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Apoptotic activity of *Angelica decursiva* extract in KB oral cancer cells

조선대학교 대학원

치의생명공학과

이 명 화

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KB 구강암세포에서 *Angelica decursiva* 추출물의
세포사멸 활성

2011년 2월 일

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지도교수 김 흥 중

이 논문을 치의학 석사학위신청 논문으로 제출함.

2010년 10월 일

조선대학교 대학원

치의생명공학과

이 명 화

이명화의 석사학위 논문을 인준함

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ABSTRACT

Apoptotic activity of *Angelica decursiva* extract in KB oral cancer cells

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The root of *Angelica decursiva* has been used the home remedy in Korean as an antitussive, an analgesic, an antipyretic, and a expectorant. However, the effects of *Angelica decursiva* on anti-cancer activity have not been clearly established. The main purpose of this study was to investigate the cytotoxic activity and the mechanism of cell death exhibited by the ethanol extract of *Angelica decursiva* (EEAD) against KB oral cancer cells.

To determine the effects of EEAD on cell growth in KB oral cancer cells and human normal oral keratinocytes (HNOK), it was examined by MTT assay. In addition, to verify the mechanism of apoptosis, DNA fragmentation analysis, immunoblotting and measuring of caspase activity were performed in KB cells.

EEAD induced the apoptotic cell death in a dose- and a time-dependent manner in KB oral cancer cells, but not in HNOK. Treatment of EEAD significantly promoted proteolytic cleavage of procaspase-7 and -9 with a decrease in the amount of procaspase-7 and -9 in the KB cells. In addition, activation of caspase-3/-7 was detected in living KB oral cancer cells by fluorescence microscopy.

Taken together, these results suggest that EEAD could induce the suppression of cell growth and the promotion of cell apoptosis in KB oral cancer cells, and therefore, that it may have potential properties for anti-cancer drug discovery.

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Key Words: *Angelica decursiva*, apoptosis, oral cancer cells, anti-cancer activity

I. INTRODUCTION

In recent years, there has been a global trend toward the use of natural substances present in fruits, vegetable, oilseeds, and herbs as medicine and functional food. Several of these substances are shown to have potential values as cancer chemopreventive or therapeutic agents within the human body. In instance, some vitamins and their derivatives have important biological roles related to cancer prevention and free radical scavenging (van Poppel and van den Berg, 1997). Some phytochemicals, such as Taxol, Oncovin, and captothecin, are spotlighted in current clinical use for cancer treatment (Pezzuto, 1997; Christou et al., 2001; Mukherjee et al., 2001). Most of these bioactive substances exert their cancer chemotherapeutic activity by blocking cell cycle progression and triggering apoptotic cell death. Therefore, induction of apoptosis in cancer cells has become an important indicator of the tumor treatment response in employing a plantbioactive substance to reduce and control human mortality due to cancer (Smets, 1994; Paschka et al., 1998).

Apoptosis, which is a major way of programmed cell death, plays an important role in the regulation of tissue development and homeostasis in eukaryotes (Green and Reed, 1998; Hengartner, 2000; Kaufmann and Hengartner, 2001). During past two decades, the molecular mechanism of apoptosis has been extensively studied. Apoptosis may occur via a death receptor-dependent extrinsic or a mitochondria-dependent intrinsic pathway. Apoptosis is induced by treatment of chemotherapeutic agents (Walker et al., 1991; Shinomiya et al., 1994; Havrilesky et al., 1995; Haschtscha et al., 1996; Kaufmann and Earnshaw, 2000; Reed, 2001). Caspase, a family of cytosolic cysteine proteases, plays an essential role in the execution of apoptosis. The procaspase-9 is activated via a mitochondrial pathway (Denecker et al., 2001). Mitochondria pathway is mediated by Bcl-2 family proteins.

Antiapoptotic Bcl-2 protein and proapoptotic Bax protein of the Bcl-2 family regulate the passage of cytochrome C from the mitochondria (Kelekar and Thompson, 1998). Caspase-9 activates executioner caspases such as caspases-3, -6 and -7 (Salvesen and Dixit, 1997).

There are several medicinal plants that are considered to possess significant anti-cancer activity and also recently, scientific attentions increased to oriental medicine for the discovery of novel drugs including anticancer agents (Hu et al., 2002; Lee et al., 2002; Cheng et al., 2005; Park et al., 2005; Tan et al., 2005). One of traditional Korean medicine, *Angelica decursiva* has been used mainly as a home remedy for treatment of antitussive, analgesic, antipyretic, and a cough. However, biological studies of apoptosis induction with this herb were reported not at all.

Therefore, this study was carried out to evaluate the cytotoxic effect and to determine the possible mechanisms of cell death elicited by the extract of *Angelica decursiva* on KB oral cancer cells. Furthermore, these results generated from this study will help to support the development of human clinical trials in the future.

II. MATERIALS AND METHODS

1. Materials

3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma (St Louis, MO, USA). Anti-caspase-7 and anti-caspase-9 antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Cell-permeable fluorogenic substrate PhiPhiLux-G_1D_2 was purchased from OncoImmunin, Inc. (Gaithersburg, MD, USA).

