J. Soc. Cosmet. Scientists Korea
Vol. 32, No. 2, June 2006, 117-121

사람 섬유아세포에서 녹차 카테킨이 노화 인자인 MMP와 type I Procollagen 발현에 미치는 영향

신 현 정† · 김 수 남* · 김 정 기 · 이 병 곤 · 장 이 섭

태평양기술연구원 피부과학연구소, *한국과학기술연구원 강릉분원

(2006년 5월 15일 접수, 2006년 6월 5일 채택)

Effect of Green Tea Catechins on the Expression and Activity of MMPs and Type I Procollagen Synthesis in Human Dermal Fibroblasts

Hyun Jung Shin†, Su Nam Kim*, Jung Ki Kim, Byeong Gon Lee, and Ih Seoup Chang

Amore Pacific Corporation R&D Center, 314-1, Bora-dong, Giheung-gu, Yongin-si, Gyeonggi-do 449-729, Korea
*KIST Gangneung Institute

(Received May 15, 2006; Accepted June 5, 2006)

요 약: collagen을 분해하여 광노화 과정에 매우 중요한 역할을 하는 것으로 알려져 있는 Matrix metallo proteinases (MMP)의 활성 및 저해 인자에 대해서는 지금까지 많은 연구가 진행되어 왔지만, 녹차 카테킨의 영향에 대한 연구는 epigallocatechin-3-gallate (EGCG) 이외에는 별로 알려진 것이 없다. 본 연구에서는 카테킨이 사람 섬유아세포에서의 MMP-1의 발현과 MMP-2의 활성 및 type I procollagen 생성에 미치는 영향을 조사하였다. 또한, 녹차의 대표적인 카테킨인 EGCG를 포함하여 자연적으로 존재하는 여덟개의 카테킨을 모두 사용하여 각각의 활성을 비교하였다. 그 결과, UVA에 의해서 사람 섬유아세포에서 발현되는 MMP-1에 대해 단백질의 양적인 변화는 EGCG 및 gallocatechin-3-gallate (GCG)에서 최대 57.4, 62.8% 감소되었으며, MMP-2의 활성 역시 감소되었다. 반면에, type I procollagen에 대해서는 생성 촉진능을 보였는데, 흥미롭게도 1 μM 이하의 저농도에서만 효능을 나타내었다. 또한 EGCG, GCG, epicatechin-gallate (ECG) 세가지 카테킨이 0.5:1.5:1.3의 비율로 조합된 경우, procollagen 합성에 가장 높은 효과를 나타내었다. 이러한 실험 결과를 통해 녹차 카테킨은 항산화능 뿐만이 아니라 자외선에 의한 MMP의 발현과 활성을 조절함으로써 콜라겐 분해를 억제함과 동시에 콜라겐 생합성을 촉진하는 것이 가능함을 확인하였다. 따라서 녹차 카테킨은 광노화의 억제 및 피부 노화 개선에 훌륭한 천연 소재로서 응용 가능할 것으로 보인다.

Abstract: Although many studies have been performed to elucidate the molecular consequence of factors that regulate skin aging, little is known about the effect of green tea catechins except EGCG. The matrix metalloproteinase (MMP), can degrade matrix proteins and results in a collagen deficiency in photodamaged skin, are known to play an important role in photoaging. This study investigated the effects of green tea catechins on the UVA-induced MMP-1 expression, activity of MMP-2 and synthesis of type I procollagen in human dermal fibroblasts. We examined eight catechins that naturally exist in green tea leaves and compared their efficacies among them. Most of catechins inhibited the expression of MMP-1 in dose dependent manner, and the levels were reduced, especially, 57.4 and 68.2% by treatment with 1 μM of epigallocatechin-3-gallate (EGCG) and gallocatechin-3-gallate (GCG), respectively. Also, catechins significantly suppressed the activities of MMP-2. Catechins also induced the expression of type I procollagen, however, they acted only at the concentration below 1 μM interestingly. Furthermore, when EGCG:GCG:ECG had the ratio of 0.5:1.5:1.3, they presented the most effective on procollagen synthesis. Therefore, we concluded that catechins significantly inhibited MMPs and induced collagen synthesis. Taken together, all these results suggested that green tea catechins might be good natural materials act as an anti-photoaging and a skin-aging inproving agent.

