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Korean J Phys Anthropol
21(2): 167～180, 2008

Apoptotic Effect of Co-Treatment with a Natural Product, Chios Gum Mastic, and a Synthetic Chenodeoxycholic Acid Derivative, HS-1200, on Human Osteosarcoma Cells

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Abstract : Chios gum mastic (CGM) is a resinous exudate obtained from the stem and the main leaves of *Pistacia lenticulus* tree native to Mediterranean areas. Recently it reported that CGM induced apoptosis in a few cancer cells *in vitro*. It has been reported that the synthetic chenodeoxycholic acid (CDCA) derivatives showed apoptosis-inducing activity on various cancer cells *in vitro*. This study was undertaken to investigate the synergistic apoptotic effect of co-treatment with a natural product, CGM and a CDCA derivative, HS-1200 on human osteosarcoma (HOS) cells.

To investigate whether the co-treatment of CGM and HS-1200 compared with each single treatment efficiently reduced the viability of HOS cells, MTT assay was conducted. Induction and augmentation of apoptosis were confirmed by DNA electrophoresis, Hoechst staining and DNA hypoploidy, Westen blot analysis and immunofluorescent staining were performed to study the alterations of the expression level and translocation of apoptosis-related proteins in co-treatment. Furthermore, proteasome activity and mitochondrial membrane potential (MMP) change were also assayed.

In this study, HOS cells co-treated with CGM and HS-1200 showed several lines of apoptotic manifestation whereas each single treated HOS cells did not.

Although the single treatment of 40 μg/mL CGM or 25 μM HS-1200 for 24 h did not induce apoptosis, the co-treatment of them induced prominently apoptosis. Therefore our data provide the possibility that combination therapy of CGM and HS-1200 could be considered as a novel therapeutic strategy for human osteosarcoma.

Key words : Chios Gum Mastic, Synthetic Chenodeoxycholic Acid (CDCA) Derivatives, HS-1200, Apoptosis, Human Osteosarcoma cells

Introduction

The plant *Pistiacia lentiscus L var chia* grows particularly and almost exclusively in the South region of Chios Island, Greece, and produces a resin, known as

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Chio gum mastic (CGM). It is obtained from the stem and leaves of *Pistacia lentiscus* trees and have been extensively used for centuries in Mediterranean and Middle Eastern countries, both as a dietary supplement and herbal remedy (Balan et al. 2007, He et al. 2007).

Bile acids are polar derivatives of cholesterol essential for the absorption of dietary lipids and regulate the transcription of genes that control cholesterol homeostasis. Different bile acids exhibit distinct biological effects. Importantly, natural bile salts were reported to inhibit cell proliferation and induce apoptosis in various cells (Blake et al. 1988, Martinez et al. 1998). Im et al. (1999, 2001) developed several ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA) derivatives, and it have been reported that they had apoptosis-inducing effect in various cancer cells (Choi et al. 2003, Jeong et al. 2003, Seo et al. 2003, Park et al. 2004, Im et al. 2005, Kim et al. 2006).

Cells undergoing apoptosis usually develop characteristic changes, including nuclear condensation and degradation of DNA into oligonucleosomal fragments (Williams 1991). Apoptotic cell death is thought to result ultimately from the proteolytic actions of caspase (Yuan 1996). and alterations in mitochondrial function play a key part in the regulation of apoptosis (Susin et al. 1999). Moreover, the proteasome system has been shown to be implicated as a negative or positive mediator of apoptosis. The proteasome pathway is mostly known to work upstream of mitochondrial alterations and caspase activation (Orlowski 1999).

Osteosarcoma is one of the most common primay malignant tumors of bone. Treatment of this tumor with systemic chemotherapy dramatically improves the prognosis. Numerous studies depicted that the therapeutic effect of a variety of chemotherapeutic agents on osteosarcoma depended on the induction of apoptosis (Lu and Yagi 1999, Fellenberg et al. 2000, Seki et al. 2000).

Recent studies have demonstrated that the co-treatment of a natural product with antitumor effect and an antitumor agent could be one of the potential therapeutic strategy reducing the extent and severity of cancer treatment-related toxicity (Adhami et al. 2007, Mai et al. 2007, Song et al. 2007, Vinall et al. 2007, Lee et al. 2008). To date, there is no report about the synergistic apoptotic effects of co-treatment with CGM and HS-1200 on human osteosarcoma cells. Therefore, this study was undertaken to investigate the synergistic apoptotic effect of co-treatment with a natural product, CGM, and a representative of CDCA derivative, HS-1200, on human osteosarcoma (HOS) cells.

Materials and Methods

1. Reagents

Chios gum mastic (CGM) was obtained from mastic Korea (Seoul, Korea). The synthetic bile acid derivative, HS-1200 was kindly provided by Professor Young-Hyun Yoo (Department of Anatomy, College of Medicine, Dong-A University, Busan, Korea). The structure and methods of the synthesis of the synthetic bile acid derivatives were described (Im EO et al. 2001). HS-1200 is a conjugate form of CDCA with β-alanine benzyl ester (N-[(3α, 5β, 7α)-3,7-dihydroxyl-24-oxocholan -yl] β-alanine benzyl ester). The structures of CDCA and its conjugate form (HS-1200) are shown in Fig. 1.

