ISSN (Online) 2287-3406
Journal of Life Science **2022** Vol. 32. No. 1. 51~55 DOI : https://doi.org/10.5352/JLS.2022.32.1.51

- Note -

Production of PMA-induced MMP-2 and MMP-9 in the HT-1080 Fibrosarcoma Cell Line is Inhibited by *Corydalis heterocarpa* via the MAPK-related Pathway

Ga Hyun Yu[1], Fatih Karadeniz[2], Jung Hwan Oh[2] and Chang-Suk Kong[1,2]*

[1]*Marine Biotechnology Center for Pharmaceuticals and Foods, Silla University, Baegyang-daero 700 beon-gil 140, Sasang-gu, Busan 46958, Korea*
[2]*Department of Food and Nutrition, College of Medical and Life Sciences, Silla University, Baegyang-daero 700 beon-gil 140, Sasang-gu, Busan 46958, Korea*

Received December 30, 2021 / Revised January 12, 2022 / Accepted January 14, 2022

Matrix metalloproteinase (MMP) enzymes are responsible for the degradation and formation of the extracellular matrix (ECM), and overproduction of MMPs is observed in several diseases, such as cancer and asthma, that progress with metastatic characteristics. Natural products, especially phytochemicals, have been an important source of MMP inhibitors with reduced side effects. Although the majority of phytochemicals inhibit the enzymatic activity of MMPs, some suppress MMP production. In this context, the current study evaluated the potential of *Corydalis heterocarpa*, a halophyte with reported bioactivities, to inhibit MMP expression in PMA-stimulated HT-1080 cells. A crude *C. heterocarpa* extract was shown to decrease the mRNA and protein expression of MMP-2 and MMP-9 while increasing the endogenous MMP inhibitors TIMP-1 and TIMP-2 which regulate MMP expression in healthy tissues. In addition, our results show that the inhibitory effects of *C. heterocarpa* might occur through suppression of the phosphorylation of MAPK signaling, the upstream activator of MMP overexpression. In conclusion, *C. heterocarpa* is a potential source of antimetastatic compounds that might serve as lead molecules to develop novel MMP inhibitors.

Key words : *Corydalis heterocarpa*, MAPK, MMP-9, TIMP-2

Introduction

Matrix metalloproteinases (MMPs) are a family of enzymes responsible for the degradation and rebuilding of the extracellular matrix components. Due to their activities on extracellular matrix (ECM) remodeling, they play crucial roles in normal biological processes such as wound healing and angiogenesis [8]. Although there are several sub-types of MMPs that act on different proteins in ECM, studies pointed out the specific involvement of the MMP-2 and MMP-9 gelatinases during the tumor cell growth and migration [3].

In healthy tissues, the expression, and activity of MMPs are regulated by endogenous tissue inhibitors such as TIMP-1 and TIMP-2 [9]. Up to date, several studies reported novel MMP inhibitors from both synthetic and natural lead molecules that can play pivotal roles in developing anti-cancer pharmaceuticals [7]. In this context, phytochemicals allocate a big portion of the researched molecules for natural MMP inhibitors. Numerous studies reported that extracts of halophytes contained MMP-2 and MMP-9 inhibitors [6, 11]. In the present study, another halophyte, *Corydalis heterocarpa*, was evaluated for its potential to inhibit upregulated MMP expression in PMA-stimulated HT-1080 cells.

***Corresponding author**
Tel : +82-51-999-5429, Fax : +82-51-999-5657
E-mail : cskong@silla.ac.kr

Materials and Methods

Materials

The *C. heterocarpa* plant material was harvested from Jeollanam-do in 2003 and subsequently sun-dried. Dried plant material was then ground to powder and immersed in 3 l MeOH for 2 days and after the MeOH was collected, remaining powder material was kept in 3 l CH_2Cl_2 for 2 more days. The crude extract of *C. heterocarpa* (CHE; 41.1 g) were obtained from the concentration of combined MeOH and CH_2Cl_2 solvents from previous step.

