Kor. J. Env. Hlth., Vol. 32, No. 6, pp 566-570(2006)

# Effects of Natural Extracts on COX-1 and COX-2 mRNA Expression on UVB-induced Skin Inflammation in C57BL/6 Mouse

**Ryoung-Me Ahn**†

*Department of Health Science, Dongduk Women's University*

(Received October 28, 2006/Accepted November 30, 2006)

**Abstract:** Exposure to ultraviolet B(UVB) radiation causes skin inflammation such as pigmentation and the induction of cyclooxygenase-2(COX-2) gene expression. In this study, we investigated the effect of natural extracts from Tea, EGb 761 and Korean red ginseng(KRG), on the pigmentation and expression of COX-1 and COX-2 mRNA in UVB-irradiated C57BL/6 mice. Before UVB irradiation, the skin color was significantly showed the lightening effect by topical application of natural compounds ($p<.05$). In the case of UVB irradiated mice, we observed a decrease in pigmentation by compounds ($p<.05$). In irradiated skin, COX-1 mRNA expression is not changed following UVB irradiation, but COX-2 gene increases. Also, natural compounds lowered mRNA levels of COX-2. Therefore, these results suggest that COX-2 mRNA increases by UVB irradiation. Also, Tea, EGb 761 and KRG as a topical application may inhibit skin pigmentation and modulate COX-2 mRNA level.

**Keywords:** UVB radiation, inflammation, pigmentation, COX-1, COX-2

## Introduction

With the acceleration of destruction of ozonosphere due to environmental contamination, there is sudden increase of ultraviolet (UV) dose reaching to the ground. Proper UV irradiation is useful to disinfection, sterilizing action, synthesis of Vit. D and prevention of rachitis. However, overdose of UV causes skin aging, pigmentation, increase of cataract, and immune suppression and promotes the onset of skin cancer, harming the health (Burke *et al.*, 2003; Wilgus *et al.*, 2000; Lee *et al.*, 1996; Choi *et al.*, 2005; Choi *et al.*, 2006).

UV irradiation of the skin induces inflammation such as edema and erythema. Inflammatory response is generated when damaged tissue (cell) or infected by internal infection source (bacteria, fungus, virus and various kinds of allergen). Also, it induces the symptoms such as enzyme activation, secretion of inflammatory mediators, invasion of body fluid, cell movement and tissue destruction related to diverse inflammatory mediators and immune cells in local vessels and body fluid. In addition, it is known that chronic inflammatory response causes various types of diseases and inducing the promotion of cancer (Afaq *et al.*, 2005).

Exposure to UV on skin epidermal cell make an increase of expression of cyclooxygenase enzyme which is involved in synthesis of pro-inflammaotory cytokine such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) and biosynthesis of prostaglandin (PG). Cyclooxygenase (COX) enzyme has a role of mediator in the process that arachidonic acid (AA) synthesized from phosphlipid of cell membrane leads inflammatory responses by inducing prostagladin (PG). Nonsteroidal anti-inflammatory drugs (NSAIDs) that are widely used as an anti-inflammatory medication suppress the action of COX, blocking the synthesis of PG and work on analgesia, antipyretic and anti-inflammatory action (Dubois *et al.*, 1998; Chun *et al.*, 2004).

These studies mainly applied to medical supplies which suppress or relief the pain for inflammatory disease and new drugs for the substance selectively controlling the action of cyclooxygenase enzyme (Kim *et al.*, 2001; Athar *et al.*, 2004a; Przybylkowski *et al.*, 2004). However, those studies is limited to the development of medication and made drugs also dose not consider the basic research for enzyme activity, causing a lot of side effects. Consequently, in the present study, we demonstrated that natural

†Corresponding author : Department of Health Science, Dongduk Women's University
Tel. 82-2-940-4481, Fax. 82-2-940-4193
E-mail : lucia@dongduk.ac.kr

compounds inhibited UVB-induced inflammatory responses in pigmented skin. In addition, we assessed the suppression of COX-2 expression by natural compounds against UVB irradiation.

## Materials and Methods

### Animals & UVB Irradiation

C57BL/6 mice (female) that were supported by Samtaco Bio Korea Co., Ltd. The animals were 8 weeks of age and were housed in temperature-controlled (23 ± 1°C), humidity-controlled (55 ± 5%) cages.

The animals were shaved on the back with clipper before 24 hours of the experiment and one group was composed of 8 mice. Test groups were put in a self-designated cage to expose only the back site, and then, put the cage into the self-made irradiator. And UVB (312 nm) was irradiated.