The following reagents were obtained commercially: Mouse monoclonal anti-human caspase-3, caspase-7, poly(ADP-ribose) polymerase (PARP), cytochrome c, apoptosis-inducing factor (AIF) antibodies, and rabbit polyclonal anti-human DFF40 (CAD), DFF45 (ICAD), β-actin antibodies, and FITC-conjugated goat anti-mouse and anti-rabbit IgGs were from Santa Cruz Biotechnology (Santa Cruz, CA, USA); HRP-conjugated sheep anti-mouse and anti-rabbit IgGs were from Amersham GE Healthcare (Little Chalfront, UK). 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetra-

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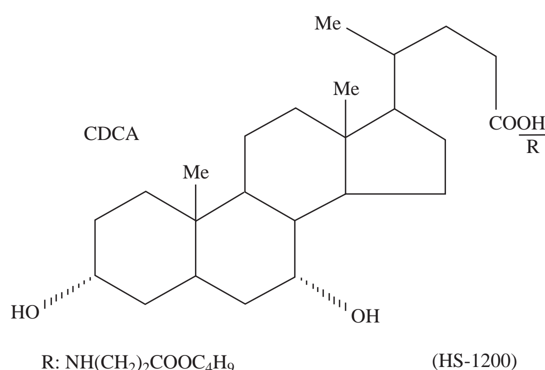

Fig. 1. Chemical structures of CDCA and its derivative, HS-1200.

ethylbenzimidazol carbocyanine iodide (JC-1) was from Molecular Probes (Eugene, USA). Suc-LLVY-AMC was from Calbiochem (EMD Biosciences, Germany). Dulbecco's modified Eagle's medium (DMEM) and FBS were from Gibco (Gaithersburg, MD, USA). Dimethyl sulfoxide (DMSO), Hoechst 33342, RNase A, proteinase K, aprotinin, leupeptin, PMSF, thiazolyl blue tetrazolium bromide and propidium iodide (PI) were from Sigma (St. Louis, MO, USA); SuperSignal West Pico enhanced chemiluminescence Western blotting detection reagent was from Pierce (Rockford, IL, USA).

2. Cell culture

The HOS human osteosarcoma cell line was purchased from ATCC (Rockville, USA). Cells were maintained at 37°C with 5% CO_2 in air atmosphere in Dulbecco's modified Eagle's medium (DMEM) with 4 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose and 1.0 mM sodium pyruvate supplemented with 10% FBS.

3. CGM and HS-1200 treatment

The stock solutions of CGM (100 mg/mL) made by dissolving the drug in DMSO and HS-1200 (100 mM) made by dissolving the drug in ethanol were kept frozen at −20°C until use. Twenty four hours after HOS cells were subcultured, the original medium was removed. The cells were washed with phosphate-buffered saline (PBS) and then incubated in the same fresh medium. Since single treatment of 40 μg/mL CGM or 25 μM HS-1200 for 24 h showed slight induction of cell death, these single concentrations were utilized for further assessment of apoptosis. The concentrations of DMSO (0.04% [vol/vol]) and ethanol (0.025% [vol/vol]) used in this study, both as a vehicle for CGM or HS-1200, and as a control, had no effect on HOS cells proliferation in our preliminary studies.

4. MTT assay

Cells were placed in a 96-well plate and incubated 24 h. Then cells treated with 40 μg/mL of CGM and/or 25 μM of HS-1200 for 24 h. And then cells were treated with 500 μg/mL of thiazolyl blue tetrazolium bromide (MTT solution). Cells were incubated at 37°C with 5% CO_2 for 4 h. And then the medium was aspirated and formed formazan crystals were dissolved in the mixture solution of 75 μL DMSO and 75 μL absolute ethanol. Cell viability was measured by an ELISA reader (Sunrise Remote Control, Tecan, Austria) at 570 nm excitatory emission wavelength.

5. Hoechst staining

Cells were harvested and cell suspension was centrifuged onto a clean, fat-free glass slide with a cytocentrifuge. The samples were stained in 4 μg/mL Hoechst 33342 for 30 min at 37°C and fixed for 10 min in 4% paraformaldehyde.

6. DNA electrophoresis

2×10^6 cells were resuspended in 1.5 mL of lysis buffer [10 mM Tris (pH 7.5), 10 mM EDTA (pH 8.0), 10 mM NaCl and 0.5% SDS] into which proteinase K (200 μg/mL) was added. After samples were incubated overnight at 48°C, 200 μL of ice cold 5 M NaCl

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was added and the supernatant containing fragmented DNA was collected after centrifugation. The DNA was then precipitated overnight at −20°C in 50% isopropanol and Rnase A-treated for 1 h at 37°C. The DNA from 10^6 cells (15 μL) was equally loaded on each lane of 2% agarose gels in Tris-acetic acid/EDTA buffer containing 0.5 μg/mL ethidium bromide at 50 mA for 1.5 h.

7. Proteasome activity

After treatment with CGM and/or HS-1200 for 24 h, cells were lysed in proteasome buffer [10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 2 mM ATP, 20% glycerol, and 4 mM dithiothreitol (DTT)], sonicated, and then centrifuged at 13,000 g at 4°C for 10 min. The supernatant (20 μg of protein) were incubated with proteasome activity buffer [0.05 M Tris-HCl, pH 8.0, 0.5 mM EDTA, 50 μM Suc-LLVY-AMC] for 1 h at 37 °C. The intensity of fluorescence of each solution was measured by a modular fluorimetric system (Spex Edison, USA) at 380 nm excitatory and 460 nm emission wavelengths. All readings were standardized using the fluorescence intensity of an equal volume of free AMC solution (50 μM).