The HT-1080 cell line was purchased from ATCC (Manassas, VA, USA), and other reagents used in cell culture, maintenance and stimulation were purchased from Gibco BRL (New York City, NY, USA) unless otherwise noted. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium

bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The primary antibodies used in Western blotting were against MMP-2 (#4022; Cell Signaling Technology, Beverly, MA, USA), MMP-9 (#393857; Cell Signaling Technology), TIMP-1 (#8946; Cell Signaling Technology), TIMP-2 (#5738; Cell Signaling Technology) , phospho (p)-p38 (#4511; Cell Signaling Technology), p-JNK (sc-293136; Santa Cruz Biotechnology), p-ERK (#4370; Cell Signaling Technology), and β-actin (sc-47778; Santa Cruz Biotechnology).

Cell culture and cytotoxicity of extracts and solvent fractions

Human HT-1080 fibrosarcoma cells were fed with Dulbecco's modified Eagle medium (DMEM). Culture medium contained 10% fetal bovine serum (FBS) and cells were grown in in 37°C temperature in the incubators with 5% CO_2 atmosphere. The HT-1080 cells were stimulated by addition of 10 ng/ml phorbol 12-myristate 13-acetate (PMA) in order to induce overexpression of MMPs. Stimulated cells were treated with or without different concentrations of CHE to evaluate its effect on PMA-stimulated MMP overexpression. The non-toxic concentrations of CHE were defined according to common MTT assay results.

Gelatin zymography

The effect of CHE on the levels of active MMP-2 and MMP-9 present in the PMA-stimulated HT-1080 culture medium was evaluated by gelatin zymography. The detailed gelatin zymography protocol was previously reported by Bae et al. [2].

Reverse transcription-polymerase chain reaction analysis

The effect of CHE on the expression of MMP-2 and MMP-9 mRNA was analyzed by known RT-PCR analysis method. The mRNA levels were quantified and normalized against β-actin levels and given as the relative percentage to that of PMA-stimulated non-treated group. Specific primer sequences for the tested genes and the detailed RT-PCR protocol were reported previously by Bae et al. [2].

Western blotting

Protein expression levels of MMP-2, MMP-9, TIMP-1, TIMP-2 and MAPK proteins were analyzed by typical Western blotting protocols. Protein levels were quantified and normalized against β-actin levels and given as the relative percentage to that of PMA-stimulated non-treated group. The method reported by Bae et al. [2] was followed to obtain the protein bands.

Statistical analysis

All numerical results were given as the mean of three different quantification of the same treatment group ± standard deviation. Any statistical importance was defined at $p<0.05$ level according to the analysis of variance (ANOVA) and post-hoc Duncan's multiple range test. The SAS v9.1 software (SAS Institute, Cary, NC, USA) was used for statistical analysis.

Results and Discussion

Unregulated MMP expression causes disrupted ECM profile and several pathological processes such as tumor metastasis, inflammation and asthma are reported to exhibit overexpressed MMP profiles [3]. Therefore, regulation of MMP expression is a well-known and accepted target to achieve prevention and treatment of aforementioned complications. Several other halophyte species such as *Atriplex gmelinii* [11], *Limonium tetragonum* [2] and *Salicornia herbacea* [5] were reported to possess phytochemicals that inhibit MMP production in stimulated cancer cells. Considering the potential upon reported studies, current study aimed to evaluate the potential of *C. heterocarpa* using PMA-stimulated Ht-1080 human fibrosarcoma cells.

Effect of CHE on active MMP-2 and MMP-9 levels

Prior to test the effects of CHE on MMP-2 and MMP-9 levels, the toxicity of CHE on HT-1080 cells was observed. Results indicated that CHE was not toxic to the cells up to 100 μg/ml (Fig. 1A) treatment which was chosen as the highest dose to be implemented. The effect of CHE on the MMP-2 and MMP-9 secretion levels was tested by gelatin zymography. The collagen degradation ability of MMP-2 and MMP-9 present in the PMA-stimulated HT-1080 culture medium was observed as hollow spaces in gels. As seen in Fig. 1B, PMA-stimulation significantly increased the active MMP-9 levels in HT-1080 medium, however, did not affect the MMP-2 levels significantly. Treatment with CHE decreased the clear zones indicating the less degradation of gelatin by MMP-9 in a dose-dependent manner.