There were 4 UVB lamps (Phillips) in the UVB irradiator and the animals were irradiated in the place departed 20 cm from the lamp. Minimal erytherma dose (MED) was determined with minimal UV dose that induced erythema on most part of irradiated site and in this experiment, 2.8 KJ/m$^2$ (2MED) was irradiated. UVB was irradiated using radiometer (6501-54 VLX-3W, France).

### Topical Application of Natural Extracts

1:2 Polyethylene Glycol(PEG) 8000 and PEG 400 mixture was slowly melted on water bath at 60°C and mixed with distilled water for base lotion (Lee *et al.*, 1999). The natural compounds used in this study were Tea (Tea extract), EGb 761 (Ginkgo biloba extract) and KRG (Korean red ginseng, Cheong-Kwan-Jang) and those were mixed with base lotion for 0.5% lotion and treated. 200 μl of those were applied twice a day (morning and afternoon) on the back of animals from a week ago of UVB irradiation. Also, 200 μl of those were applied on the back immediately after irradiation. To minimize the error due to loss of hair during experiment, the back was checked in every treatment and the back was carefully shaved not to hurt.

### UVB-induced Pigmentation in C57BL/6 Mice

200 μl of the lotion containing 0.5% natural compound was pretreated on the back from a week ago, and then, a total of 6 times UVB irradiation were performed 3 times a week in concentration of 2MED (2.8 KJ/m$^2$) for 2 weeks. After 24 hours of last irradiation, the degree of pigmentation was measured using chromameter (CR400, Minolta, Tokyo, Japan) and evaluated by calculating $\Delta L^*$ value. After irradiation, the lotion containing 0.5% natural compound was applied twice a day for 2 weeks and skin color change was measured 1 week interval.

### RT-PCR Analysis

Total RNA was extracted using TRIzol and stored at −80°C before use. All primers used in PCR was purchased or synthesized from Bioneer (Korea). RT-PCR premix (K-2057) was purchased from Bioneer (Korea). cDNAs were synthesized using RT reaction at 42°C, 50 min and 99°C, 5 min in Peltier thermal cycler (PTC-200). The amplification was performed at 94°C for 60s, at 58-68°C for 60s, and at 72°C for 90s with 30cycles for COX-1, COX-2 and 32 cycles for GAPDH, in 50 μl reaction

**Table 1.** Cytokine-specific primer pair sequences used in PCR

| CYT[a] | Oligonucleotide sequence 5'-3'[b] | Size[c] |
|---|---|---|
| COX-1 | F 5'-TGC ATG TGG CTG TGG ATG TCA TCA A-3' | 450 |
| | R 5'-CAC TAA GAC AGA CCC GTC ATC TCC A-3' | |
| COX-2 | F 5'-ACT CAC TCA GTT TGT TGA GTC ATT C-3' | 583 |
| | R 5'-TTT GAT TAG TAC TGT AGG GTT AAT G-3' | |
| GAPDH | F 5`-GTC ATT GAG AGC AAT GCC AG-3` | 215 |
| | R 5`-GTG TTC CTA CCC CCA ATG TG-3` | |

[a]: cytokine.
[b]: F, foward sequence. R, reverse sequence.
[c]: predicted size of PCR product (base pair).

mixture. The result of PCR was loaded using 1% agarose gel electrophoresis containing ethidium bromide and PCR product was examined on UV transilluminatior. Primer sequences were presented on Table 1.

### Statistical Analysis

Data was analyzed using SPSS 10.0 for statistics and t-test was performed. Statistical significance was evaluated ≤0.05.

## Results

Before UVB irradiation, 200 μl of the lotion containing 0.5% extracts of red ginseng, green tea or ginkgo was applied twice a day for a week on the back of animals and the change in skin color was evaluated by natural compound. The results presented in Fig. 1. Compared to control group, in test groups, the change of skin color was significantly reduced ($p < .05$). Among them, compared to leave of ginkgo and green tea, the reduction degree in red ginseng group showed 2-fold effectiveness higher.

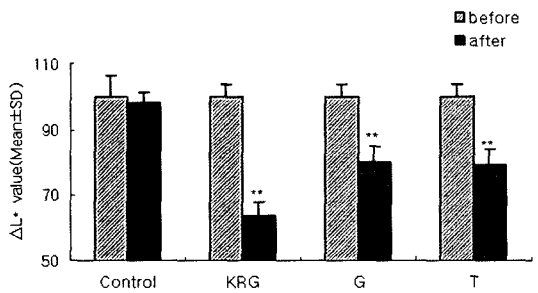


**Fig. 1.** The degree of pigmentation ($\Delta L^*$-value) control and compounds (0.5% Korean red ginseng, EGb 761 and Tea) Dorsal skin of mice was topically applied for 7 days. **p<0.05 : significantly different from control.