8. Western blot analysis

Cells (2×10^6) treated with CGM and/or HS-1200 were washed twice with ice-cold PBS, resuspended in 200 μL ice-cold solubilizing buffer [300 mM NaCl, 50 mM Tris-Cl (pH 7.6), 0.5% Triton X-100, 2 mM PMSF, 2 μL/mL aprotinin and 2 μL/mL leupeptin] and incubated at 4°C for 30 min. The lysates were centrifuged at 14,000 revolutions per min for 15 min at 4°C. Protein concentrations of cell lysates were determined with Bradford protein assay (Bio-Rad, USA) and 50 μg of proteins were loaded onto 7.5～15% SDS/ PAGE. The gels were transferred to Nitrocellulose membrane (Amersham Pharmacia Biotech, UK) and reacted with each antibody. Immunostaining with antibodies was performed using SuperSignal West Pico enhanced chemiluminescence substrate and detected with Alpha Imager HP (Alpha Innotech, USA).

9. Immunofluorescent staining

Cells were cytocentrifuged and fixed for 10 min in 4% paraformaldehyde, incubated with each primary antibody for 1 h, washed 3 each for 5 min, and then incubated with FITC-conjugated secondary antibody for 1 h at room temperature. Cells were mounted with PBS. Fluorescent images were observed and analyzed under Zeiss LSM 510 laser-scanning confocal microscope (Göettingen, Germany).

10. Assay of mitochondrial membrane potential (MMP)

JC-1 was added directly to the cell culture medium (1 μM final concentration) and incubated for 15 min. The medium was then replaced with PBS. Flow cytometry to measure MMP was performed on a CYTOMICS FC500 flow cytometry (Beckman Coulter, FL, CA, USA). Data were acquired and analyzed using CXP software version 2.2.

11. Quantification of DNA hypoploidy and cell cycle phase by flow cytometry

After treatment for 24 h, cells were harvested by trypsinization and ice cold 95% ethanol with 0.5% Tween 20 was added to the cell suspensions to a final concentration of 70% ethanol. Fixed cells were pelleted, and washed in 1% BSA-PBS solution. Cells were resuspended in 1 mL PBS containing 20 μg/mL RNase A, incubated at 4°C for 30 min, washed once with BSA-PBS, and resuspended in PI solution (10 μg/mL). After cells were incubated at 4°C for 5 min in the dark, DNA content were measured on a CYTOMICS FC500 flow cytometry system (Beckman Coulter, FL, CA, USA) and data was analyzed using the Multicycle software which allowed a simultaneous estimation of

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cell-cycle parameters and apoptosis.

12. Statistical analysis

Three independent experiments were performed for each experimental group and each experiment was performed in triplicate. The results of the experimental and control groups were compared for statistical significance ($p < 0.001$, 0.01 and 0.05) using paired T-test statistical method by SPSS for Win 12.0 for summary data.

Results

1. Co-treatment of CGM and HS-1200 augmented the reduction in viability of HOS cells

To investigate co-treatment of CGM and HS-1200 reduced the viability of HOS cells, MTT assy was conducted. Single treatment of CGM at 40 µg/mL or HS-1200 at 25 µM for 24 h reduced viability of HOS cells, slightly (CGM, 85.3%; HS-1200, 89.9%). Co-treatment of CGM and HS-1200 significantly reduced cell viability compared to the effect of each single treatment (co-treatment, 49.6%) (Fig. 2).

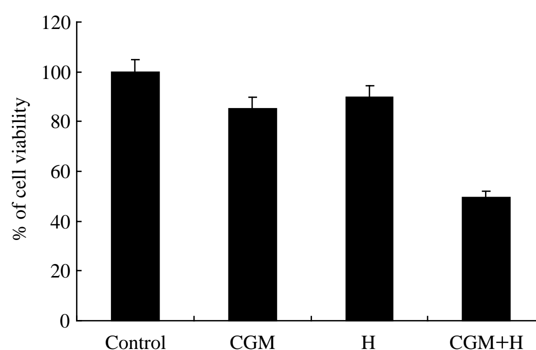

Fig. 2. Co-treatment of CGM and HS-1200 significantly reduced cell viability in HOS cells. Cell viability was determined by MTT assay (CGM, $p < 0.05$; CGM+H, $p < 0.001$). Three independent assays were performed. Values are means ± SD of triplicates of each experiment (CGM, cells treated with 40 µg/mL Chios gum mastic for 24 h; H, cells treated with 25 µM HS-1200 for 24 h; CGM+H, cells treated with 40 µg/mL Chios gum mastic plus 25 µM HS-1200 for 24 h).

2. Co-treatment of CGM and HS-1200 augmented the nuclear condensation and fragmentation in HOS cells

To explore whether nuclear condensation and fragmentation were induced, Hoechst staining which is a hallmark of apoptosis, was conducted. The co-treatment of CGM and HS-1200 showed a variety of condensed and fragmented nuclei compared to the single treatment (Fig. 3).