Keywords: *green tea catechin, skin aging, MMP-1, MMP-2, collagen*

† 주 저자 (e-mail: shin316@amorepacific.com)

1. Introduction

Aging of skin is a complex biological phenomenon consisting of two components; intrinsic aging and photoaging caused by environmental exposure, primarily UV light. Radical oxygen species (ROS) have been shown to play an important role in the response to UV radiation[1,2]. The decreased metabolic function and accumulated oxidative damage induced by ROS are responsible for many cutaneous disorders and skin aging. Increased matrix metalloproteinases (MMPs) that stimulated by UV irradiation and ROS has been suggested to be responsible for the characteristic collagen degradation in the dermis making wrinkles in aged skin[3]. For that reason, the agents that can decrease the level of MMPs production and increase the level of collagen synthesis have been the main focus of recent search including antioxidants.

It's well known that green tea catechins are not only a group of ROS scavengers that function as antioxidants in the epidermis, but also act as modulators of different gene groups and signal pathways[4,5]. The most abundant naturally occurring tea catechins are (-)-epicatechin ((-)-EC), (-)-epigallocatechin ((-)-EGC), (-)-epicatechin-3-gallate ((-)- ECG), (-)-epigallocatechin-3-gallate ((-)-EGCG) and their stereospecific epimers. Epigallocatechin-3-gallate (EGCG) is the most powerful antioxidant among green tea catechins and shows other beneficial effects[6]. The influence of EGCG on MMPs expression and activity in dermal fibroblast cells against UVR damage was reported recently[7,8]. However, the effects of other catechins in skin are unknown yet.

In this study, we examined the radical scavenging effects, the influences on MMP inhibition and collagen synthesis of eight catechins. In addition, their synergic effects and ratio constants for collagen synthesis with other green tea components were discussed.

2. Materials and Methods

2.1. Cell Culture and UVA Irradiation

Human dermal fibroblasts from the infant foreskin were grown under proper culture condition (37℃, 5% CO_2) in Dulbecco's Modified Eagle's Medium (DMEM) containing 0.48 mg/mL glutamine, 100 IU/mL penicillin, 50 mg/mL streptomycin, and 10% fetal bovine serum. Between the fourth and seventh passage, cells were used for the experiment.

UVA irradiation was provide by high-intensity UVA source (Dermlight cube 401 equipped with UVA filters) emitting wavelengths of the 320~400 nm range at a distance of 40 cm. The amount of UVA administered was assessed with UVA meter (UVATEC, Inc. Sherman Oaks, CA). After the UV exposure, fresh culture medium containing various sample was added and incubated before proper assays. Negative controls consisted of cells incubated with no UV irradiation.

2.2. Measurement of Antioxidant Activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was used to determine free radical-scavenging potential of each sample. The samples were added to DPPH solution (0.025 g/L). The absorbance was measured at 517 nm after 30 min of reaction at 37℃. The antiradical activity was calculated as a percentage of DPPH decoloration versus control.

2.3. Evaluation of MMP-1 Synthesis by Enzyme Linked Immunosorbent Assay

After UVA irradiation, dermal fibroblasts were cultured with serum free medium containing samples for 24 h. Then, the supernatants were transferred to a 96 well plate and coating buffer was added with the same volume and incubated for 24 h. The expression level of MMP-1 was determined by ELISA kit (Amersharm pharmacia, UK) following the manufacturer's instruction.

