

Proanthocyanidins Suppresses Lipopolysaccharide-stimulated Inflammatory Responses via Heme Oxygenase-1 Induction in RAW264.7 Macrophages

Hye-Jin Cheon¹, Sun Young Park¹, Hee-Ji Jang¹, Da-Young Cho¹, Jiwon Jung², Gimin Park², Kyeong Mi Jeong² and Jin-Kyung Kim^{1*}

¹Department of Biomedical Science, Daegu Catholic University, 38430 Gyeongsan-si, Korea

²Yeongcheon Girl's High School, 38841 Yeongcheon-si, Korea

Received January 22, 2019 / Revised February 19, 2019 / Accepted February 20, 2019

Proanthocyanidins are naturally occurring polyphenolic compounds abundant in many vegetables, plant skins (rind/bark), seeds, flowers, fruits, and nuts. Numerous *in vitro* and *in vivo* studies have demonstrated myriad effects potentially beneficial to human health, such as antioxidation, immunomodulation, DNA repair, and antitumor activity. Among immune cells, macrophages are crucial players in a variety of inflammatory responses to environmental conditions. However, it has been widely reported that macrophages cause chronic inflammation and are involved in a variety of diseases, such as obesity, diabetes, metabolic syndrome, and cancer. In this study, we report the suppressive effect of proanthocyanidins *via* the heme oxygenase-1 (HO-1)-related system, on the immune response of the LPS-stimulated mouse macrophage cell line RAW264.7. Increased HO-1 expression at mRNA and protein levels were found in proanthocyanidins-treated RAW264.7 cells. Further, proanthocyanidins enhanced nuclear factor-erythroid 2-related factor 2 translocation into the nucleus. RAW264.7 cells were treated with lipopolysaccharide (LPS) with or without proanthocyanidins, and inflammatory mediator expression levels were assessed. Proanthocyanidins treatment resulted in the attenuation of nitric oxide production and inducible nitric oxide synthase expression in LPS-stimulated RAW264.7 cells. In addition, mRNA and protein expression of proinflammatory cytokines, such as tumor necrosis factor- α and interleukin-6, was inhibited by proanthocyanidins treatment in LPS-stimulated RAW264.7 cells. These findings support proanthocyanidins as a promising anti-inflammatory agent.

Key words : Heme oxygenase-1, inducible nitric oxide synthase, inflammation, macrophages, proanthocyanidins

Introduction

Among immune cells, macrophages are one of the most widely studied pleiotropic cells in the immune system. Macrophages play critical and plastic roles in host defense, immune regulation, and wound healing, adapting to their local environments and adopting diverse phenotypes [20]. When the sophisticated balance of macrophage activity collapses, macrophages can cause various diseases [20]. For example, several reports have indicated the important relationship between the particular steps of colon cancer development and inflammation due to obesity, which is mediated

by inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) secreted by macrophages [6, 15]. Therefore, it is important to prevent abnormal activation and to keep macrophages within their proper range of activity to avoid lifestyle-related diseases caused by macrophages.

One of the antioxidant enzymes, heme oxygenase (HO), is a rate-limiting enzyme catalyzing the degradation of heme to biliverdin, free iron, and carbon monoxide (CO). In particular, HO-1 is one of three mammalian HO isozymes and is a stress-responsive protein induced by various stimuli including oxidative stress, ischemia-reperfusion, heavy metals, and cytokines [6, 15]. It is generally assumed that these heme-related catabolites have antioxidant activity [6, 15, 17]. For example, CO mediates potent anti-inflammatory effects [27]. Otterbein et al., revealed that CO at low concentrations inhibited the expression of the lipopolysaccharide (LPS)-induced inflammatory cytokines TNF- α , IL-1 β , and others [14].

Proanthocyanidins are a group of polyphenolic bioflavonoids that are naturally occurring in many vegetables,

*Corresponding author

Tel : +82-53-850-3774, Fax : +82-53-850-3774

E-mail : toto0818@cu.ac.kr

This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

flowers, fruits, and nuts [25]. A number of pharmacological effects have been reported for proanthocyanidins, such as antiviral, antimicrobial, anti-HIV, anti-oxidative and anti-tumor-promoting properties, as well as cardiotoxic and anti-arteriosclerotic activities [1, 11, 16, 19, 22]. In this study, we examined the effects of proanthocyanidins on lipopolysaccharide (LPS)-induced inflammatory responses in murine RAW264.7 macrophages. LPS is a component of the outer membrane of gram-negative bacteria. Since LPS is widely used in studies of inflammation and chronic inflammation can be modeled by administration of LPS *in vivo* [12], we used LPS to study inflammation in an *in vitro* model.