2. Plant material and extract preparation

Dried root parts of *Angelica decursiva* were purchased from Jeonnam herbal medicine farmer's cooperative, Korea. The botanical identification was made by Prof. Su-In Cho, School of Oriental Medicine, Pusan National University, Korea. The roots were ground with a Wiley mill to pass a 1-㎜ screen and were extracted with 95% ethyl alcohol (EtOH) at 40℃ for 5 hours. The extract was then filtered through a Advantec No. 1 filter paper. The collected filtrate was dried by evaporation under vacuum at 40℃ using a rotary evaporator (N-1000V-W, Eyela, Japan). After evaporation, the concentrated extract was freeze-dried at -40℃ for 3 days and stored in a refrigerator at 2℃ until used.

3. Cell cultures

The KB cells were grown in MEM containing NEAA (non-essential amino acids) at a ratio of 100:1, and the media was supplemented with 5% FBS. Cells were maintained as monolayers in plastic culture plates at 37℃ in a humidified

atmosphere containing 5% CO_2.

Healthy oral mucosa was obtained with proper informed consent from healthy human subjects who were patients undergoing dental surgery for crown lengthening at the Dental Hospital, Chosun University, Gwangju, Korea. The surgical materials were approved by the Chosun University Institutional Research Board to be used for medical study. In this study, 15 tissue specimens came from healthy subjects aged between 15 and 40 years. Occasionally, more than one sample of tissue came from a single subject. After disinfecting in povidone iodine solution, the tissue samples, which had a size of approximately 5 × 10 mm, were immersed for 2 hours at 4℃ in 0.25% trypsin/Hank's balanced salt solution (HBSS), supplemented with 100 U/㎖ streptomycin and 1% amphotericin B, to facilitate the removal of connective tissue. The tissue samples were kept at room temperature for 30 min and the epithelium was separated using fine forceps. The epithelium was dissociated with 0.025% trypsin in EDTA for 10 min and centrifuged at 400 × g. The cells were collected and fed with keratinocyte growth medium (KGM-SFM) containing 50 ㎍/㎖ of gentamycin, 5 ng/㎖ of EGF (epidermal growth factor) and 50 ㎍/㎖ of pituitary gland extract as culture media. The seeding density of cells was at least 0.5 × 10^3 cells/㎠. The human normal oral keratinocytes (HNOK) from passages 1 to 3 were used for this study.

4. Inhibition of cell growth (MTT assay)

The cell viability test was performed according to the previously-described method (Keum et al., 2002; Kim et al., 2004), with minor modifications. The cells were seeded at a concentration of 5 x 10^3 cells/well in 24-well plates. After 24 hours growth, the cells were treated with the ethanol extract of *Angelica decursiva* (EEAD) at various concentrations for 24 hours. The cell viability was assessed

using MTT assay. Three separate experiments were performed for each concentrations.

5. DNA fragmentation analysis

Following treatment with 0.3 ㎍/㎖ EEAD for various incubation times (0, 8 and 24 hours) approximately 5 x 10^6 cells were collected and transferred to lysis buffer containing 100 mM NaCl, 10 mM EDTA, 300 mM Tris-HCl, pH 7.5, 200 mM sucrose, 0.5% SDS and 0.5 ㎎/㎖ proteinase K and incubated at 65℃. DNA was extracted with an equal volume of phenol/chloroform/isoamylalcohol (25:24:1, v/v) and precipitated with ethanol. The DNA was resuspended in Tris-EDTA buffer, pH 8.0 containing 5 ㎍/㎖ DNase-free RNase and incubated at 37℃ for 1 hour. The DNA was visualized on 2% agarose gel in the presence of 0.5 ㎍/㎖ ethidium bromide.

6. Immunoblotting

The cells were treated with 0.3 ㎍/㎖ EEAD for various incubation times (0, 8 and 24 hours). For western blot analysis, cells were washed twice with PBS at 4°C, and 0.1 ㎖ of lysis buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 0.25% sodium deoxycholate, 0.1% Nonidet P-40, 0.1% Triton X-100, 50 mM NaF, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 50 mM sodium pyrophosphate, 10 mM sodium vanadate and 1 x protease inhibitor cocktail (Roche; Indianapolis, IN)) was added. After centrifugation, the supernatant was transferred to a new tube and SDS-PAGE sample buffer was added to the supernatant. Approximately 30 ㎍ of protein from each lysate was resolved by SDS-PAGE and transferred to a polyvinylidene difluride membrane for immunoblotting. Membranes were blocked 1

hour with 10% milk, followed by incubation for 1 hour with the anti-caspase-7, anti-caspase-9 antibody (1:1000 dilution, Cell Signaling Technology, Inc., Danvers, MA, USA), or anti-β-actin (sc-47778, Santa Cruz Biotechnology) as primary antibodies. After washing 2 times with TTBS (0.1% Tween 20, 50 mM Tris-HCl [pH 7.4], 150 mM NaCl), secondary antibodies conjugated with a horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse antibody were added. They were then incubated for 1 hour before development with ECL Western blotting reagents (Amersham, NJ, USA). The intensity of individual bands was determined with ImageQuant analysis software (Amersham, NJ, USA).

7. Determination of caspase activation

The activity of caspase-3/-7 was determined using the cell-permeable fluorogenic substrate PhiPhiLux-G_1D_2 (OncoImmunin, Inc. Gaithersburg, MD, USA), which was used according to the manufacturer's instructions. The KB cells were treated with 0.3 ㎍/㎖ of EEAD for 24 hours and incubated with PhiPhiLux-G_1D_2. The activity of caspase-3/-7 was visualized by fluorescence microscopy (IX71, Olympus, Japan).