3. Co-treatment of CGM and HS-1200 showed the DNA fragmentation in HOS cells

To explore whether DNA fragmentation was induced, DNA electrophoresis was conducted. DNA electrophoresis did not show a ladder pattern of DNA fragments in the single treatment of CGM or HS-1200 whereas showed a ladder pattern of DNA fragments in the co-treatment (Fig. 4).

4. Augmentation of apoptosis by co-treatment of CGM and HS-1200 was demonstrated by proteasome activity in HOS cells

Single treatment of CGM slightly reduced proteasome activity compared to the control group. The co-treatment of CGM and HS-1200 significantly reduced proteasome activity compared to the single treatment (Fig. 5).

5. Augmentation of apoptosis by co-treatment of CGM and HS-1200 was demonstrated by reduction of mitochondrial membrane potential (MMP) in HOS cells

The single treatment of CGM and HS-1200 did not show the loss of MMP compared to control group. But the co-treatment of CGM and HS-1200 remarkably reduced MMP compared to the single treatment (Fig. 6).

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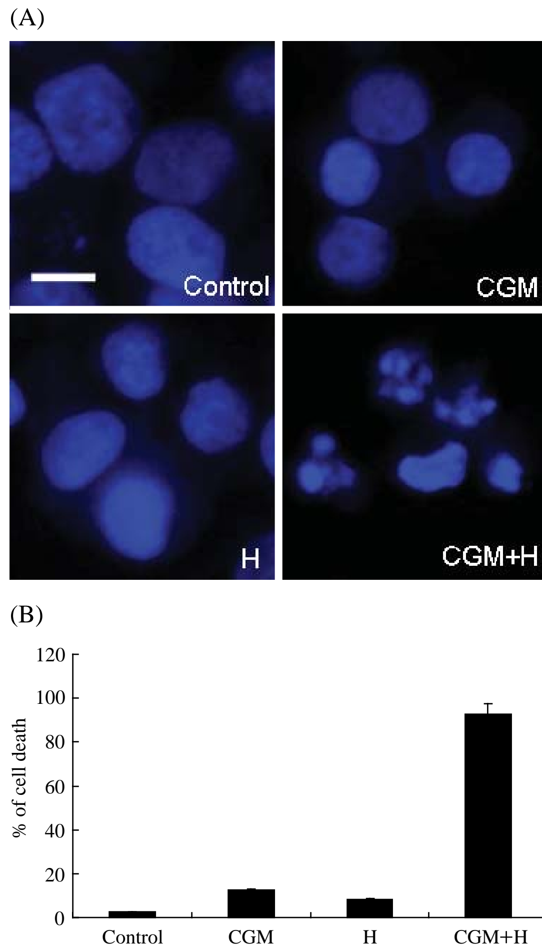

Fig. 3. Co-treatment of CGM and HS-1200 showed numerous condensed and fragmented nuclei in HOS cells compared to the single treatment (CGM, cells treated with 40 μg/mL Chios gum mastic for 24 h; H, cells treated with 25 μM HS-1200 for 24 h; CGM+H, cells treated with 40 μg/mL Chios gum mastic plus 25 μM HS-1200 for 24 h). (A) Immunofluorescent micrographs showing nuclear morphology after Hoechst staining. Scale bar, 10 μm. (B) The values below micrographs are the mean ± SD of the means of apoptotic cells as determined by Hoechst staining. The results presented are representative of three independent experiments (CGM+H, $p < 0.001$).

6. Augmentation of apoptosis by co-treatment of CGM and HS-1200 was demonstrated by the decrease of DNA content in G361 cells

The flow cytometry showed that co-treatment of CGM and HS-1200 remarkably increased apoptotic cells with DNA hypoploidy compared to the single treatment (Fig. 7).

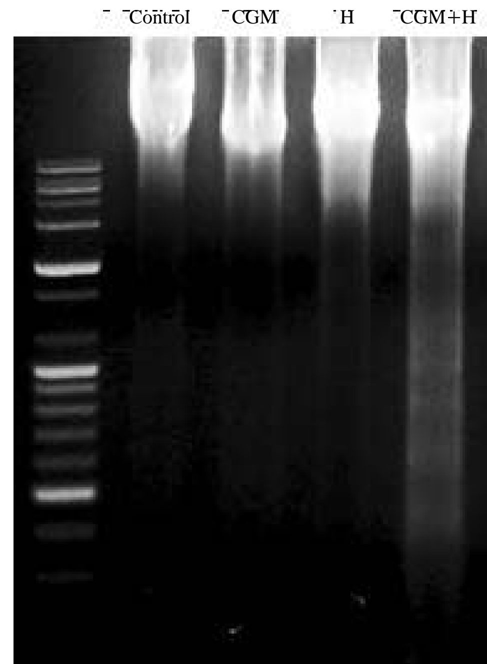

Fig. 4. Co-treatment of CGM and HS-1200 efficiently showed DNA fragmentation in HOS cells. DNA fragmentation analysis was determined by the agarose gel electrophoresis. Whereas single treated cells showed no DNA fragmentation, co-treated cells showed DNA degradation characteristic of apoptosis with a ladder pattern of DNA fragments (CGM, cells treated with 40 μg/mL Chios gum mastic for 24 h; H, cells treated with 25 μM HS-1200 for 24 h; CGM+H, cells treated with 40 μg/mL Chios gum mastic plus 25 μM HS-1200 for 24 h).