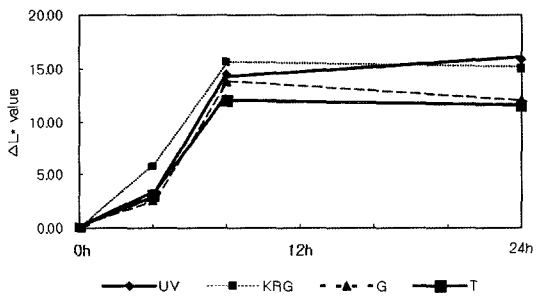


**Fig. 2.** Inhibition of UVB-induced pigmentation by topical application of 0.5% KRG (Korean red ginseng), G (EGb 761), T (Tea) in mice dorsal skin. Mice were irradiated once with 2MED (2.8 KJ/m$^2$) of UVB.

After one UVB irradiation in concentration of 2MED (2.4 KJ/m$^2$) on the back of mouse, the skin color change was examined. Fig. 2 shows the skin pigmentation according to time course. After UVB irradiation, the degree of pigmentation was continuously increased and the treatment group with compounds showed visible decrease in pigmentation compared to the control group.

To determine the effect of natural compound application on UVB-induced skin inflammation, the change of skin color was observed by generating pigmentation on the skin of mice. The animals were exposed to 2MED of UVB radiaiton 3 times a week for 2 consecutive weeks (Fig. 3). In the group treated with natural compound, the change was significantly decreased ($p < .05$) and it showed that the effectiveness of red ginseng was greatest just like the result of Fig. 1.

To determine whether the effect of natural compound for pigmentation decrease is related to the expression of inflammation-related enzyme, COX-2, the amount of COX-2 production was evaluated on the back of mice using RT-PCR (Fig. 4). The expression of COX-2 was not altered and the production rate of COX-2 induced by UVB was

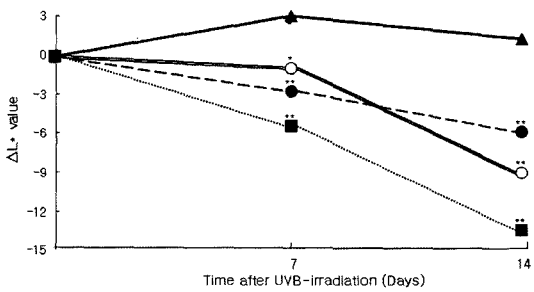


**Fig. 3.** Inhibition of UVB-induced hyperpigmentation by topical application of 0.5% KRG (Korean red ginseng), G (EGb 761), T (Tea) in mice dorsal skin. Hyperpigmentation was induced by six UVB irradiations. The results were expressed as $\Delta L^*$, which represented the amount of decrease in $L^*$ values.
▲: UVB, ○: T(Tea), ●: G(EGb 761), ■: KRG(Korean red ginseng).
**p<0.05 : significantly different from control.

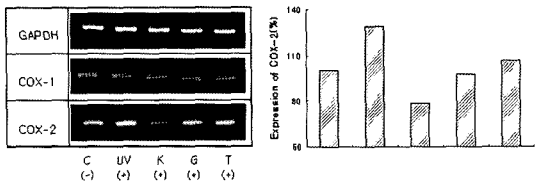


**Fig. 4.** Inhibition of UVB-induced COX-2 expression. $^{**}p<0.05$ : significantly different from control.

reduced by natural compound. Especially, Korean red ginseng suppressed the gene expression of COX-2 about 50%. These results suggests that natural compound may modulate the inflammatory response.

## Discussion

Our domestic research for the body's inflammatory response is mostly about development of anti-tumor promoter that chronic inflammation blocks the step of cancer promotion and the development of drug relieving pain due to chronic inflammatory disease is in progress actively. In research for UV among various factors causing inflammation, Athar *et al.* studied the expression of COX-2 due to UVB in pathological aspect and Wilgus *et al.* compared the expression of COX-1 and COX-2 in mouse and sunburn cell using commercially used anti-inflammatory and analgesic drug, ibupropen and celecoxib (Athar *et al.*, 2001b; Wilgus *et al.*, 2000).