8. Data Analysis

All experiments were performed in triplicate. Results are presented as mean ± SEM. Statistical significance was analyzed by using Student's *t*-test for two groups and one way analysis of variance for multi-group comparisons. $P<0.05$ is considered statistically significant.

Ⅲ. RESULTS

1. Cytotoxic activity of EEAD

To analyze the effect of EEAD on the viability of oral cancer cells, the cells were treated with EEAD at various concentrations for 24 hours, and then the MTT assay was performed. From 0.01 to 30 ㎍/㎖ treatment of EEAD, the inhibition of KB cells growth depended on the EEAD concentrations (Fig. 1). As shown in Fig. 1, cytotoxicity of EEAD on KB cells were shown in a dose dependent manner. The IC_{50} value of EEAD treatment was 0.21 ± 0.032 ㎍/㎖. In contrast, the cytotoxocity of EEAD in HNOK was not detected. These results suggest that EEAD has a specific effect for inhibition of KB oral cancer cell growth.

2. DNA fragmentation assay

Increased cellular apoptosis is only one among several possible mechanisms involved in reduced cell proliferation. To determine if apoptosis is indeed the underlying mechanism for the reduced cell proliferation we had observed, the KB cells treated with EEAD were subjected to DNA fragmentation. As shown in Fig. 2, the formation of a DNA ladder in the KB cells treated with 0.3 ㎍/㎖ of EEAD was observed in a time-dependent manner.

3. Activation of caspases by EEAD

The levels of procaspase-7 and procaspase-9 were examined by Western blot analysis and detected by fluorescence microscopy using a selective fluorogenic

substrate since caspase-7 and caspase-9 are effector caspases of apoptotic cell death. Treatment (0.3 ㎍/㎖) of EEAD significantly promoted proteolytic cleavage of procaspase-7 and -9 with a decrease in the amount of procaspase-7 and -9 in the KB cells (Fig. 3 and 4). Furthermore, activation of caspase-3/-7 in EEAD treated KB cells was confirmed by fluorescence microscopy using fluorogenic substrate. As shown in Fig. 5, EEAD treatment led to activate the caspase-3/-7 in the KB cells.

Ⅳ. DISCUSSION

Chemotherapeutic drugs are known to induce cytotoxicity in tumor cells through diverse mechanisms, in which signaling events play an important role depending upon the cell type and stimulus (Hoshino et al., 1991; Tian et al., 2006). There is a need to find new anti-cancer drugs that can kill cancerous cells with minimal toxicity. The cytostatic effect of whole plant extracts on cancer cells is often much better than the effect of their particular biologically active compounds (Yano et al., 1994; Vickers, 2002). The main purpose of this study was to investigate the effect of *Angelica decursiva* extract on cell growth and apoptosis induction in KB human oral cancer cell line.

In the MTT assay, EEAD inhibited cell growth in a dose-dependent manner in the KB cells, but not in HNOK (Fig. 1). Results have shown that EEAD remarkably reduced proliferation of KB cells at 1 ㎍/㎖. However, EEAD has not shown such anti-proliferative effects in HNOK. Even in high concentration (30 ㎍/㎖), EEAD is not cytotoxic as judged by MTT assay (Fig. 1). This corresponded with the results of several extracts (*Echinacea root, toona sinensis, willow bark)* that have anti-cancer effects via the suppression of cancer cell growth in a dose-dependent manner (Yang et al., 2006; Chicca et al., 2007). As shown in Fig. 1, relatively low concentration (1 ㎍/㎖) of EEAD was enough to suppress the cell growth and apoptosis compared with other extracts (Yang et al., 2006; Chicca et al., 2007; Hostanska et al., 2007). These results indicate that EEAD has specific cytotocity for only cancer cells and potential value for anti-cancer drug discovery.

Apoptosis is an important way to maintain cellular homeostasis between cell division and cell death (Green and Reed, 1998; Hengartner, 2000; Kaufmann and Hengartner, 2001). So, induction of apoptosis in cancer cells is one of useful strategies for anti-cancer drug development (Hu and Kavanagh, 2003). In this

respect, many studies were performed for screening of apoptosis including compounds from plants. In this study, treatment with EEAD induced internucleosomal DNA fragmentation in a time-dependent manner in KB cells, suggesting apoptotic cell death (Fig. 2). These results indicate that EEAD inhibited the growth of this cell by activating cell apoptosis.

Furthermore, the activation of a family of intracellular cysteine proteases, called caspases, is known to play an important role in the initiation and execution of apoptosis induced by various stimuli (Datta et al., 1997; Liu et al., 1997). Among the caspases identified in mammalian cells, caspase-7 and caspase-9 may serve as effector caspases of apoptotic cell death (Datta et al., 1997; Liu et al., 1997). Caspase-7 and caspase-9 are synthesized as inactive proenzymes (of sizes 35 kDa and 47 kDa, respectively), which require proteolytic activation (Cohen, 1997; Datta et al., 1997; Liu et al., 1997). Our results showed that high levels of procapase-7 and low level of procaspase-9 were present in EEAD-untreated cancer cells, and the amount of procaspase-7 and -9 was decreased after EEAD treatment in the KB cells (Fig. 3 and 4). In addition, the activity of caspase-3/-7 was increased by EEAD treatment in the KB cells compared with DMSO treatment as a control (Fig. 5).