7. Co-treatment of CGM and HS-1200 showed to lead to the translocation of AIF from mitochondria onto the nuclei

The confocal microscopy showed that AIF was located at mitochondria in the single treatment of CGM or HS-1200 whereas AIF was evidently translocated onto nuclei in the co-treatment (Fig. 8).

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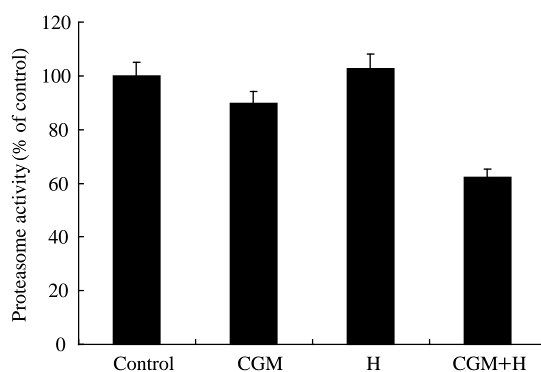

Fig. 5. Co-treatment of CGM and HS-1200 significantly showed the reduction of proteasome activity in HOS cells compared to the single treatment (CGM, $p<0.05$; CGM+H, $p<0.001$). Three independent assays were performed. Values are means±SD of triplicates of each experiment. (CGM, cells treated with 40 μg/mL Chios gum mastic for 24 h; H, cells treated with 25 μM HS-1200 for 24 h; CGM+H, cells treated with 40 μg/mL Chios gum mastic plus 25 μM HS-1200 for 24 h).

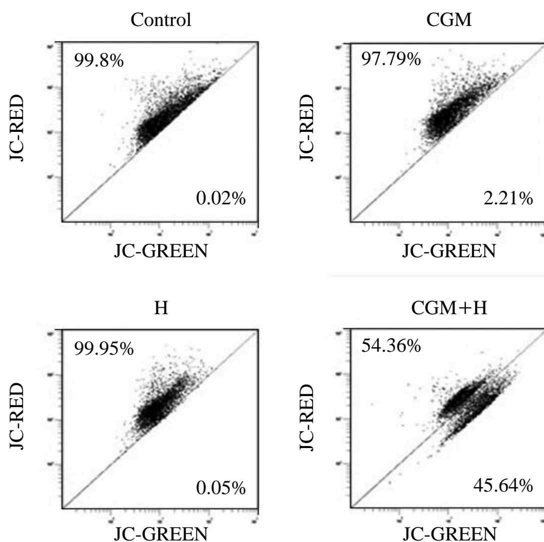

Fig. 6. Co-treatment of CGM and HS-1200 remarkably showed the loss of MMP (Δψm) compared to the single treatmemt (CGM+H, $p<0.001$). MMP was measured by JC-1 with flow cytometry (CGM, cells treated with 40 μg/mL Chios gum mastic for 24 h; H, cells treated with 25 μM HS-1200 for 24 h; CGM+H, cells treated with 40 μg/mL Chios gum mastic plus 25 μM HS-1200 for 24 h).

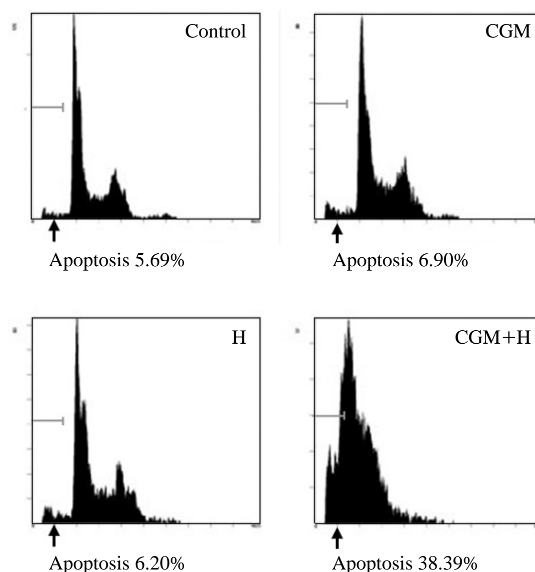

Fig. 7. The kinetic analysis of the effect of co-treatment on HOS cell cycle progression and induction of apoptosis. Co-treatment remarkably showed the increase of apoptotic cells with DNA hypoplpoidy compared to the single treatment (CGM+H, $p<0.001$), (CGM, cells treated with 40 μg/mL Chios gum mastic for 24 h; H, cells treated with 25 μM HS-1200 for 24 h; CGM+H, cells treated with 40 μg/mL Chios gum mastic plus 25 μM HS-1200 for 24 h).

8. Co-treatment of CGM and HS-1200 showed to lead to the release of cytochrome c from mitochondria into the cytosol

The confocal microscopy showed that cytochrome c was located at mitochondria in the single treatment of CGM or HS-1200 whereas cytochrome c was evidently released into the cytosol in the co-treatment (Fig. 9).