Materials and Methods

Chemicals and reagents

All reagents were from obtained Sigma-Aldrich (St. Louis, MO) unless otherwise indicated. Proanthocyanidins was obtained from Avention (Incheon, Korea). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin and streptomycin were obtained from Hyclone (Logan, UT, USA). TNF- α and IL-6 ELISA kits were purchased from eBioscience (San Diego, CA, USA). PRO-PREPTM Protein Extraction Solution and NE-PERTM Nuclear and Cytoplasmic Extraction Reagents were obtained from iNtRON Biotechnology (Seongnam, Korea) and Thermo ScientificTM (Waltham, MA, USA), respectively. Antibodies against inducible nitric oxide synthase (iNOS) and HO-1 were purchased from BD Biosciences (San Jose, CA) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively.

Cell culture and cell viability assay

Murine RAW264.7 macrophages were obtained from Korea Cell Bank (Seoul, Korea) and cultured in DMEM containing 10% FBS, 100 U/ml penicillin, and 100 μ g/ml streptomycin at 37°C in 5% CO₂. A D-PlusTM CCK cell viability assay kit (Dongin Biotech, Seoul, Korea) was used for the cell viability assay. A defined number of cells were centrifuged after collection, resuspended with fresh complete medium and then seeded into 96-well plates at a density of 2×10^4 cells per well. Proanthocyanidins was added to each plate at indicated concentrations. After 24 hr incubation, the number of viable cells was counted according to the manufacturer's instructions.

Measurement of nitrite

The amount of nitric oxide (NO) produced by the mouse macrophages was measured in the RAW264.7 cell culture supernatant. RAW264.7 cells were plated at a density of 2.5×10^5 cells in a 48-well cell culture plate with 500 μ l of culture medium and incubated for 12 hr. They were then treated with indicated concentrations of proanthocyanidins plus LPS (500 ng/ml) and incubated for another 24 hr. The amount of nitrite produced was measured using the Griess reagent system (Promega, Madison, WI, USA).

Cytokine measurement

The amount of IL-6 and TNF- α in the cell culture supernatant was measured using an ELISA kit. RAW264.7 cells were plated in a 48-well cell culture plate at a density of 2.5×10^5 cells and incubated with indicated concentrations of proanthocyanidins in 500 ng/ml LPS for 24 hr. The culture supernatant was collected and assayed according to the manufacturer's instructions to determine the amount of IL-6 and TNF- α released from the cells.

Quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR)

Total RNA was isolated from RAW264.7 cells using Trizol Reagent (Invitrogen, Carlsbad, CA, USA). DNA was eliminated from total RNA using RNA Qualified RNase-Free DNase (Promega) and cDNA was synthesized by GoScriptTM Reverse Transcription System (Promega). qRT-PCR assay was carried out with LightCycler (Roche Diagnostics, Basel, Switzerland) using LightCycler FastStart DNA Master SYBR Green I (Roche Diagnostics). All the experiments were repeated twice in triplicate each time. Transcripts of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a house-keeping gene were quantified as endogenous RNA of reference to normalize each sample. Relative quantities were estimated by the $-\Delta\Delta$ Ct method. The primers used in this study corresponded to mouse iNOS: F 5'-CCT CCT CCA CCC TAC CAA GT-3', R 5'-CAC CCA AAG TGC TTC AGT CA-3', mouse IL-6: F 5'-CAT CCA GTT GCC TTC TTG GGA-3', R 5'-CTG AAG GAC TCT GGC TTG TC-3', mouse TNF- α : F 5'-TGT CTC AGC CTC TTC TCA TT-3' R 5'-AGA TGA TCT GAG TGT GAG GG-3', mouse HO-1: F 5'-GCT TGT TGC GCT CAT TCT CC-3' R 5'-GCC ACC AAG GAG GTA CAC ATA -3' and mouse GAPDH: F 5'-TCT TGC TCA GTG TCC TTG C-3'. R 5'-CTT TGT CAA GCT CAT TTC CTG G-3'

Western blotting analysis

Whole cell, cytosolic or nuclear extracts were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The separated proteins were electrophoretically transferred to nitrocellulose membranes. The membranes were incubated with the indicated Abs and specific bands were visualized using an enhanced chemiluminescence kit (Amersham Biosciences, Piscataway, NJ, USA).

Data analysis

The data were presented means \pm SEM. The values were evaluated by one-way ANOVA with Bonferroni multiple comparison range tests using GraphPad Prism 4.0 software (GraphPad Software Inc, San Diego, CA). Statistically significant differences were considered at a P -value < 0.05 .

Results

Cell viability of proanthocyanidins-treated RAW 264.7 macrophages

Murine RAW264.7 macrophages were chosen for use in an investigation of the anti-inflammatory effects of proanthocyanidins. We first analyzed the effects of proanthocyanidins on the viability of RAW 264 macrophages by D-Plus™ CCK cell viability assay. Cells were seeded and incubated with medium containing 0, 10, 50, 100, 200, 400 μ M proanthocyanidins for 24 hr. No notable cytotoxicity was observed when the cells were exposed up to 200 μ M proanthocyanidins (Fig. 1B). Since proanthocyanidins showed no cytotoxicity with concentrations up to 200 μ M in RAW264.7 macro-

phages, we used up to 200 μ M proanthocyanidins for the rest of the experiments.