In conclusion, this study clearly demonstrates that the EEAD strongly inhibits cell proliferation and induces apoptosis in KB oral cancer cell. EEAD induced apoptosis through activation of caspase-7 and -9 and degradation of chromosomal DNA. Because apoptosis was regarded as a new target in discovery of anti-cancer drugs, these results confirm the potential of *Angelica decursiva* as an agent of chemotherapeutic and cytostatic activity in KB oral cancer cell. However, to elaborate this nascent possibility, further investigation of its activity including *in vivo* and purification of bioactive compounds is now in progress.

V. REFERENCES

Cheng YL, Lee SC, Lin SZ, Chang WL, Chen YL, Tsai NM, Liu YC, Tzao C, Yu DS, Harn HJ: Anti-proliferative activity of *Bupleurum scrozonerifolium* in A549 human lung cancer cells *in vitro* and *in vivo*. Cancer Lett 222: 183 - 193, 2005.

Chicca A, Adinolfi B, Martinotti E, Fogli S, Breschi MC, Pellati F, Benvenuti S, Nieri P: Cytotoxic effects of Echinacea root hehanic extracts on human cancer lines. J Ethnopharmacol 110: 148-153, 2007.

Christou L, Hatzimichael E, Chaidos A, Tsiara S, Bourantas KL: Treatment of plasma cell leukemia with vincristine, liposomal doxorubicin and dexamethasone. Eur J Haematol 67: 51 - 53, 2001.

Cohen GM: Caspases: the executioners of apoptosis. Biochem J 326: 1-16, 1997.

Datta R, Kojima H, Yoshida K, Kufe D: Caspase-3-mediated cleavage of protein kinase C theta in induction of apoptosis. J Biol Chem 272: 20317-20320, 1997.

Denecker G, Vercammen D, Declercq W, Vandenabeele P: Apoptotic and necrotic cell death induced by death domain receptors. Cell Mol Life Sci 58: 356 - 370, 2001.

Green DR, Reed JC: Mitochondria and apoptosis. Science 281: 1309 - 1312, 1998.

Havrilesky LJ, Elbendary A, Hurteau JA, Whitaker RS, Rodriguez GC, Berchuck A: Chemotherapy-induced apoptosis in epithelial ovarian cancers. Obstet Gynecol 85: 1007 - 1010, 1995.

Hengartner MO: The biochemistry of apoptosis. Nature 407: 770 - 776, 2000.

Hoshino T, Hara A, Inoue M, Honda J, Imai Y, Oizumi K, Yokoyama MM: Flow cytometric measurement of NK cell cytotoxicity. J Clin Lab Immunol 36: 39 - 43, 1991.

Hostanska K, Jurgenliemk G, Abel G, Nahrstedt A, Saller R: Willow bark extract (BNO1455) and its fractions suppress growth and induce apoptosis in human colon and lung cancer cells. Cancer Detect Prev 31: 129-139, 2007.

Hu H, Ahn NS, Yang X, Lee YS, Kang KS: *Ganoderma lucidum* extract induces cell cycle arrest and apoptosis in MCF-7 human breast cancer cell. Int J Cancer 102: 250 - 253, 2002.

Hu W, Kavanagh JJ: Anticancer therapy targeting the apoptotic pathway. Lancet Oncol 4: 721 - 729, 2003.

Huschtscha LI, Bartier WA, Ross CE, Tattersall MH: Characteristics of cancer cell death after exposure to cytotoxic drugs in vitro. Br J Cancer 73: 54 -

60, 1996.

Kaufmann SH, Earnshaw WC: Induction of apoptosis by cancer chemotherapy. Exp Cell Res 256: 42 - 49, 2000.

Kaufmann SH, Hengartner MO: Programmed cell death: alive and well in the new millennium. Trends Cell Biol 11: 526 - 534, 2001.

Kelekar A, Thompson CB: Bcl-2 family proteins: the role of the BH3 domain in apoptosis. Trends Cell Biol 8: 324 - 330, 1998.

Keum YS, Kim J, Lee KH, Park KK, Surh YJ, Lee JM, Lee SS, Yoon JH, Joo S Y, Cha IH, Yook JI: Induction of apoptosis and caspase-3 activation by chemopreventive [6]-paradol and structurally related compounds in KB cells. Cancer Lett 177: 41-47, 2002.

Kim DK, Kim IJ, Hwang S, Kook JH, Lee MC, Shin BA, Bae CS, Yoon JH, Ahn SG, Kim SA, Kanai Y, Endou H, Kim JK: System L-amino acid transporters are differently expressed in rat astrocyte and C6 glioma cells. Neurosci Res 50: 437-446, 2004.

Lee SM, Li ML, Tse YC, Leung SC, Lee MM, Tsui SK, Fung KP, Lee CY, Waye MM: Paeoniae Radix, a Chinese herbal extract, inhibit hepatoma cells growth by inducing apoptosis in a p53 independent pathway. Life Sci 71: 2267 - 2277, 2002.

Liu X, Zou H, Slaughter C, Wang X: DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. Cell 89: 175-184, 1997.

Mukherjee AK, Basu S, Sarkar N, Ghosh AC: Advances in cancer therapy with plant based natural products. Curr Med Chem 8: 1467 - 1486, 2001.

Park DI, Lee JH, Moon SK, Kim CH, Lee YT, Cheong J, Choi BT, Choi YH: Induction of apoptosis and inhibition of telomerase activity by aqueous extract from *Platycodon grandiforum* in human lung carcinoma cells. Pharmacol Res 51: 437 - 443, 2005.