Effect of CHE on MMP-2 and MMP-9 expression

The expression of MMP-2, MMP-9 at the mRNA and protein levels was analyzed by RT-PCR and Western blotting, respectively. PMA stimulation significantly increased the MMP-2 and MMP-9 expression at mRNA and protein levels (Fig. 1C, Fig. 1D). Treatment with CHE dose-dependently suppressed the MMP-2 and MMP-9 levels. At the concentration of 100 μg/ml, CHE treatment decreased the MMP-9 mRNA levels to 15.4% and MMP-2 mRNA levels to 19.0% of the untreated PMA-stimulated group (Fig. 1C). Similar effects were observed in MMP-2 and MMP-9 protein expression (Fig. 1D).

The effect of CHE was also tested on the protein levels TIMP-1 and TIMP-2, the intracellular inhibitors of MMP expression. PMA-stimulation of HT-1080 cells resulted in suppressed levels of TIMP-1 and TIMP-2 protein as a result of MMP overexpression (Fig. 2A). The release of expressional control on MMPs via downregulation of TIMP levels is observed in tumor metastasis [13]. Therefore, in addition to suppression of MMP expression, reverting the TIMP expression to normal levels were expected to act against harmful effects of MMP overexpression [12]. The cells exhibited significant decrease in TIMP-1 and TIMP-2 levels following PMA-stimulation. Treatment with 50 μg/ml CHE was able