9. Co-treatment of CGM and HS-1200 results in DFF45 activation (Western blot assay) and translocation of DFF40

Western blot assay and confocal microscopy were conducted to examine whether DNA fragmentation factor (DFF) degrading chromosomal DNA, is involv-

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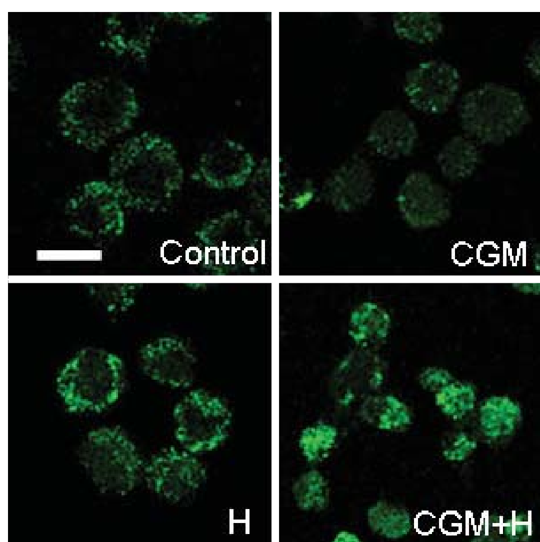

Fig. 8. The confocal microscopy showed that AIF was evidently translocated onto nuclei in HOS cells co-treated with CGM and HS-1200. Scale bar, 10 µm (CGM, cells treated with 40 µg/mL Chios gum mastic for 24 h; H, cells treated with 25 µM HS-1200 for 24 h; CGM+H, cells treated with 40 µg/mL Chios gum mastic plus 25 µM HS-1200 for 24 h).

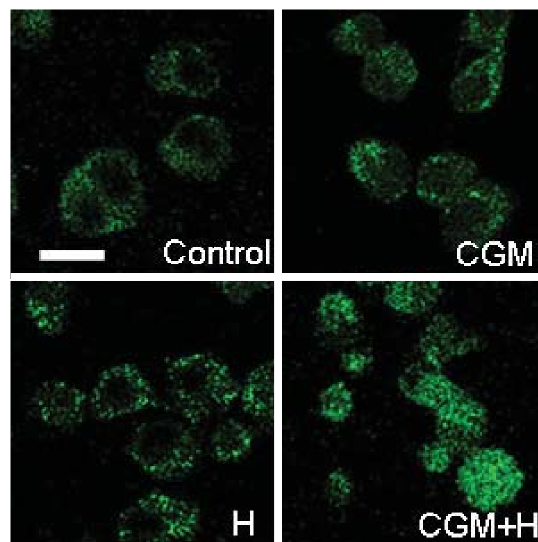

Fig. 9. The confocal microscopy showed that cytochrome c was evidently released to the cytosol in HOS cells co-treated with CGM and HS-1200. Scale bar, 10 µm (CGM, cells treated with 40 µg/mL Chios gum mastic for 24 h; H, cells treated with 25 µM HS-1200 for 24 h; CGM+H, cells treated with 40 µg/mL Chios gum mastic plus 25 µM HS-1200 for 24 h).

ed with the co-treatment. The co-treatment of CGM and HS-1200 induced the degradation of DFF45 (ICAD) and produced DFF45 30 kDa and 11 kDa cleaved products. And confocal microscopy showed that the co-treatment led to the translocation of DFF40 (CAD) from cytosol onto nuclei (Fig. 10).

10. Efficient apoptotic effect of co-treatment of CGM and HS-1200 was demonstrated by Western blot assay

The co-treatment of CGM and HS-1200 induced the degradation of caspase-7, caspase-3 and PARP, and produced the processed caspase-7 20 kDa, caspase-3 17 kDa and PARP 85 kDa cleaved products whereas the single treatment did not (Fig. 11).

Discussion

Chios gum mastic (CGM) is a resinous exudate obtained from the stem and the main leaves of *Pistacia lenticulus* tree native to Mediterranean areas. Previous studies have demonstrated that CGM is effective in the treatment of benign gastric and duodenal ulcers and it have definite antibacterial activity against *Helicobacter pylori* (Al-Habbal et al. 1984, Huwez and Al-Habbal 1986, Al-Said et al. 1986, Hawez et al. 1998). It has also been shown to have antimicrobial properties (Aksoy et al. 2006). Recently it was reported that CGM induces cell cycle arrest and apoptosis in human prostate and colon cancer cells (Balan et al. 2007, He et al. 2007). We also demonstrated that CGM induces G1 the cell cycle arrest via the modu-