Paschka AG, Butler R, Young CY: Induction of apoptosis in prostate cancer cell lines by the green tea component, (−)-epigallocatechin-3-gallate. Cancer Lett 130: 1 - 7, 1998.

Pezzuto JM: Plant-derived anticancer agents. Biochem Pharmacol 53: 121 - 133, 1997.

Reed JC: Apoptosis-regulating proteins as targets for drug discovery. Trends Mol Med 7: 314 - 319, 2001.

Salvesen GS, Dixit VM: Caspases: intracellular signaling by proteolysis. Cell 91: 443 - 446, 1997.

Shinomiya N, Shinomiya M, Wakiyama H, Katsura Y, Rokutanda M: Enhancement of CDDP cytotoxicity by caffeine is characterized by apoptotic cell death. Exp Cell Res 210: 236 - 242, 1994.

Smets LA: Programmed cell death (apoptosis) and response to anti-cancer drugs. Anticancer Drugs 5: 3 - 9, 1994.

Tan ML, Sulaiman SF, Najimuddin N, Smian MR, Muhammad TS: Methanolic extract of *Pereskia bleo* (Kunth) DC. (Cactaceae) induces apoptosis in breast carcinoma, T47-D cell line. J Ethnopharmacol 96: 287 - 294, 2005.

Tian Z, Chen S, Zhang Y, Huang M, Shi L, Huang F, Fong C, Yang M, Xiao P: The cytotoxicity of naturally occurring styryl lactones. Phytomedicine 13: 181 - 186, 2006.

van Poppel G, van den Berg H: Vitamins and cancer. Cancer Lett 114: 195 - 202, 1997.

Vickers A: Botanical medicines for the treatment of cancer: rationale, overview of current data, and methodological considerations for phase I and II trials. Cancer Invest 20: 1069 - 1079, 2002.

Walker PR, Smith C, Youdale T, Leblanc J, Whitfield JF, Sikorska M: Topoisomerase II-reactive chemotherapeutic drugs induce apoptosis in thymocytes. Cancer Res 51: 1078 - 1085, 1991.

Yang HL, Chang WH, Chia YC, Huang CJ, Lu FJ, Hsu HK., Hseu YC: Toona sinensis extracts induces apoptosis via reactive oxygen species in human premyelocytic leukemia cells. Food Chem Toxicol 44: 1978-1988, 2006.

Yano H, Mizoguchi A, Fukuda K, Haramaki M, Ogasawara S, Momoski S, Kojiro M: The herbal medicine sho-saiko-to inhibits proliferation of cancer cell lines by inducing apoptosis and arrest at G0/G1 phase. Cancer Res 54: 448 - 454, 1994.

Ⅵ. FIGURE LEGENDS

Fig. 1. Cytotoxic effects of EEAD on KB cells and HNOK. Cytotoxic activity detected by MTT assay. The cells were exposed to various concentrations of EEAD for 24 hours. The KB (■) and HNOK (●) were treated with 0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, and 30 ㎍/㎖ EEAD for 24 hours. Cell viabilities were determined by the MTT assay. The percentage of cell viability was calculated as a ratio of A570 ㎚ of EEAD treated cells and untreated control cells. Each data point represents the mean ± SEM for three experiments. ***$P<0.001$ vs. control (the control cells were measured in the absence of EEAD treatment).

Fig. 2. Analysis of DNA fragmentation using agarose gel electrophoresis. Apoptosis induced by EEAD in KB cells. KB cells were incubated in the presence of 0.3 ㎍/㎖ of EEAD for 0, 8, and 24 hours. Genomic DNA was prepared as described in "MATERIALS AND METHODS" and analyzed by 2% agarose electrophoresis followed by ethidium bromide staining. The figure is a representative of results from three independent experiments.

Fig. 3. EEAD treatment induces apoptosis via proteolytic cleavage of procaspase-7. (A) Western blot analysis of procaspase-7 in KB cells. The cells were treated with 0.3 ㎍/㎖ of EEAD for the indicated time periods. The cell lysate was prepared and analyzed by western blot analysis as described in "MATERIALS AND METHODS". (B) Quantitative analyses of the blots in (A).

Fig. 4. EEAD treatment induces apoptosis via proteolytic cleavage of procaspase-9. (A) Western blot analysis of procaspase-9 in KB cells. The cells

were treated with 0.3 ㎍/㎖ of EEAD for the indicated time periods. The cell lysate was prepared and analyzed by western blot analysis as described in "MATERIALS AND METHODS". (B) Quantitative analyses of the blots in (A).

Fig. 5. Activation of caspase-3/-7 by EEAD treatment in living KB cells. The cells were treated with 0.3 ㎍/㎖ of EEAD for 24 hours and added specific cell-permeable substrate PhiPhiLux G_1D_2. Active of caspase-3/-7 was visualized by fluorescence microscopy.