HO-1 protein expression analysis of proanthocyanidins-treated RAW264.7 macrophages

We next examined whether proanthocyanidins induces HO-1 mRNA and protein expression in RAW264.7 cells. Cells were challenged with different proanthocyanidins concentrations and the levels of HO-1 mRNA and protein were quantified by qRT-PCR and Western blotting analysis, respectively. HO-1 mRNA and protein expression levels of the proanthocyanidins-added group were significantly up-regulated compared with those of the control group in a dose-dependent (Fig. 2A, Fig. 2B). These results suggest that proanthocyanidins addition induces HO-1 expression at mRNA and protein levels without noticeable cell damage in RAW264.7 cells.

The transcription of HO-1 is mainly regulated by transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf-2) [6, 15]. Under resting condition, the cytosolic Nrf-2 is combined with Kelch-like ECH-associated protein 1 (Keap1), a repressor of Nrf-2, followed by Nrf-2 degradation through proteasome. Upon activation, Nrf-2 is dissociated from Keap1, leading to translocation into the nucleus, where it activates the transcription of phase II genes including HO-1 by binding to the antioxidant response element (ARE) sequence in their promoter regions of target genes [6, 15]. Therefore, we examined the nuclear translocation of the Nrf-2 protein in proanthocyanidins-treated RAW264.7 cells. To determine whether proanthocyanidins induced the nuclear translocation of Nrf-2 in macrophages, RAW264.7 cells

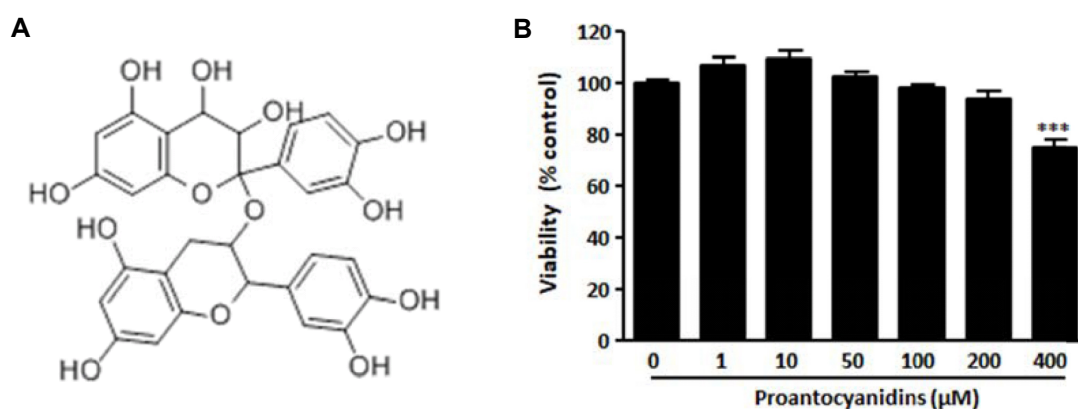


Fig. 1. Effect of proanthocyanidins on cell viability in RAW264.7 macrophages. (A) The chemical structure of proanthocyanidins. (B) The viability of RAW264.7 cells were tested by CCK-8 assay after 24 hr incubation in medium containing indicated concentration of proanthocyanidins. Triplicate experiments were used to evaluate the data, and the mean value is expressed as \pm SEM. Significant compared to 0 μ M proanthocyanidins-treated cells, *** $p < 0.001$.