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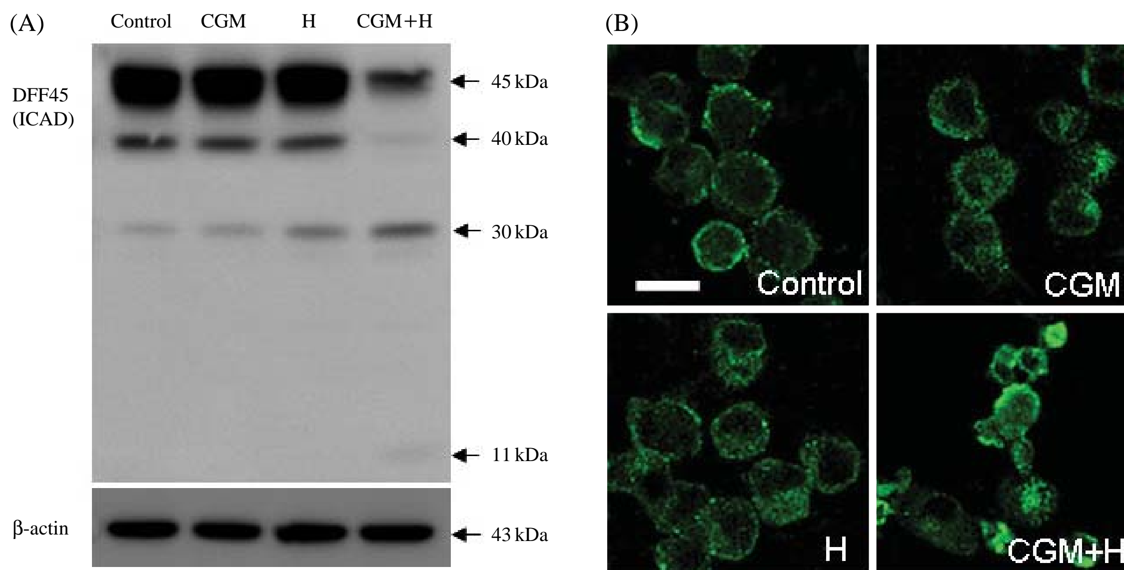

Fig. 10. Western blot analysis and confocal microscopy showing the efficient apoptotic effects in HOS cells co-treated with CGM and HS-1200 (CGM, cells treated with 40 µg/mL Chios gum mastic for 24 h; H, cells treated with 25 µM HS-1200 for 24 h; CGM+H, cells treated with 40 µg/mL Chios gum mastic plus 25 µM HS-1200 for 24 h). (A) The co-treatment remarkably induced DFF45 (ICAD) degradation and produced the processed DFF45 30 kDa and 11 kDa cleaved product. β-actin, a loading control. (B) The confocal microscopy showed that DFF40 (CAD) was translocated onto the nuclei in the co-treatment of CGM and HS-1200. Scale bar, 10 µm.

lation of cell cycle-related proteins, and apoptosis via mitochondria and caspase pathway in oral squamous carcinoma cells (Park et al. 2008).

It has been reported the antiproliferative efficacy of synthetic CDCA derivatives in various cancer cells by inducing apoptosis. Those studies demonstrated the decrease of proteasome activity, mitochondrial events, and nuclear condensation (Choi et al. 2001, Yoon et al. 2001, Choi et al. 2003, Jeong et al. 2003, Seo et al. 2003) in synthetic CDCA derivatives-induced apoptosis. In addition, it has been demonstrated that a synthetic CDCA derivative, HS-1200 shows the strongest apoptosis-inducing effect among the synthetic CDCA derivatives (Choi et al. 2003, Seo et al. 2003, Kim et al. 2004, Baek et al. 2007).

Proteasome is a fundamental non-lysosomal tool that cells use to process or degrade a variety of short-lived proteins. Proteolysis mediated by the ubiquitin-proteasome system has been reported to be implicated in the regulation of apoptosis (Drexler et al. 2000). The proteasome pathway is mostly known to work upstream of the mitochondrial alterations and caspase activation (Orlowski 1999). In this study, the co-treatment with CGM and HS-1200 in HOS cells causes the significant reduction of proteasome acitivity compared to the single treatment.

Mitochondria plays an important role in apoptosis. And induction of the mitochondrial permeability transition play a key part in the regulation of apoptosis (Kroemer et al. 1997, Green and Reed 1998, Susin et al. 1999). Outer mitochondrial membrane becomes permeable to intermembrane space proteins such as cytochrome c and AIF (apoptosis inducing factor) during apoptosis (Golab et al. 2000). Cytochrome c release and disruption of mitochondrial membrane potential (MMP) are in fact known features in apoptosis

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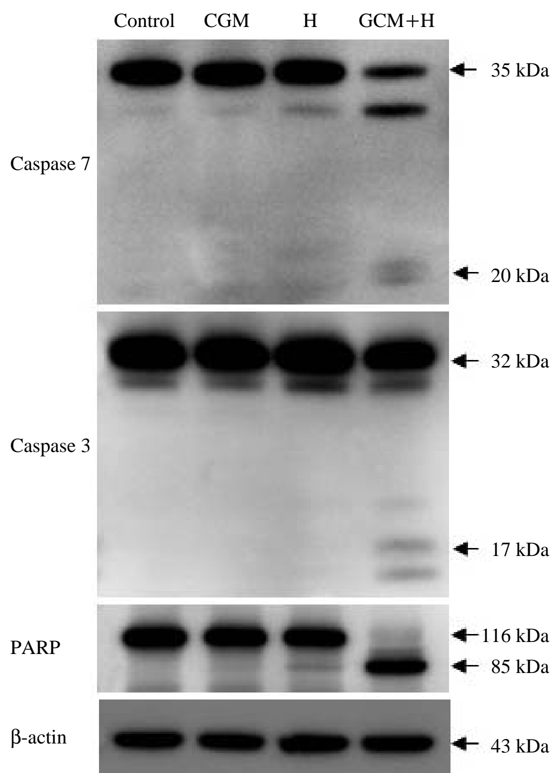