Ⅶ. FIGURES

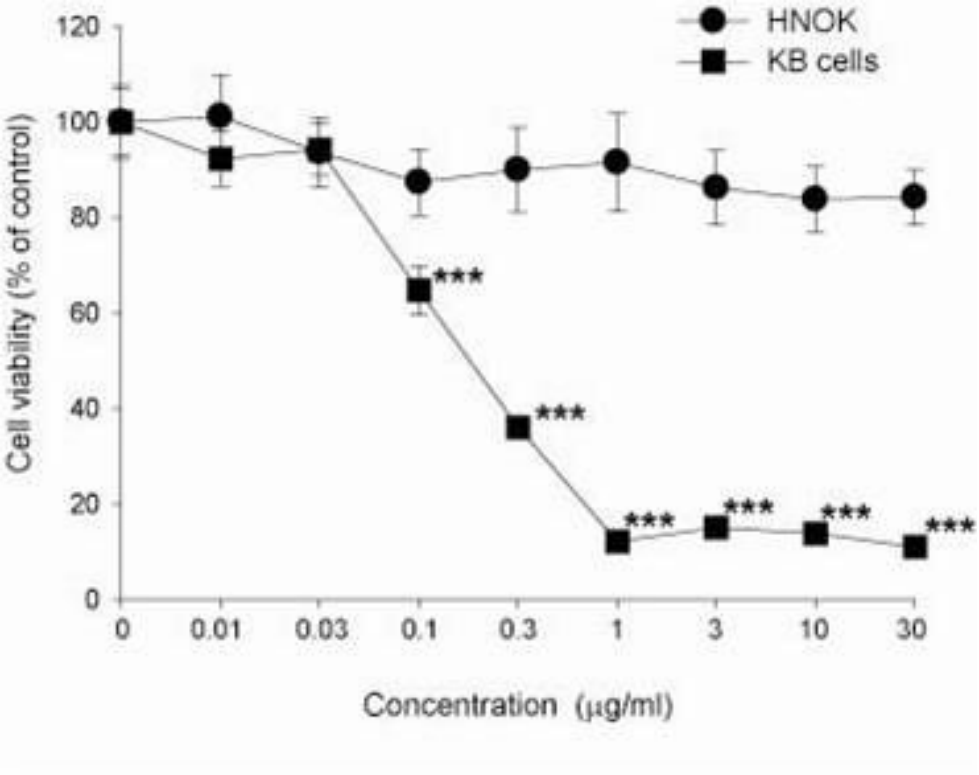

Fig. 1. Cytotoxic effects of EEAD on KB cells and HNOK.

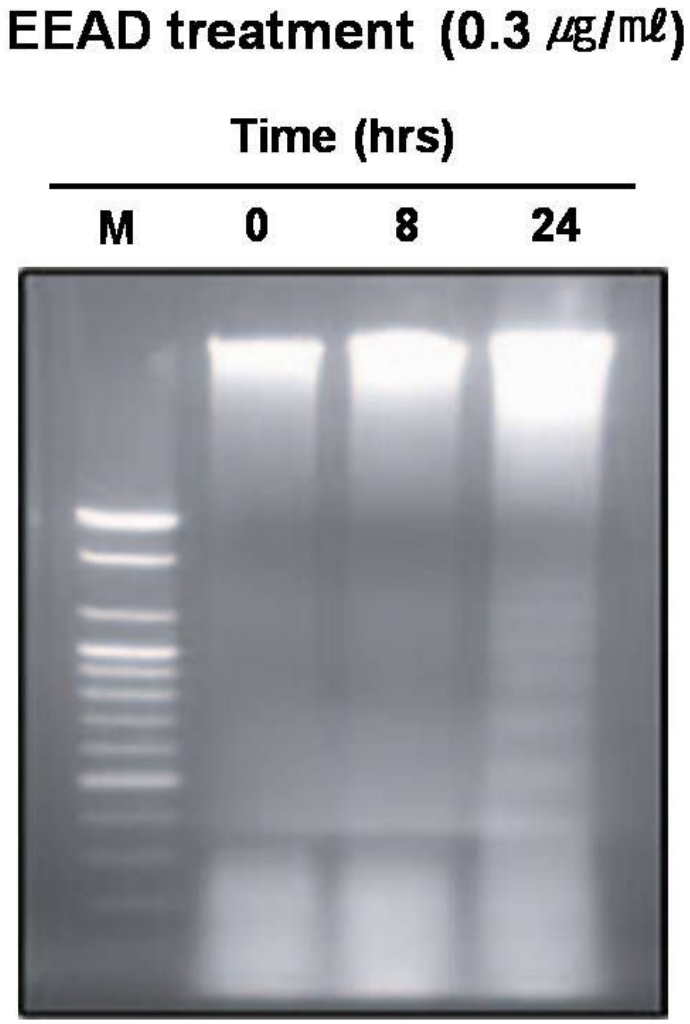

Fig. 2. Analysis of DNA fragmentation using agarose gel electrophoresis.

