq (RSeQC)[5]. Also, the expression level of transcripts was quantified with Cufflinks and comparison was made between the samples treated with the microdust dispersion and a normal sample[6]. By applying a strict cutoff of false discovery rate (FDR) adjusted p-value < 0.05 and ≥ 2.0 fold-change, the genes which showed significant change in expression upon treatment with the dispersion of microdust with a particle diameter of 2.5 μm were determined.

3. Results and Discussion

The green tea root powder obtained from the 70% ethanol extraction, and the yield was 16.8%. From the analysis of amino acids results, especially GABA (γ-aminobutyric acid) contents were found to have higher concentrations. GABA is well known as a neurotransmitter, but various efficacies on human skin have been also reported[7]. Most GABA productions from plants were known to be increased during seed germination, but in green tea root, substantial amount of GABA was found even without the germination process (Figure 1).

Using the vanillin-sulfuric acid colorimetric methods, the results of the total crude saponin contents of the green tea root extract were as follows (Table 1). As a result, total crude saponin contents in green tea root extract was 54.37%, it is higher than that of ginseng and soybean extract. The saponins of green tea root were triterpenoid series, although they were different from that of ginseng and soybean, but the total contents of green tea root was

Table 1. Comparison of the Crude Saponin Contents with Ginseng and Soybean Known to Be Rich in Saponins (All extracts were extracted with 70% ethanol)

	Green tea root	Ginseng root	Soybean
Total crude saponins Mean ± SD (%)	54.37 ± 0.00	20.64 ± 0.02	21.12 ± 0.02

Three independent experiments were performed.

Table 2. NGS Analysis for Upregulated Cytokine Genes by PM2.5 in NHEKs

Name	Gene	Description	Fold change (N=3)	
			AVR	SD
NM_000576	IL1B	interleukin 1, beta	10.7	1.35
NM_019618	**IL36G**	**interleukin 36, gamma**	**7.1**	**0.62**
NM_000575	IL1A	interleukin 1, alpha	4	0.75
NM_012275	IL36RN	interleukin 36 receptor antagonist	3.9	1.49
NM_004633	IL1R2	interleukin 1 receptor, type Ⅱ	3.8	2.15
NM_001270507	TNFAIP3	tumor necrosis factor, alpha-induced protein 3	2.5	0.19
NM_173841	IL1RN	interleukin 1 receptor antagonist	2.4	0.3
NM_006291	TNFAIP2	tumor necrosis factor, alpha-induced protein 2	2.3	0.12

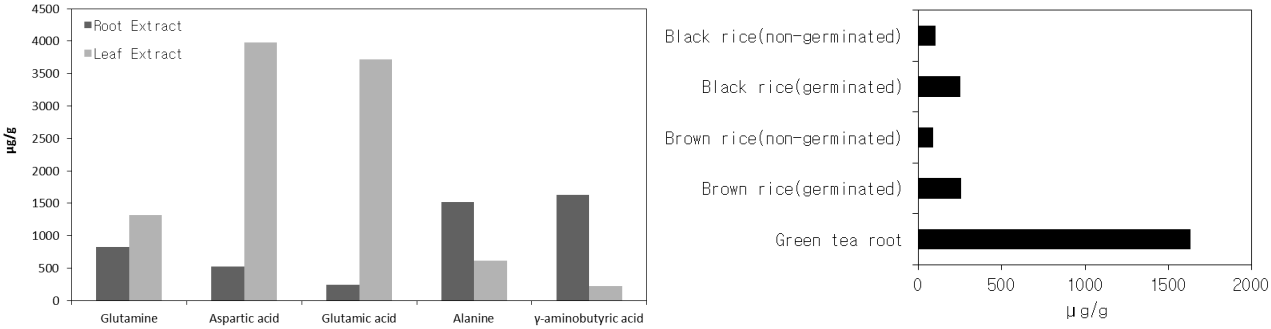

Figure 1. Amino acid and GABA of the green tea root and leaf (left) / Comparison between plants known for its high GABA content and green tea root (right). The experiment was performed once.

twice as high as that of ginseng and soybean. In addition, the foam stability of green tea root was better than products on the market (data not shown). These mean that green tea root can be used as a natural surfactant agent in cosmetics.

NGS-based RNA-Seq method with transcriptome and gene ontology (GO) enrichment analysis was performed to demonstrate the harmful influences of PM2.5 in NHEKs. The cause and effect of PM2.5-induced changes in NHEKs were predicted by ingenuity pathway analysis (IPA) upstream regulators and downstream effects analysis.