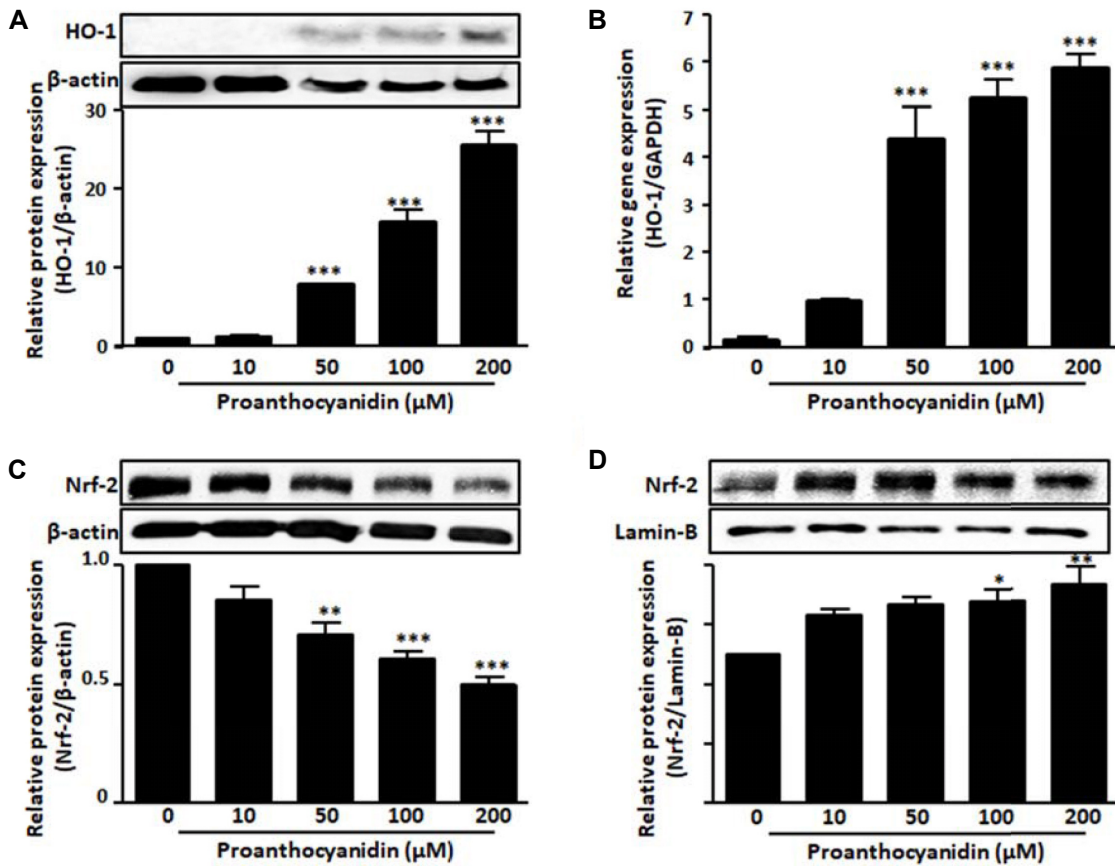


Fig. 2. Proanthocyanidins induced HO-1 protein expression. RAW264.7 cells were treated with indicated concentration of proanthocyanidins for 18 hr or 8 hr to measure (A) HO-1 protein and (B) mRNA expression levels, respectively. After cells were treated with indicated concentrations of proanthocyanidins for 2 hr, Nrf-2 was measured both cytoplasmic (C) and nuclear fraction (D). β-actin and lamin B1 were used as loading controls of cytoplasmic and nuclear protein, respectively. Triplicate experiments were used to evaluate the data, and the mean value is expressed as ± SEM. Significant compared to 0 μM proanthocyanidins-treated cells, **p*<0.05, ***p*<0.01, ****p*<0.001.

were exposed to proanthocyanidins for different concentrations, and then Western blot analysis was performed using nuclear and cytoplasmic extracts. As shown in Fig. 2C and Fig. 2D, the Nrf-2 nuclear accumulation increased by proanthocyanidins treatment, whereas the cytosolic accumulation of Nrf-2 was decreased. These results suggest that proanthocyanidins activates the Nrf-2 nuclear translocation that mediate HO-1 expression.

NO production and iNOS expression in RAW264.7 macrophages under LPS stimulation with proanthocyanidins

Since we evaluated the effect of proanthocyanidins alone on HO-1 and nuclear translocation of Nrf-2, we investigated the effect of proanthocyanidins on the activity of the RAW 264.7 cells. NO, a free radical produced by iNOS, has been shown to have a number of important biological functions,

including tumor cell killing and host defense against intracellular pathogens [10]. iNOS is generally not present in inactive cells but is induced by various stimuli such as LPS [10]. Therefore, NO releases and iNOS expression of RAW 264.7 macrophages under LPS stimulation with proanthocyanidins was performed to assess the effect of proanthocyanidins on the immune response.

To evaluate the effects of proanthocyanidins on the production of NO, Griess reagent was used to detect the level of LPS-induced NO release in the supernatant media of RAW 264.7 cells. The results showed that LPS treatment caused significant increase of NO release compared to blank, while proanthocyanidins dose-dependently inhibited LPS-induced NO production in RAW264.7 cells as shown in Fig. 3A. Subsequently, to investigate whether these effects of proanthocyanidins are related to iNOS expression of the gene and protein, we used qRT-PCR and Western blot analy-