Such studies for anti-inflammatory substances is mostly limited to the level of anti-inflammatory drug development and side effects for the drugs are getting worse because of lack of basic research for the body's enzyme activity. In 1990's, it was clear that there are 2 types of COX isomerase in the body. It is known that COX-1 is expressed in normal condition, existing mainly in stomach and kidney relating to protection of gastrointestinal tract and kidney function control and COX-2 is generally induced by stimulation such as inflammation, mediating inflammatory response (Kwon, 2001; Kim *et al.*, 2001). Therefore, recently, there are a lot of researches for the development of drug selectively inhibiting only COX-2 enzyme and many researchers try to find such substance in natural compound. In addition, it is clarified that COX-2 has a key role in cancer formation; therefore, COX inhibitor is expected to be useful in the prevention of cancer (Brecher, 2002). In this study, environmental factor, UV causing skin inflammation was selected as a subject and it examined inflammatory response in skin tissue which may be easily exposed to UV. Unlike previous researches, this is for inflammatory activity of certain organ called skin and by inducing inflammation with UV which the harmful effect is continuously increase among environmental toxins, it is thought that acute inflammatory response and the expression of inflammatory mediators by UV may be a fine indicator representing inflammatory damage to skin. Besides, the result of inhibition effect of red ginseng, tea, EGb 761 which inhibit the expression of COX-2 may be used to basic data for the development of drug targeted inflammatory disease and applied to antipyrotic, functional cosmetics, and the development of immune regulating substance for pigmentation, wrinkle, aging and oncogenesis induced by UV.

## Acknowledgement

This study was suppoted by a Dongduk Women's University Research Grant (2005-2006).

## References

1. Burke, K.E., Clive, J., Combs, G.F. and Nakamura, R.M. : Effects of topical L-selenomethionine with topical and oral vitamin E on pigmentation and skin cancer induced by ultraviolet irradiation in Skh:2 hairless mice. *Journal of the American Academy of Dermatology,* **49**(3), 458-472, 2003.
2. Wilgus, T.A., Ross, M.S., Parrett, M.L. and Oberyszyn, T.M. : Topical application of a selective cyclooxygenase inhibitor suppresses UVB mediated cutaneous inflammation. *Prostaglandin & Other lipid mediators,* **62**, 367-384, 2000.
3. Lee, M.K., Yi, S.Y., Ann, H.S. and Ahn, R.M. : Lipid peroxidation and antioxidase activities on hairless mouse induced by UVB irradiation and effects of tannic acid. *Korean Journal of Environmental Health,* **22**(4), 102-108, 1996.
4. Choi, W.H., Ann, H.S. and Ahn, R.M. : Prevention of Ultraviolet B radiation (280-320 nm) induced contact hypersensitivity by EGb 761. *Korean Journal of Environmental Health,* **31**(1), 7-14, 2005.
5. Choi, W.H., Ann, H.S., Choi, T.Y., Jin, S.Y. and

Ahn, R.M. : Effects of natural extracts on UVB-induced pigmentation and inflammation in C57BL/6 mouse skin. *Korean Journal of Environmental Health,* **32**(5), 492-498, 2006.
6. Kim, E.J., Oh, O.J., Lee, S.K. and Yang, K.S. : Inhibitory effect of Astragali Radix on COX-2 activity. *The Korean Society of Pharmacognosy,* **32**(4), 311-315, 2001.
7. Athar, M., An, K.P., Morel, K.D., Kim, A.L., Aszterbaum, M., Longley, J., Epstein, E.H. and Bickers, D.R. : Photoprotective effects of sulindac against ultraviolet B-induced phototoxicity in the skin of SKH-1 hairless mice. *Toxicology and Applied Pharmacology,* **195**, 370-378, 2004a.
8. Przybylkowski, A., Jastrzebska, I.K., Joniec, I., Ciesielska, A., Czlonkowska, A. and Czlonkowski, A. : Cyclooxygenases mRNA and protein expression in striata in the experimental mouse model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration to mouse. (Reasearch report) *Brain Research,* **1019**, 144-151, 2004.
9. Lee, C.K., Han, S.S., Shin, Y.K., Chung, M.H., Park, Y.I., Lee, S.K. and Kim, Y.S. : Prevention of ultraviolet radiation-induced suppression of contact hypersensitivity by Aloe vera gel components. *International Journal of Immunopharmacology,* **21**, 303-310, 1999.
10. Athar, M., An, K.P., Morel, K.D., Kim, A.L., Aszterbaum, M., Longley, J., Epstein, E.H. and Bickers, D.R. : Ultraviolet B(UVB)-induced COX-2 expression in murine skin : an immunohistochemical study. *Biochemical and Biophysical research communications,* **280**, 1042-1047, 2001b.
11. Kwon, S.K. : SAR of COX-2 inhibitors. *The Journal of Applied Pharmacology,* **9**, 69-78, 2001.
12. Brecher, A.R. : The role of cyclooxygenase-2 in the pathogenesis of skin cancer. *Journal of Drugs in Dermatology,* **1**(1), 44-47, 2002.