Fig. 11. Western blot analysis showing that the co-treatment of CGM and HS-1200 in HOS cells remarkably induced caspase-7, caspase-3 and PARP degradations and produced the processed caspase-7 20 kDa, caspase-3 17 kDa, and PARP 85 kDa cleaved products. β-actin, a loading control (CGM, cells treated with 40 μg/mL Chios gum mastic for 24 h; H, cells treated with 25 μM HS-1200 for 24 h; CGM+H, cells treated with 40 μg/mL Chios gum mastic plus 25 μM HS-1200 for 24 h).

triggered by proteasome inhibition (Wagenknecht et al. 2000, Marshansky et al. 2001). On induction of apoptosis, AIF translocates to the nucleus, resulting in chromatin condensation and large-scale DNA fragmentation (Daugas et al. 2000). This study evidently showed that co-treatment with CGM and HS-1200 in HOS cells results in remarkable decrease of MMP, the release of cytochrome c into cytosol and the translocation of AIF onto nuclei whereas the single treatment does not.

A common final event of apoptosis is nuclear condensation, which is controlled by caspases, DFF, and PARP. Caspases, the cysteinyl aspartate-specific intracellular proteinase, play an essential role during apoptotic death (Acehan et al. 2002). Once activated, the effector caspases (caspase-3, caspase-6 or caspase-7) are responsible for the proteolytic cleavage of a broad spectrum of cellular targets, leading ultimately to cell death. The known cellular substrates include structural components (such as actin and nuclear lamin), inhibitors of deoxyribonuclease (such as DFF45 or ICAD) and DNA repair proteins (such as PARP) (Gross et al. 1999, Porter 1999). In apoptotic cells, activation of DFF40 (CAD), also a substrate of caspase-3, occurs with the cleavage of DFF45 (ICAD). Once DFF40 is activated and released from the complex of DFF45 and DFF40, it can translocate to the nucleus and then degrade chromosomal DNA and produce DNA fragmentation (Cheng 2007). This study demonstrated that co-treatment with CGM and HS-1200 in HOS cells results in the degradation and the cleavage of caspase-3, caspase-7, PARP and DFF45 (ICAD), and the translocation of DFF40 (CAD) onto nuclei whereas the single treatment does not.

In the study of co-treatment with a natural product, CGM and an antitumor agent, HS-1200, HOS cells co-treated with CGM and HS-1200 showed several lines of apoptotic manifestation such as nuclear condensations, DNA fragmentation, reduction of proteasome activity and MMP, the decrease of DNA content, the release of cytochrome c into cytosol, the translocation of AIF and DFF 40 (CAD) onto nuclei, degradation and activation of caspase-7, caspase-3, PARP and DFF45 (ICAD) whereas each single treated HOS cells did not.

In conclusion, combination therapy of CGM and HS-1200 could be considered, in the future, as an alternative therapeutic strategy for human osteosarcoma (HOS). Its clinical application awaits further extensive studies.

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Korean J Phys Anthropol
21(2): 167～180, 2008

천연물질 Chios gum mastic과 합성 chenodeoxycholic acid 유도체 HS-1200의 병용처리가 사람골육종세포에 미치는 세포자멸사 효과에 대한 연구

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간추림 : Chios gum mastic(CGM)은 그리이스 지역에서만 서식하는 *Pistacia lenticulus* 나무의 줄기와 잎에서 추출한 수지상의 천연 추출물이다. 합성 chenodeoxycholic acid (CDCA) 유도체가 여러 가지 암세포에 유도한 세포자멸사 연구들이 보고되어져 왔다. 본 연구는 천연물질인 CGM과 합성 CDCA 유도체인 HS-1200의 병용처리가 사람골육종세포에 효과적인 상승 세포자멸사 효과가 있는지를 알기 위해서 수행되었다.

CGM과 HS-1200의 병용처리가 단독처리에 비해서 효과적인 세포생존율 감소가 있는지 확인하기 위해서 MTT 법을 시행하였고, 세포자멸사의 유도와 증가를 확인하기 위해서 Hoechst 염색법과 DNA 전기영동법을 사용하였다. 병용처리 때, 세포자멸사에 관계하는 단백질의 발현 변화와 세포내에서의 이동을 밝혀내기 위해서 Western bot 분석과 면역형광염색법을 수행하였다. 더 나아가서 proteasome 활성도와 사립체막 전위 변화를 측정하였다.

병용처리 된 사람골육종세포는 단독처리 된 사람골육종세포에서 거의 관찰할 수 없었던 많은 핵 응축, DNA 조각남, 사립체막 전위와 proteasome 활성도의 감소, DNA 양의 감소, cytochrome c의 세포질로의 유리, AIF와 DFF40 (CAD)의 핵으로의 이동, caspase-7, caspase-3 그리고 PARP의 활성화와 같은 세포자멸사 증거를 보였다.

24시간 동안 40 μg/mL CGM과 25 μM HS-1200을 각기 단독처리 한 결과에서는 세포자멸사를 유도 못했으나, 병용처리한 결과에는 아주 탁월한 세포자멸사의 유도를 보였다. 이러한 병용처리 결과는 사람골육종의 새로운 치료적 전략으로 응용될 수 있다고 생각한다.

찾아보기 낱말 : Chios Gum Mastic (CGM), 합성 chenodeoxycholic acid(CDCA) 유도체, HS-1200, 세포자멸사, 사람골육종세포

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