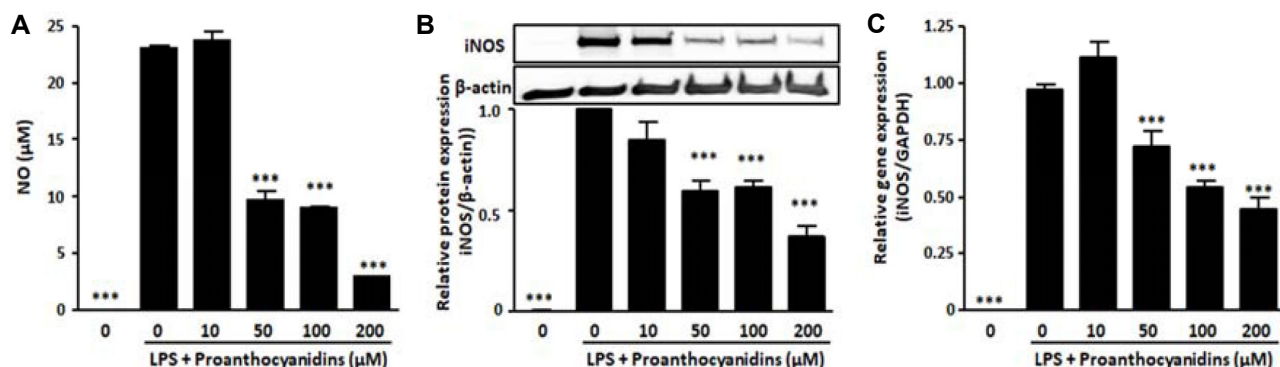


Fig. 3. Proanthocyanidins significantly reduced LPS-induced NO production and iNOS expression. RAW264.7 cells were incubated in 0.5 μg/ml LPS condition with or without proanthocyanidins for 24 hr. (A) After incubation, the concentrations of nitrite were measured as described in Materials and Methods. (B) iNOS protein and (C) mRNA levels were analyzed by Western blotting and qRT-PCR analysis, respectively. β-actin was used as control. Triplicate experiments were used to evaluate the data, and the mean value is expressed as ± SEM. Significant compared to LPS-treated cells, *** $p < 0.001$.

sis, respectively. The protein levels of iNOS significantly increased in response to LPS treatment, whereas proanthocyanidins inhibited this effect in a dose-dependent manner (Fig. 3B). Our results also showed that proanthocyanidins significantly reduced the expression of *iNOS* gene induced by LPS (Fig. 3C). These data demonstrate proanthocyanidins can restrain the production of NO by down-regulating the expression of iNOS.

Proinflammatory cytokine production in RAW264.7 macrophages under LPS stimulation with proanthocyanidins

In addition to NO production investigation, we further analyzed the action of proanthocyanidins in the production of proinflammatory cytokines, such as TNF-α and IL-6, known to be induced by LPS stimulation. After LPS stimulation with or without proanthocyanidins, the mRNA expression levels of *TNF-α* and *IL-6* were assessed by qRT-PCR analysis (Fig. 4A, Fig. 4B). The mRNA levels of each of the proinflammatory cytokines were upregulated by LPS treatment. In contrast, these LPS-inducible cytokines were significantly reduced with proanthocyanidins addition compared with the LPS-alone condition. In addition, the ELISA analysis of TNF-α and IL-6 showed a significant reduction when RAW264.7 cells were treated with LPS and proanthocyanidins, compared with the LPS-treated group (Fig. 4C, Fig. 4D). These results are consistent with Otterbein et al. [14] and they suggested that CO, which is the by-product of heme degradation by HO-1, inhibits the expression of LPS-induced proinflammatory cytokines. Thus, our data indicated that the addition of proanthocyanidins leads to HO-1

expression and a downregulative effect on proinflammatory mediators.

Discussion

In this study, we evaluated the functions of proanthocyanidins-induced expression of HO-1 by macrophage cells under LPS treatment. First, we showed that proanthocyanidins addition induces HO-1 mRNA and protein expression without obvious cell damage. Second, we revealed that proanthocyanidins causes changes in several proinflammatory factors induced by LPS stimulation. In particular, proanthocyanidins treatment induced the attenuation of iNOS expression and NO production and the downregulation of proinflammatory cytokines, such as TNF-α and IL-6, were shown. These data indicate that proanthocyanidins, as a HO-1 inducer, has promise as a novel anti-inflammatory agent.

HO-1 is well-known as a member of the heat-shock protein group, which is involved in responses to a number of cellular injuries, including thermal and oxidant stresses [6, 15]. HO-1 catalyzes the rate-limiting step in the degradation of cellular heme, producing equimolar quantities of iron, CO, and biliverdin [6, 15]. It is generally assumed that this HO-1 and a bi-product-related system may act as a crucial regulator in inflammatory processes, and regulates the balance between proinflammatory and anti-inflammatory mediators [15]. The beneficial effects of HO-1 induction have been attributed to several factors, including the degradation of pro-oxidant heme [24], formation of biliverdin and bilirubin with their antioxidant properties [23], as well as the release of CO, which has anti-apoptotic and anti-inflammatory