2.4. Determination of MMP-2 Activity

To induce the secretion of MMPs into culture media, human dermal fibronlasts were treated with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) for three days. The activity of the gelatinases (MMP-2) in the supernatants was determined by gelatin zymography. Briefly, zymography was carried out by running same volume of aliquots of the supernatants on 10% zymogram gel (Novex, San Diego, USA). After electrophoresis, the gels were incubated for 30 min in 2% Triton X-100 (2% Triton X-100, 50 mM TrisHCl, pH 7.4) for renaturation. After washed out with reaction buffer (50 mM TrisHCl, pH 8.0, 5 mM $CaCl_2$, 0.02% NaN_3), they were reacted for 24 and 72 h at 37℃ in a reaction buffer containing each catechins. The gels were stained with

Table 1. Antioxidant Effects of Green Tea Catechins on Radical Scavenging

Compounds	SC_{50}[a] values (μM) of DPPH[b]
(-)-EGCG	3.5
(-)-GCG	3.5
(-)-ECG	4.1
(-)-CG	4.5
(-)-EGC	6.8
(-)-GC	7.1
(-)-GC	7.1
(-)-C	8.3
L-Ascorbic Acid	6.4

[a] Concentration giving a 50% decrease of DPPH radicals. The value are the means of triplicate experiments with SD.

[b] 1,1-diphenyl-2-picrylhydrazyl radical

a Coomassie brilliant blue solution for 10 min and de-stained with 10% acetic acid until the bands of the proteolytic activities were visualized. For quantification of the bands, the gels were scanned using an image-analysis system and analyzed using a densitometric program.

2.5. ELISA for type I Procollagen Protein

Type I procollagen protein levels in supernatant were determined by ELISA (Takara, Shiga, Japan). Supernatants of cultured human dermal fibroblast treated with green tea components were harvested and quantified following manufacturer's instruction.

2.6. Statistics

Statistical analysis was performed with Student's t-test and ANOVA. A P value of 0.05 was selected as the limit of statistical significance.

3. Results and Discussions

3.1. Radical Scavenging Effects of Catechins

Green tea catechins exhibited a potent scavenging activity against the DPPH radicals with SC_{50} values as shown in Table 1. Furthermore, EGCG appeared to be the most efficient in comparison with the other catechins and reference compounds, ascorbic acid.

3.2. Inhibitory Effects of Catechins on the Expression of MMP-1

The effect of eight catechins on the viability of

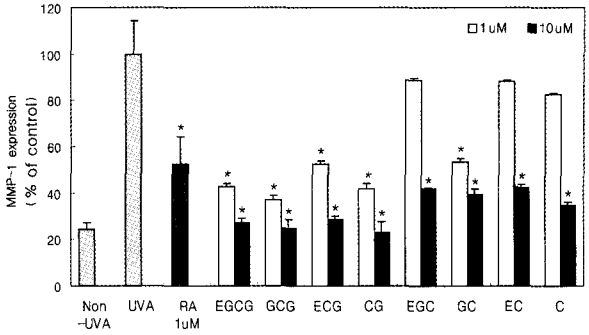

Figure 1. Effect of green tea catechins on the production of MMP-1 by UVA-irradiated human dermal fibroblast. The cells were treated with various concentration of catechins for 24 h. The MMP-1 contents in culture medium were determined by ELISA as detailed under Materials and Methods. The results were expressed as mean value of triplicate samples with SD. *p < 0.05 compared with UVA-irradiated control cell.

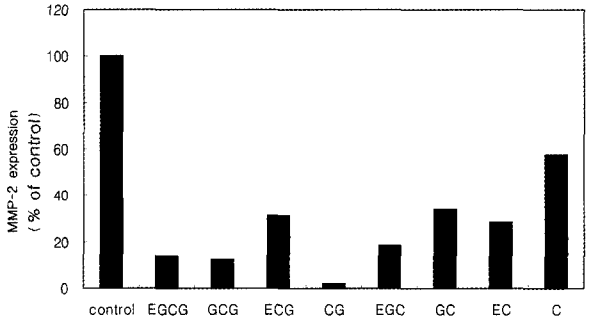

Figure 2. Effect of green tea catechins on MMP-2 activity. Human dermal fibroblasts were treated with TPA for 3 days and cultured media were analyzed on gelatin zymography. Used concentration of green tea catechins was 100 μM.

fibroblast was investigated for photo-protective activities in the protein levels by MTT test. All catechins did not show cytotoxicity against fibroblast cells in tested dose compared to control (data not shown).