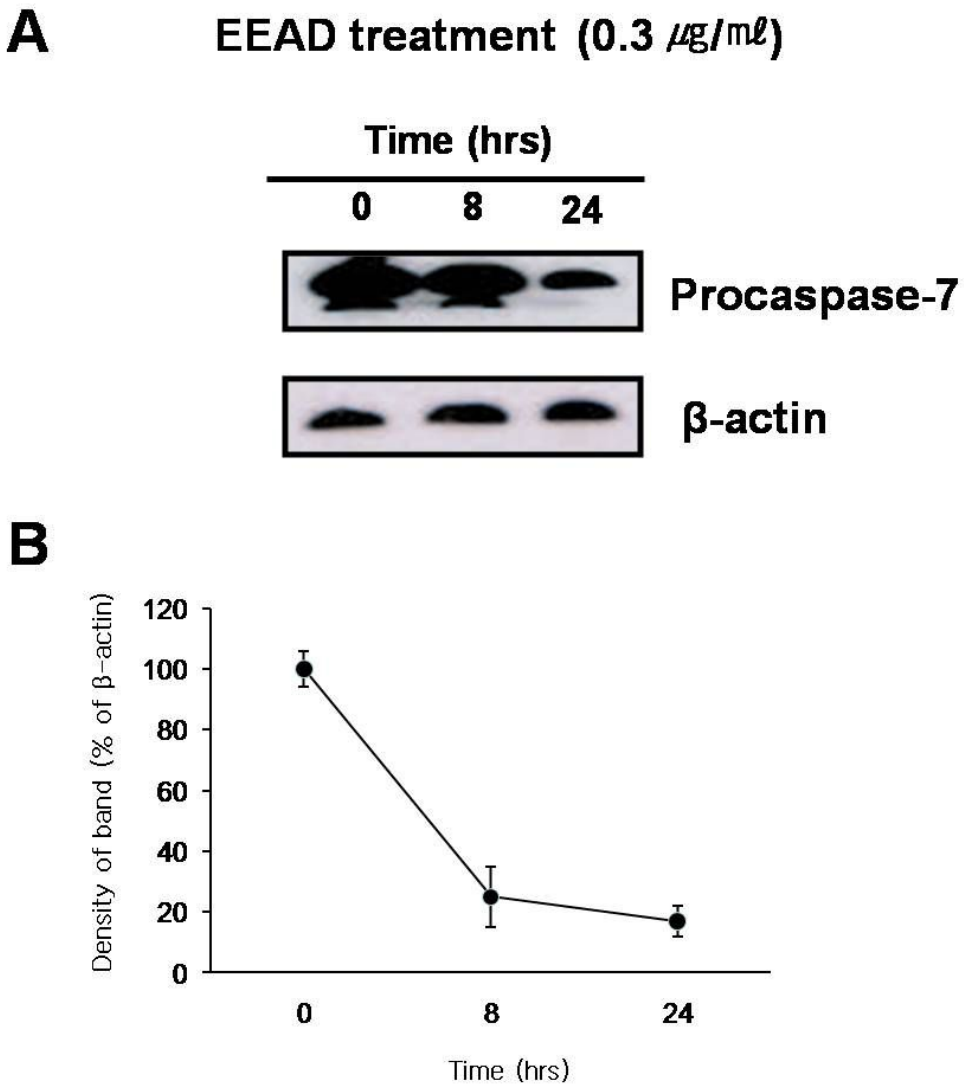

Fig. 3. EEAD treatment induces apoptosis via proteolytic cleavage of procaspase-7.

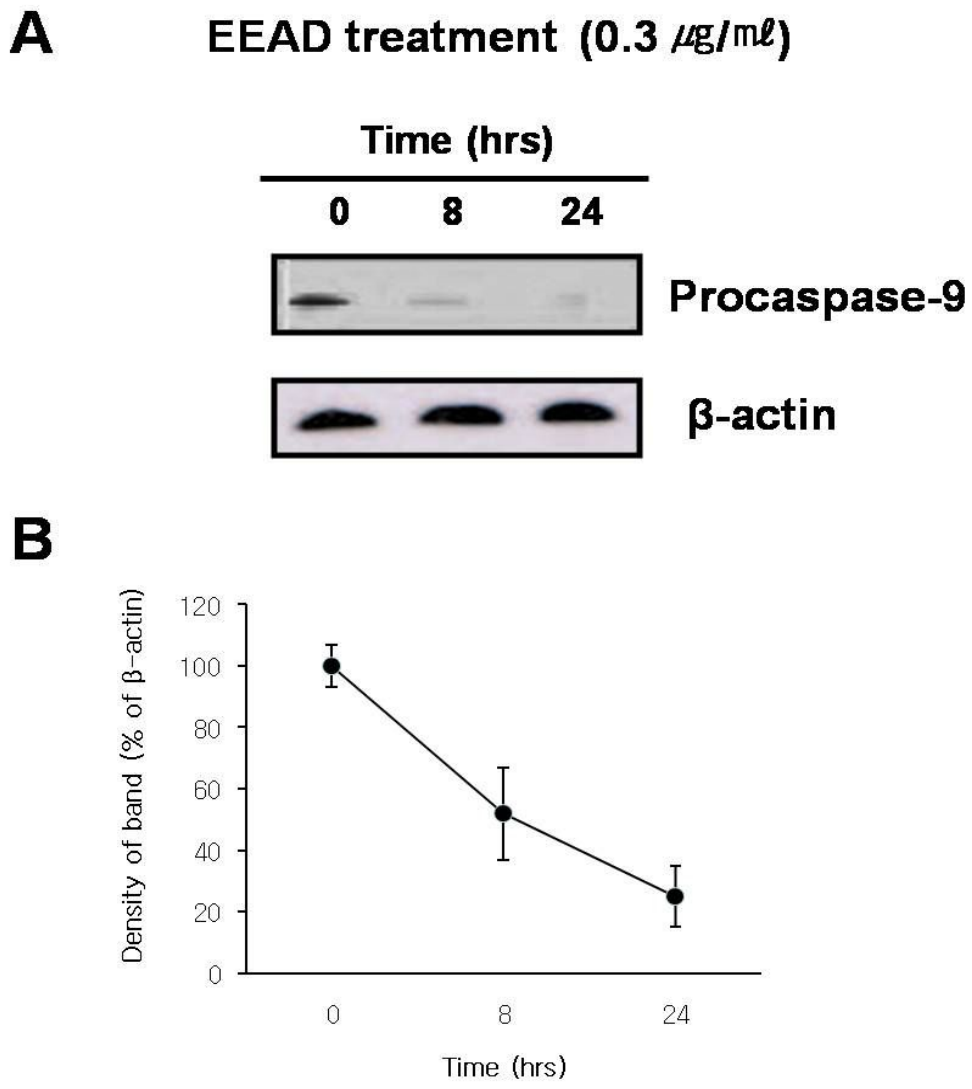

Fig. 4. EEAD treatment induces apoptosis via proteolytic cleavage of procaspase-9.