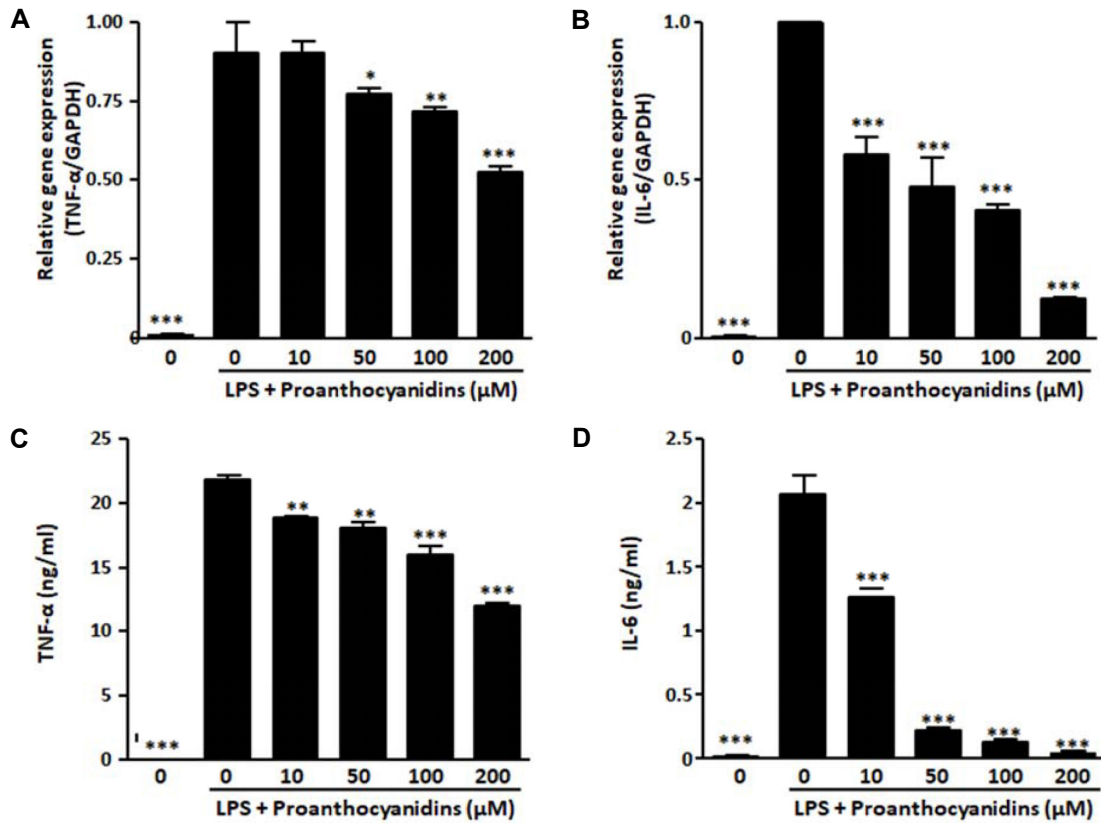


Fig. 4. Proanthocyanidins treatment shown significant inhibitory effect on proinflammatory cytokines. RAW264.7 cells were treated with indicated concentration of proanthocyanidins for 6 hr to measure the mRNA levels of (A) TNF- α and (B) IL-6 by qRT-PCR. RAW264.7 cells was incubated in 0.5 μ g/ml LPS condition with or without proanthocyanidins for 24 hr. The production of (C) TNF- α and (D) IL-6 in cell culture medium was measured by ELISA. Triplicate experiments were used to evaluate the data, and the mean value is expressed as \pm SEM. Significant compared to LPS-treated cells, $p < 0.05$, $**p < 0.01$, $***p < 0.001$.

properties [14, 15, 26, 27]. Although the exact mechanisms involved in anti-inflammatory actions of the HO-1 system have not been fully elucidated, one or more of the HO-1 reaction products have been evaluated as possible factors. For example, CO has been shown to exert significant anti-inflammatory effects in several models of inflammatory tissue injury [14, 17, 27]. CO has been shown to decrease the production of IL-6 [13] and NO [21], and increase the production of IL-10 [9] in LPS-stimulated macrophages. It should also be noted that biliverdin and/or bilirubin are capable of blocking key events in inflammation. Using the well-described rat model of LPS-induced shock, Otterbein et al. [14] demonstrated that exposure to biliverdin imparts a potent defense against lethal endotoxemia systemically, as well as in the lungs, and effectively abrogates the inflammatory response [18]. From the above consideration, we hypothesize the following mechanism of the anti-inflammatory effect of proanthocyanidins. Proanthocyanidins may upregulate heme synthesis, which in turn increases HO-1, which then acts to

degrade heme. At the same time, the catabolites of heme increase and show a suppressive effect on iNOS and other proinflammatory cytokines.

Proanthocyanidins are ubiquitous in fruits, seeds, cereals, bark, flowers, nuts, and vegetables. Proanthocyanidins function as powerful scavengers of oxygen free radicals, with potency comparable to vitamins C and E [2, 3, 9]. In addition, emerging evidence indicates that proanthocyanidins target deleterious signaling pathways activated downstream of free radical production [7, 8]. Therefore, proanthocyanidins are of great interest in nutrition and medicine because of their potent antioxidant capacity and possible protective effects on human health. We believe that our data help to interpret the beneficial effects of proanthocyanidins in reducing the risk of inflammation.

Acknowledgement

This study was supported by the Future Scientist Training

program of the Gyeongsangbuk-do Office of Education.