We further examined the inhibitory effects of catechins on MMP-1 expression in UVA irradiated fibroblasts (15 J/cm^2). The cells were treated with various concentrations of catechins for 24 h and then, the MMP-1 contents in the culture medium were determined by ELISA. The treatment of UVA-irradiated fibroblasts with catechins significantly suppressed MMP-1 production at the protein levels in a dose dependent manner (Figure 1). Interestingly, the inhibitory effect of

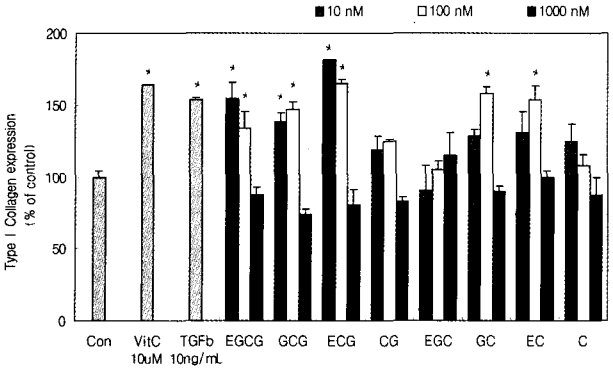

Figure 3. Effect of green tea catechins on type I procollagen synthesis. The presence level of type I collagen after treatment with catechins for 48 h was analyzed by ELISA. Ascorbic acid (VitC) and TGF-beta (TGFb) were used as a positive control. The results were expressed as mean value of triplicate samples with SD. *p < 0.05 compared with non-treated control (Con) cell.

EGCG on MMP-1 production was much higher than that of all-trans retinoic acid (RA), which is well known as an inhibitor of UVA-induced MMPs.

3.3. Effects of Catechins on MMP-2 Activities

In order to investigate the activities of MMP-2, the protein samples from culture medium treated with TPA that stimulated secretion of MMPs were subjected to gelatin zymographic assay. The MMP-2 activities observed by zymogram after all catechins treatment showed a decreased manner. EGCG, GCG and CG decreased the gelatinolytic activity of MMP-2 by over 85% (Figure 2). Interestingly, at the same concentrations, the group of catechins with a gallate moiety (EGCG, GCG, ECG and CG) had a slightly higher effect on the gelatinolytic activity than the others.

3.4. Promotion of Type I Procollagen Protein Expression

We examined whether or not catechins affect type I procollagen expression in cultured fibroblast. After 24 h serum starvation, the cells were subsequently incubated for 48 h in the presence or absence of the indicated doses of catechins. The levels of type I procollagen were determined using ELISA. They were induced maximally 154 and 182% (% of control) respectively by treatment with 10 nM EGCG and ECG, but the response was decreased when 1000 nM catechins were treated as well as EGCG, interestingly (Figure 3). All catechins in this experiment had shown the inhibitory effects on type I procollagen synthesis at the concentration of 1, 10 μM without cell cytotoxicity rather than promotion of procollagen (data not shown). It's very interesting that catechins stimulated type I procollagen expression only at the concentration less than 1 μM especially.