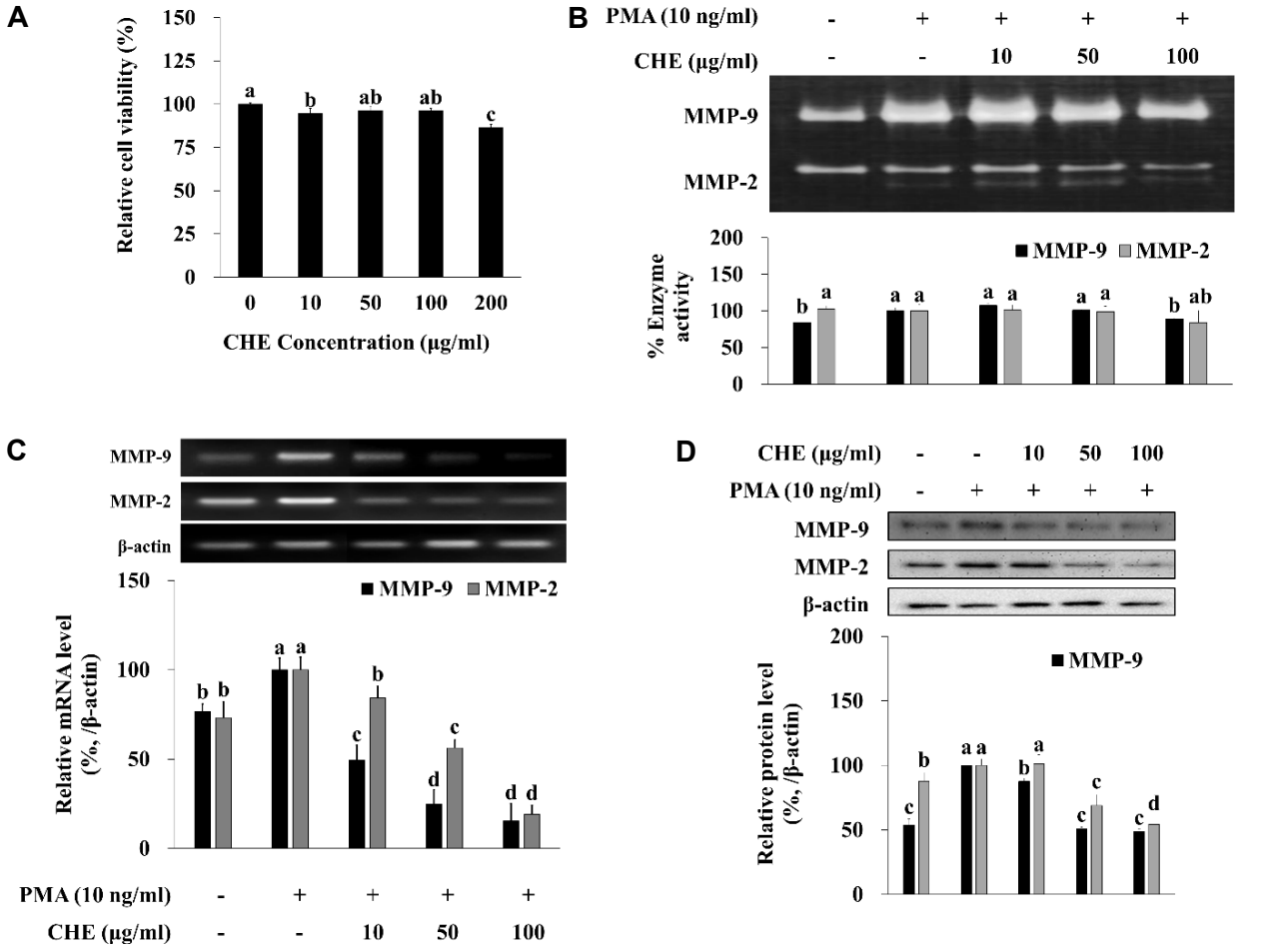

Fig. 1. Effect of *Corydalis heterocarpa* crude extract (CHE) on MMP-2 and MMP-9 levels in PMA-stimulated HT-1080 cells. (A) Effect of SKI-MC and SKI-MeOH fractions on the viability of HT1080 human fibrosarcoma cells analyzed by MTT assay. (B) Effect of CHE on the MMP-2 and MMP-9 activity levels in PMA-stimulated HT-1080 cell culture conditioned media. HT-1080 cells were stimulated with PMA and treated with indicated concentrations of CHE and CHE fractions for 24 hr. Active MMP-2 and MMP-9 levels were evaluated by electrophoresis of enzymes on gelatin containing polyacrylamide gel. (C, D) Effect of CHE on the mRNA (C) and protein (D) expression of MMP-2 and MMP-9. The mRNA expression was quantified via the density of the bands and normalized against β-actin. The mRNA expression levels were given as the relative percentage of the PMA-stimulated untreated group. Values are mean ± SD. [a-e]Means with different letters are significantly different at $p<0.05$ level.

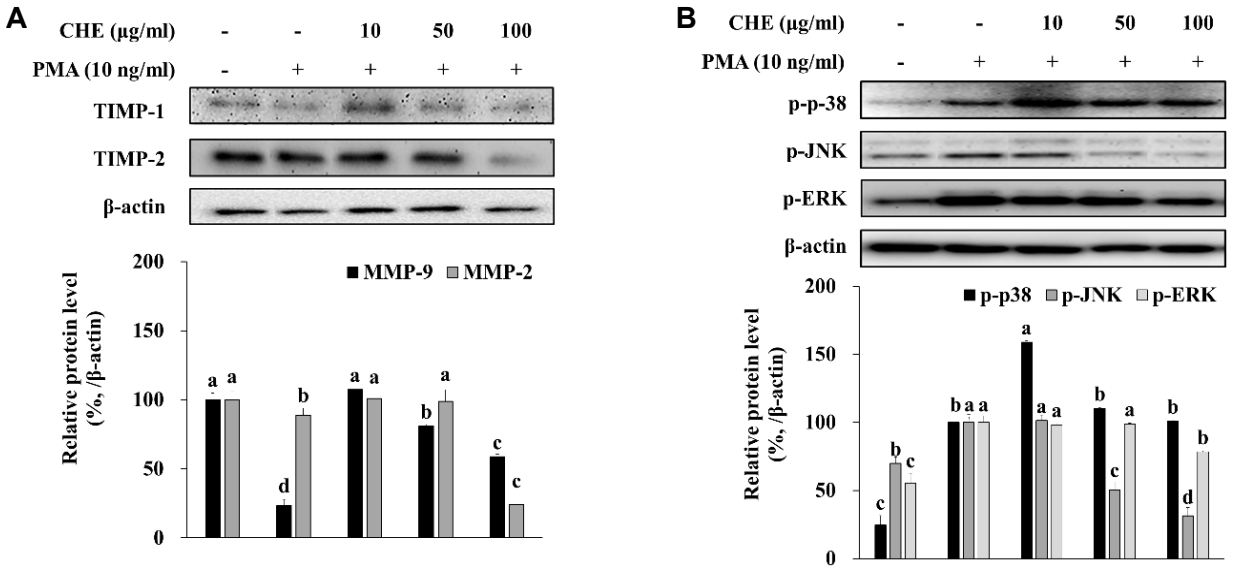

Fig. 2. Effect of CHE on the protein levels of (A) TIMPs and (B) phosphorylated (p-) MAPKs. The protein expression levels in PMA-stimulated HT-1080 cells treated with or without CHE were analyzed by Western blotting and β-actin was used as loading control.