References

1. Ammar el, S. M., Said, S. A., El-Damarawy, S. L. and Suddek, G. M. 2013. Cardioprotective effect of grape-seed proanthocyanidins on doxorubicin-induced cardiac toxicity in rats. *Pharm. Biol.* **51**, 339-344.
2. Bagchi, D., Bagchi, M., Stohs, S. J., Das, D. K., Ray, S. D., Kuszynski, C. A., Joshi, S. S. and Pruess, H. G. 2000. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. *Toxicology* **148**, 187-197.
3. Bagchi, D., Garg, A., Krohn, R. L., Bagchi, M., Tran, M. X. and Stohs, S. J. 1997. Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract *in vitro*. *Res. Commun. Mol. Pathol. Pharmacol.* **95**, 179-189.
4. Braune, J., Weyer, U., Hobusch, C., Mauer, J., Brüning, J. C., Bechmann, I. and Gericke, M. 2017. IL-6 Regulates M2 Polarization and local proliferation of adipose tissue macrophages in obesity. *J. Immunol.* **198**, 2927-2934.
5. De Simone, V., Franzè, E., Ronchetti, G., Colantoni, A., Fantini, M. C., Di Fusco, D., Sica, G. S., Sileri, P., MacDonald, T. T., Pallone, F., Monteleone, G. and Stolfi, C. 2015. Th17-type cytokines, IL-6 and TNF- α synergistically activate STAT3 and NF- κ B to promote colorectal cancer cell growth. *Oncogene* **34**, 3493-503.
6. Dennery, P. A. 2014. Signaling function of heme oxygenase proteins. *Antioxid. Redox. Signal.* **20**, 1743-1753.
7. El-Shitany, N. A. and Eid, B. 2017. Proanthocyanidin protects against cisplatin-induced oxidative liver damage through inhibition of inflammation and NF- κ B/TLR-4 pathway. *Environ. Toxicol.* **32**, 1952-1963.
8. He, L., Li, P., Yu, L. H., Li, L., Zhang, Y., Guo, Y., Long, M., He, J. B. and Yang, S. H. 2018. Protective effects of proanthocyanidins against cadmium-induced testicular injury through the modification of Nrf2-Keap1 signal path in rats. *Environ. Toxicol. Pharmacol.* **57**, 1-8.
9. Lee, T. S. and Chau, L. Y. 2002. Heme oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice. *Nat. Med.* **8**, 240-246.
10. Lind, M., Hayes, A., Caprnda, M., Petrovic, D., Rodrigo, L., Kruzliak, P. and Zulli, A. 2017. Inducible nitric oxide synthase: Good or bad? *Biomed. Pharmacother.* **93**, 370-375.
11. Long, M., Yang, S., Zhang, Y., Li, P., Han, J., Dong, S., Chen, X. and He, J. 2017. Proanthocyanidin protects against acute zearalenone-induced testicular oxidative damage in male mice. *Environ. Sci. Pollut. Res. Int.* **24**, 938-946.
12. Matzneller, P., Strommer, S., Drucker, C., Petroczi, K., Schörghofer, C., Lackner, E., Jilma, B. and Zeitlinger, M. 2017. Colistin reduces LPS-triggered inflammation in a human sepsis model *in vivo*: A randomized controlled trial. *Clin. Pharmacol. Ther.* **101**, 773-781.
13. Morse, D., Pischke, S. E., Zhou, Z., Davis, R. J., Flavell, R. A., Loop, T., Otterbein, S. L., Otterbein, L. E. and Choi, A. M. 2003. Suppression of inflammatory cytokine production by carbon monoxide involves the JNK pathway and AP-1. *J. Biol. Chem.* **278**, 36993-36998.
14. Otterbein, L. E., Bach, F. H., Alam, J., Soares, M., Tao Lu, H., Wysk, M., Davis, R. J., Flavell, R. A. and Choi, A. M. 2000. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nat. Med.* **6**, 422-428.
15. Paine, A., Eiz-Vesper, B., Blasczyk, R. and Immenschuh, S. 2010. Signaling to heme oxygenase-1 and its anti-inflammatory therapeutic potential. *Biochem. Pharmacol.* **80**, 1895-1903.
16. Ravindranathan, P., Pasham, D., Balaji, U., Cardenas, J., Gu, J., Toden, S. and Goel, A. 2018. Mechanistic insights into anticancer properties of oligomeric proanthocyanidins from grape seeds in colorectal cancer. *Carcinogenesis* **39**, 767-777.
17. Rochette, L., Cottin, Y., Zeller, M. and Vergely, C. 2013. Carbon monoxide: mechanisms of action and potential clinical implications. *Pharmacol. Ther.* **137**, 133-152.
18. Sarady-Andrews, J. K., Liu, F., Gallo, D., Nakao, A., Overhaus, M., Ollinger, R., Choi, A. M. and Otterbein, L. E. 2005. Biliverdin administration protects against endotoxin-induced acute lung injury in rats. *Am. J. Physiol. Lung Cell Mol. Physiol.* **289**, L1131-L1137.
19. Shahat, A. A., Ismail, S. I., Hammouda, F. M., Azzam, S. A., Lemièrre, G., De Bruyne, T., De Swaef, S., Pieters, L. and Vlietinck, A. 1998. Anti-HIV activity of flavonoids and proanthocyanidins from *Crataegus sinaica*. *Phytomedicine* **5**, 133-136.
20. Shapouri-Moghaddam, A., Mohammadian, S., Vazini, H., Taghadosi, M., Esmaeili, S. A., Mardani, F., Seifi, B., Mohammadi, A., Afshari, J. T. and Sahebkar, A. 2018. Macrophage plasticity, polarization, and function in health and disease. *J. Cell. Physiol.* **233**, 6425-6440.
21. Srisook, K., Han, S. S., Choi, H. S., Li, M. H., Ueda, H., Kim, C. and Cha, Y. N. 2006. CO from enhanced HO activity or from CORM-2 inhibits both O₂- and NO production and downregulates HO-1 expression in LPS-stimulated macrophages. *Biochem. Pharmacol.* **71**, 307-318.
22. Su, X., Howell, A. B. and D'Souza, D. H. 2010. Antiviral effects of cranberry juice and cranberry proanthocyanidins on foodborne viral surrogates—a time dependence study *in vitro*. *Food Microbiol.* **27**, 985-991.
23. Takeda, T. A., Sasai, M., Adachi, Y., Ohnishi, K., Fujisawa, J. I., Izawa, S. and Taketani, S. 2017. Potential role of heme metabolism in the inducible expression of heme oxygenase-1. *Biochim. Biophys. Acta Gen. Subj.* **1861**, 1813-1824.
24. Yang, G., Li, Y., Wu, W., Liu, B., Ni, L., Wang, Z., Miao, S., Wang, L. and Liu, C. 2015. Anti-oxidant effect of heme oxygenase-1 on cigarette smoke-induced vascular injury. *Mol. Med. Rep.* **12**, 2481-2486.
25. Yang, L., Xian, D., Xiong, X., Lai, R., Song, J. and Zhong, J. 2018. Proanthocyanidins against oxidative stress: From molecular mechanisms to clinical applications. *Biomed. Res. Int.* **2018**, 8584136.
26. Wang, Y. R., Chen, K. L., Li, C. M., Li, L. and Wang, G.