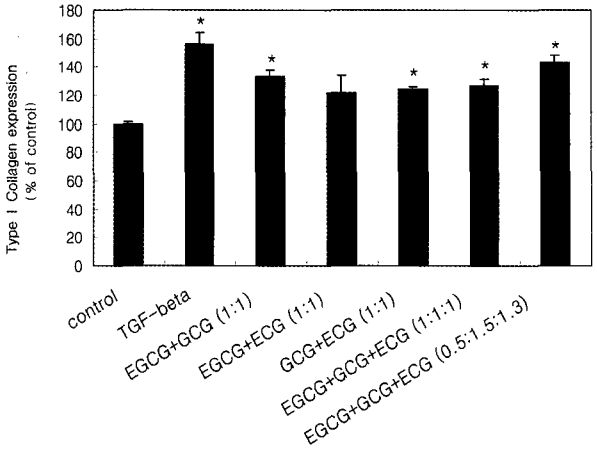

Figure 4. Synergic effect of EGCG, GCG and ECG combination on type I procollagen synthesis. To determine the most effective ratio of catechins for collagen production, human dermal fibroblasts were treated with various combinations for 48 h with the total molar concentration remained uniform, 1 μM. The procollagen levels were anayzed by ELISA. TGF-beta was used as a positive control. The results were expressed as mean value of triplicate samples with SD. *p < 0.05 compared with non-treated control cell.

3.5. Synergic Effects of Type I Procollagen Synthesis of Catechins.

To determine the optimum concentration of tea catechin for the stimulation of type I procollagen synthesis, we investigated the effect of EGCG, GCG and ECG with a various combination using ELISA. The experiment was done at the ratio of 1 : 1 for the combination of two catechins, and 1 : 1 : 1 for three, with the total molar concentration remained uniform. Results in Figure 4 showed that EGCG + GCG + ECG was most effective at the ratio of 0.5 : 1.5 : 1.3 than the application of two-catechins combination.

4. Conclusion

EGCG, one of the green tea catechins, has been reported that it is effective in reducing the gelatinase activities, inhibiting of collagenase expression and increasing the TIMP-1, a tissue inhibitor of MMPs, expression even *in vivo* models [7]. However, the effects of other catechins in green tea on skin are unknown.

In this study, eight green tea catechins exhibited significant DPPH radical scavenging activity. Also, they had significantly reduced the expression of collagenase, MMP-1, at the protein levels in dose dependent manner. Especially, EGCG, GCG, ECG and CG showed a powerful inhibitory effects on MMP-1 expression as well as MMP-2 activity in human dermal fibroblasts. Moreover, these catechins increased Type I procollagen contents in a dose-dependent manner only at the range of low concentration in cultured human dermal fibroblasts. Furthermore, when EGCG : GCG : ECG had the ratio of 0.5 : 1.5 : 1.3, they presented the most effective on procollagen synthesis than other combination among catechins.

Therefore, we suggest that green tea catechins can be developed as a promising photoaging-improving components and may be potential natural components to protect skin aging.

References

1. A. Oikarinen, The aging of skin: chronoaging versus photoaging, *Photodermatol. Photoimmunol. Photomed.*, **7**, 3 (1990).
2. B. A. Gilchrest, Skin aging and photoaging: an overview, *J. Am. Acad. Dermatol.*, **21**, 610 (1989).
3. J. Varani, R. L. Warner, M. Gharaee-Kermani, S. H. Phan, S. Kang, J. H. Chung, Z. Q. Wang, S. C. Datta, and G. J. Fisher, Voorhees, Vitamin A antagonizes decreased cell growth and elevated collagen-degrading matrix metalloproteinases and stimulates collagen accumulation in naturally aged human skin, *J. Invest. Dermatol.*, **114**, 480 (2000).
4. S. K. Katiyar, B. M. Bergamo, P. K. Vyalil, and C. A. Elment, Green tea polyphenols: DNA photodamage and photoimmunology, *J. Photochem. Photobiol.*, **65**, 109 (2001).
5. S. K. Katiyar and C. A. Elment, Green tea polyphenolic antioxidants and skin protection, *Int. J. Oncol.*, **18**, 1307 (2001).
6. J. D. Lambert and C. S. Yang, Cancer chemopreventive activity and bioavailability of tea and tea polyphenols, *Mutat. Res.*, **523**, 201 (2003).
7. J. H. Lee, J. H. Chung, and K. H. Cho, The effect of epigallocatechin-3-gallate on extracellular matrix metabolism, *J. Dermatol. Sci.*, **40**, 195 (2005).
8. S. Hsu, Green tea and the skin, *J. Am. Acad. Dermatol.*, **52**, 1049 (2005).