to relieve the effect of MMP overexpression on TIMP-1 and TIMP-2 levels. However, 100 μg/ml treatment exhibited further suppressed levels for both TIMP-1 and TIMP-2, indicating that the at higher concentrations the inhibitory effect of CHE on MMPs was not fully dependent on TIMP mechanism.

Effect of CHE on the phosphorylation of MAPKs

Some studies reported that the expressional regulation of MMPs is carried out by the transcriptional activities of AP-1 protein [14]. AP-1 protein is activated by the phosphorylated MAPK proteins which in turn stimulate the nuclear translocation of AP-1 protein and consequent MMP-2 and MMP-9 expressions. Therefore, any effect of CHE on the phosphorylated p38, ERK and JNK MAPKs were analyzed by Western blotting. As expected, PMA-stimulation increased the phosphorylated MAPK levels (Fig. 2B). Results suggested that CHE might intervene with the MAPK activation in order to suppress the MMP expression.

Previously, Kang et al. [4] reported that coumarins isolated from *C. heterocarpa* expressed anti-inflammatory effects partially through MAPK inhibition. Similarly, anti-photoaging properties of *C. heterocarpa* were credited to its suppressive effect on MAPK activation among others [10]. All these reports were in accordance with current results, further suggesting that *C. heterocarpa* extract might suppress MMP production via MAPK suppression. A study by Anuar et al. [1] also indicated that most of the MMP inhibitors from natural sources act through MAPK signaling and reduce the cancer cell characteristics such as proliferation and migration.

In conclusion, *C. heterocarpa* was suggested to possess bioactive ingredients that suppresses over-stimulated MMP production in fibrosarcoma cells. Further studies focusing on isolation and characterization of active MMP inhibitors from *C. heterocarpa* might yield novel natural MMP inhibitors to be utilized in pharmaceutical and nutraceutical industries.

Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (2020R1I1A3070750) and was supported by the BB21plus funded by Busan Metropolitan City and Busan Institute for Talent & Lifelong Education (BIT).