- L. 2019. Heme oxygenase 1 regulates apoptosis induced by heat stress in bovine ovarian granulosa cells via the ERK1/2 pathway. *J. Cell. Physiol.* **234**, 3961-3972.
27. Zhang, R. G., Pan, K., Hao, Y., Yip, C. Y. and Ko, W. H.

2019. Anti-inflammatory action of HO-1/CO in human bronchial epithelium in response to cationic polypeptide challenge. *Mol. Immunol.* **105**, 205-212.

초록 : 프로안토시아니딘의 항염증효과

천혜진¹ · 박선영¹ · 장희지¹ · 조다영¹ · 정지원² · 박기민² · 정경미² · 김진경^{1*}
(¹대구가톨릭대학교 의생명과학과, ²영천여자고등학교)

프로안토시아니딘(proanthocyanidins)은 식물계에 가장 풍부한 폴리페놀성 화합물로 다양한 고등식물의 뿌리, 잎, 열매, 나무껍질 등에 널리 존재할 뿐만 아니라 이러한 원료로 만들어진 차, 와인, 맥주 등과 같은 식품에도 상당량 함유되어 있다. 세포 및 실험동물을 이용한 다수의 연구보고에 의하면 프로안토시아니딘은 항산화활성 및 면역조절활성, DNA 복구 및 항종양 작용과 같은 인체 건강에 유익한 무수한 효과를 가지고 있는 것으로 밝혀졌다. 면역 세포 중 대식세포(macrophage)는 염증반응을 매개하는 중요한 세포로 외부 병원체 제거에 중요한 역할을 수행하고 있다. 그러나 대식세포가 만성 염증을 유발하고 비만, 당뇨병, 대사 증후군 및 암과 같은 다양한 질병에 관여한다는 것 또한 널리 보고되어왔다. 본 연구에서는 마우스의 대식세포주인 RAW264.7세포를 이용하여 프로안토시아니딘의 항염증활성의 일단이 Heme oxygenase-1 (HO-1)의 유도에 의해서 매개됨을 밝혔다. RAW264.7세포에 프로안토시아니딘을 처리한 결과 세포독성을 보이지 않은 농도에서 HO-1의 발현을 증강시켰다. 또한 프로안토시아니딘의 처리는 HO-1의 발현을 조절하는 핵심 전사인자인 Nrf (nuclear factor-erythroid 2-related factor)-2의 핵으로의 이동을 유의적으로 증가시켰다. 프로안토시아니딘의 처리는 LPS (lipopolysaccharide)에 의해 유도된 NO (nitric oxide)의 생성 및 iNOS (inducible NO synthase)의 발현과 염증성 사이토카인의 생성 및 발현도 유의적으로 억제 하였다. 이러한 결과는 프로안토시아니딘의 항염증제제로서의 개발 가능성을 제시하는 결과이다.