IL-36G mRNA expression level showed remarkable changes by PM2.5 treatment in NHEKs. IL-36G, alternatively called interleukin-1 family member 9 (IL-1F9), was also considerably augmented among the cytokines which were altered their expressions by PM2.5 (Table 2). It was recently reported that IL-36G is a biomarker in psoriasis[8]. In the report, IL-36G was expressed in only psoriasis lesions among inflammatory skin diseases (psoriasis, atopic dermatitis, lichen planus and contact eczema) markers. Therefore IL-36G is a therapeutic target

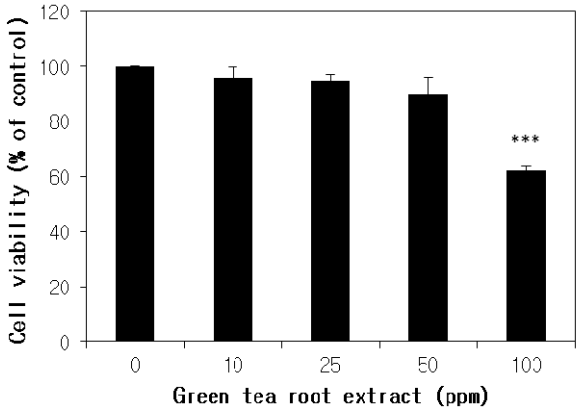

Figure 2. Viability of cells treated with green tea root extract. The graphs indicate the means ± S.D. of three independent experiments. ***p < 0.001 vs control.

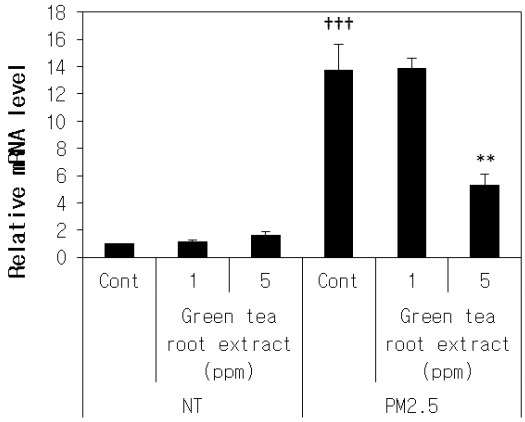

Figure 3. Quantitative Real-time PCR (graph). The graphs indicate the means ± S.D. of three independent experiments. **p < 0.05 vs control, †††p < 0.001 vs without PM2.5.

for the detrimental effects of PM2.5 in NHEKs.

The viability of green tea root extract treated NHEKs was checked. Green tea root extract did not affect NHEKs viability at below 50 ppm (Figure 2). In results, the mRNAs of inflammatory factor, IL-36G was induced by the external stimulant, PM2.5, but this was significantly decreased by green tea root extracts (Figure 3), and thus, improvement of psoriasis-related anti-inflammatory effects was observed. Green tea root extracts have a protective effect against the damage induced by the external stimulant (PM2.5). Therefore green tea root extracts can help in maintaining a healthy skin against PM2.5.

4. Conclusion

30-year-old green tea root has different amino acid compositions compared to green tea leaf, especially GABA, even without germination, it has more GABA than other plants. And green tea root has a lot of triterpenoid saponins, twice as high as that of ginseng and soybean. This means that green tea root can be used as a natural surfactant agent in cosmetics.

According to IL-36G mRNA expression assay of green tea root, it has a protective effect against the damage induced by the external stimulant. Therefore green tea root can be a good cosmetic reagent suitable for a lot of air pollution.

Reference

1. P. Chattopadhyay, S. E. Besra, A. Gomes, M. Das, P. Sur, S. Mitra, and J. R. Vedasiromoni, Anti-inflammatory activity of tea (*Camellia sinensis*) root extract, *Life Sci*., **74**, 1839-1849 (2004).
2. S. Hiai, H. Oura, Y. Odaka, and T. Nakajima, A colorimetric estimation of Ginseng saponins, *Planta Med*., **28**, 363-369 (1975).
3. C. Trapnell, A. Roberts, L. Goff, G. Pertea, D. Kim, D. R. Kelley, H. Pimentel, S. L. Salzberg, J. L. Rinn, and L. Pachter, Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks, *Nat. Protoc*., **7**(3): 562-78 (2012).
4. C. Trapnell, L. Pachter, and S. L. Salzberg, TopHat: discovering splice junctions with RNA-Seq, *Bioinformatics*, **25**(9), 1105-1111 (2009).
5. L. Wang, S. Wang, and L. Wei, RSeQC: quality control of RNA-seq experiments, *Bioinformatics*, **28**, 2184-2185 (2012).
6. C. Trapnell, B. A. Williams, G. Pertea, A. Mortazavi, G. Kwan, M. J. van Baren, S. L. Salzberg, B. J. Wold, and L. Pachter, Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation, *Nat.*

Biotechnol., **28**(5), 511-515 (2010).
7. E. Uehara, H. Hokazono, M. Hida, T. Sasaki, H. Yoshioka, and N. Matsuo, GABA promotes elastin synthesis and elastin fiber formation in normal human dermal fibroblasts (HDFs), *Biosci., Biotechnol., Biochem*., **81**, 1198-1205 (2017).
8. A. M. D'Erme, D. Wilsmann-Theis, J. Wagenpfeil, M. Hölzel, S. Ferring-Schmitt, S. Sternberg, M. Wittmann, B. Peters, A. Bosio, T. Bieber, and J. Wenzel, IL-36 γ (IL-1F9) is a biomarker for psoriasis skin lesions, *J. Invest. Dermatol*., **135**, 1025-1032 (2015).