The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

References

1. Anuar, N. N. M., Zulkafali, N. I. N. and Ugusman, A. 2021. Modulation of matrix metalloproteinases by plant-derived

products. *Curr. Cancer Drug Targets* **21**, 91-106.
2. Bae, M. J., Karadeniz, F., Oh, J. H., Yu, G. H., Jang, M. S., Nam, K. H., Seo, Y. and Kong, C. S. 2017. MMP-inhibitory effects of flavonoid glycosides from edible medicinal halophyte *Limonium tetragonum*. *Evid. Based Complement. Altern. Med.* **2017**, 6750274.
3. Gonzalez-Avila, G., Sommer, B., Mendoza-Posada, D. A., Ramos, C., Garcia-Hernandez, A. A. and Falfan-Valencia, R. 2019. Matrix metalloproteinases participation in the metastatic process and their diagnostic and therapeutic applications in cancer. *Crit. Rev. Oncol. Hematol.* **137**, 57-83.
4. Kang, K. H., Kong, C. S., Seo. Y., Kim, M. M. and Kim, S. K. 2009. Anti-inflammatory effect of coumarins isolated from *Corydalis heterocarpa* in HT-29 human colon carcinoma cells. *Food Chem. Toxicol*. **47**, 2129-2134.
5. Kong, C. S., Kim, Y. A., Kim, M. M., Park, J. S., Kim, J. A., Kim, S. K., Lee, B. J., Nam, T. J. and Seo, Y. 2008. Flavonoid glycosides isolated from *Salicornia herbacea* inhibit matrix metalloproteinase in HT1080 cells. *Toxicol. In Vitro* **22**, 1742-1748.
6. Kong, C. S., Um, Y. R., Lee, J. I., Kim, Y. A., Yea, S. S. and Seo, Y. 2010. Constituents isolated from *Glehnia littoralis* suppress proliferations of human cancer cells and MMP expression in HT1080 cells. *Food Chem.* **120**, 385-394.
7. Kumar, G. B., Nair, B. G., Perry, J. J. P. and Martin, D. B. C. 2019. Recent insights into natural product inhibitors of matrix metalloproteinases. *Medchemcomm* **10**, 2024-2037.
8. Malemud, C. 2006. Matrix metalloproteinases (MMPs) in health and disease: an overview. *Front. Biosci.* **11**, 1696-1701.
9. Murphy, G. 2011. Tissue inhibitors of metalloproteinases. *Genome Biol.* **12**, 233.
10. Oh, J. H., Karadeniz, F., Lee, J. I., Kim, H. R., Seo, Y. and Kong, C. S. 2020. Antiphotoaging effect of (2′S)-columbianetin from *Corydalis heterocarpa* in UVA-irradiated human dermal fibroblasts. *Appl. Sci*. **10**, 2568.
11. Park, M. J., Kong, C. S. and Seo, Y. 2019. Inhibition of MMP-2 and -9 by crude extracts and their solvent-partitioned fractions from the halophyte *Atriplex gmelinii*. *Ocean Polar Res.* **41**, 79-88.
12. Raeeszadeh-Sarmazdeh, M., Do, L. D. and Hritz, B. G. 2020. Metalloproteinases and their inhibitors: Potential for the development of new therapeutics. *Cells* **9**, 1313.
13. Safranek, J., Pesta, M., Holubec, L., Kulda, V., Dreslerova, J., Vrzalova, J., Topolcan, O., Pesek, M., Finek, J. and Treska, V. 2009. Expression of MMP-7, MMP-9, TIMP-1 and TIMP-2 mRNA in lung tissue of patients with non-small cell lung cancer (NSCLC) and benign pulmonary disease. *Anticancer Res.* **29**, 2513-2517.
14. Tombulturk, F. K., Soydas, T., Yaprak Sarac, E., Tuncdemir, M., Coskunpinar, E., Polat, E., Sirekbasan, S. and Kanigur-Sultuybek, G. 2019. Regulation of MMP 2 and MMP 9 expressions modulated by AP-1 (c-jun) in wound healing: improving role of *Lucilia sericata* in diabetic rats. *Acta Diabetol.* **56**, 177-186.

초록 : PMA로 자극된 HT-1080 세포에서 염주괴불주머니 추출물의 MAPK 경로를 통한 MMP-2, MMP-9 발현 억제 효과

유가현[1] · 카라데니즈 파티[2] · 오정환[2] · 공창숙[1,2]*
([1]신라대학교 의생명과학대학 식품영양학과, [2]신라대학교 해양식의약소재융합기술연구소)

Matrix metalloproteinases (MMPs)는 세포의 기저막 분해에 관여하는 효소로 과발현된 MMPs는 암세포 침윤과 전이에 직접적인 영향을 주는 것으로 알려져 있다. 본 연구에서는 항산화, 항염증, 항균활성 있는 것으로 보고되어 있는 염주괴불주머니 추출물을 이용하여 PMA로 유도된 인간 섬유육종세포 HT-1080 세포에서 MMP-2, MMP-9의 발현 조절에 미치는 영향을 확인하였다. 그 결과 염주괴불주머니 추출물은 TIMP-1 및 TIMP-2를 증가시키면서 MMP-2 및 MMP-9의 mRNA 및 단백질 발현 수준을 모두 감소시키는 것으로 나타났다. 또한 p38, JNK, ERK의 인산화를 억제하였으며, 이를 통해 염주괴불주머니 추출물은 MAPKs 신호 전달 경로 조절에 영향을 줌으로써 MMPs 발현을 감소시키는 것을 확인할 수 있었다. 따라서 이러한 연구의 결과는 염주괴불주머니를 이용한 암 전이 억제 소재 개발을 위한 기초자료로 활용될 수 있을 것으로 기대된다.