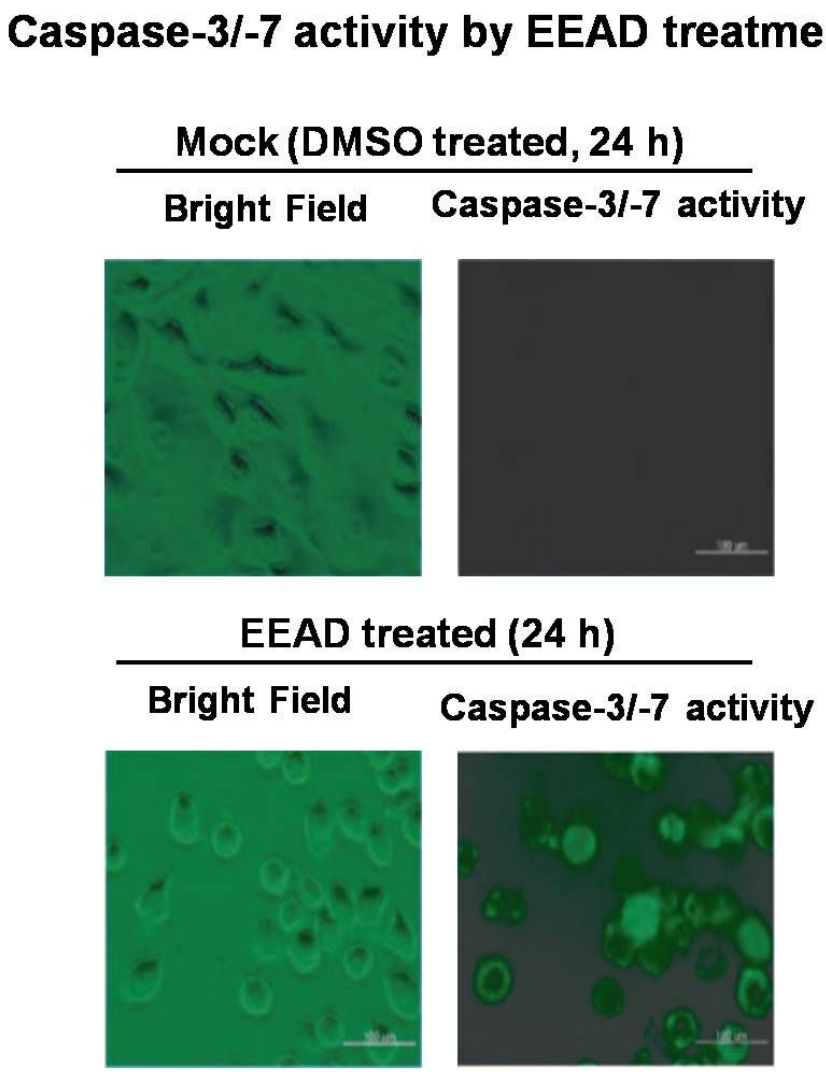

Fig. 5. Activation of caspase-3/-7 by EEAD treatment in living KB cells

ABSTRACT in KOREAN

KB 구강암세포에서 *Angelica decursiva* 추출물의 세포사멸 활성

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우리나라에서 민간요법으로 사용되는 바디나물(*Angelica decursiva*) 뿌리는 진해제, 진통제, 해열제 또는 거담제의 효능을 가진 것으로 알려져 있다. 그러나 *Angelica decursiva*의 항암효과에 대해 알려진 바는 거의 없다. 따라서 본 연구에서는 KB 구강암세포에서 *Angelica decursiva*의 에탄올 추출물(EEAD)에 의한 구강암세포 성장억제 효과와 그 기전을 분석하고자 하였다.

EEAD에 의한 구강암세포 성장억제 효과와 그 기전을 밝히기 위해 KB 구강암세포에서 MTT 분석, DNA fragmentation 분석, immunoblotting 및 caspase 활성분석 등

을 시행하였다.

사람정상구강각화세포(HNOK)에서 EEAD는 세포성장의 억제를 유도하지 않았으나 KB 구강암세포에서는 시간과 농도에 의존적으로 세포사멸을 유도하는 것을 확인할 수 있었다. KB 구강암세포에 EEAD를 처리한 실험군에서 procaspase-7, procaspase-9의 proteolytic cleavage 현상과 caspase-3/-7의 활성화를 확인할 수 있었다.

위의 결과들을 종합해보면 *Angelica decursiva*는 KB 구강암세포에서 apoptosis를 유도하여 암세포 성장을 억제시키는 것으로 생각되며, 본 연구의 결과로 EEAD를 이용한 암세포 성장억제의 하나의 방향을 제시할 수 있을 것으로 사료된다.

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중심어 : *Angelica decursiva*, 세포사, 구강암세포, 항암효과

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본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가
   없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(O) 반대( )

2011년 2월 일

저작자: 이 명 화 (서명 또는 인)

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