농학석사 학위논문

Cinnamaldehyde enhances cytotoxic effect of cisplatin by ROS-induced autophagy in cisplatin-resistant ovarian cancer cells.

cisplatin에 저항성을 지닌 난소암세포에서
cinnamaldehyde와 cisplatin의 조합에 의해
발생한 ROS로부터 유도된 자가포식 기전 연구

2017년 2월

서울대학교 대학원

농생명공학부 바이오모듈레이션

정 재 영

ABSTRACT

Ovarian cancer is the most lethal gynecologic cancer. A major obstacle to the current therapy for ovarian cancer is the platinum-resistance. Cisplatin (CDDP) is representatively platinum-based drug and has anti-cancer effect by triggering reactive oxygen species (ROS) in various cancer cells. Cinnamaldehyde (CA), extracted from the stem bark of *Cinnamomum cassia*, has been shown to possess anticancer effects in various cancers and to induce apoptotic cell death by ROS generation. According to previous studies, induction of ROS by cellular stressors promotes autophagic cell death as well as apoptosis in many cancers. So, we tested whether CA and CDDP boost ROS-mediated apoptosis and autophagy and also have synergistic effect in ovarian cancer cells. We chose two types of ovarian cancer cell lines (A2780/s sensitive to CDDP and A2780/cis resistant to CDDP). Low dose (1 μM) of CDDP appeared cytotoxicity and induced ROS-mediated apoptosis and autophagy in A2780/s. But high dose (10 μM) of CDDP was not affected in A2780/cis. In this condition, co-treatment of CA remarkably increased synergistic growth-inhibitory effect and induced ROS-mediated apoptosis and autophagy in A2780/cis. Thus, excessive ROS by combination of CA and CDDP might be proposed to a way to overcome the chemoresistance in ovarian cancer.

Keywords: Ovarian cancer, chemoresistance, autophagy, Cinnamaldehyde, Reactive oxygen species

Student Number: 2015-20522

CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

ATG5: Autophagy protein 5

CA: Cinnamaldehyde

CDDP: Cisplatin

DHE: dihydroethidium

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

H_2DCFDA: 6-carboxy-2,7-dichlorodihydrofluorescein diacetate

LC3B: Light chain 3B

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

NAC: N-acetyl-cysteine

PBMC: Peripheral blood mononuclear cells

ROS: Reactive oxygen species

‘

INTRODUCTION

Ovarian cancer remains the highest mortality rate from gynecologic tumors amongst women [1]. For the past several decades, the standard therapy for women with advanced ovarian cancer has consisted of cytoreductive surgical approaches followed by platinum-based chemotherapy. [2] To date, patient prognosis has significantly improved for many types of solid tumors. Whereas, survival of epithelial ovarian cancer patients has changed little due to the development of platinum-resistance [3]. This poor prognosis in ovarian cancer likely owing to chemoresistance proves an urgent need for an alternative chemotherapy.

Autophagy is a self-destructive process, by which intracellular components isolated inside double-membrane vesicles (autophagosomes) are fused to the lysosome and degraded by lysosomal hydrolases, for recycling amino acids and other substrates [4]. Under basal conditions, autophagic flux helps eliminate damaged organelles within cells and plays an important role in cellular equilibrium [5]. Intriguingly, researchers have reported that this process of self-destruction is involved in pathogenesis of a number of disease states including cancer [6]. Deficiency of autophagy has been found in various cancers including breast, prostate and ovarian cancer. Also, a

low level of autophagy has been associated with tumor progression and development [7]. Many anticancer agents and natural products have been reported to induce autophagic cell death through reactive oxygen species (ROS) generation [8, 9]. A high level of ROS promotes cell death by activating various mechanisms such as apoptosis and autophagic cell death. Furthermore, *in vitro* studies have shown that the accumulation of excessive ROS induces cell death in cancer [10].

Cinnamaldehyde (CA), an aromatic α,β-unsaturated aldehyde, is the major component (> 80%) of essential oil extracted from some cinnamon species [11, 12]. CA is a bioactive compound that has been identified to have anti-tumorigenic activity in various types of human cancer cells [13-15]. Recent studies have shown that CA induces apoptosis via ROS-induced DNA damage and mitochondrial permeability transition [16]. Cisplatin (CDDP), a representative platinum-based drug used to treat ovarian cancer, also triggers ROS generation through direct damage to mitochondrial DNA [17]. Recently, combination therapies of conventional chemotherapeutic agents with phytochemicals have shown to be effective at inducing apoptosis in lung [18], colorectal, hepatocellular [19], and ovarian cancers [20]. Similarly, our results show that combination treatment of CA and CDDP augments generation of ROS, which in turn induces apoptosis

and autophagic cell death in chemoresistant ovarian cancer cells. Herein, we present a potential strategy that could improve the current conventional chemotherapy.

MATERIALS AND METHODS

Cell Culture of Ovarian cancer cells

Human ovarian cancer cell lines A2780/s sensitive to CDDP and A2780/cis resistant to CDDP were grown in RPMI1640 (WelGENE, Seoul, Korea) at 37℃ in humidified atmosphere of 5% CO_2. RPMI1640 was supplemented with 10% fetal bovine serum (Life Technologies) and 100 μg/mL penicillin-streptomycin (Life Technologies).

MTT Assay

A total 3,000 cells of A2780/s and A2780/cis were plated in each well of 96-well plates. After incubation for 24 h, various concentrations of cinnanmaldehyde (0, 5, 10, 20, 40, and 80μM) were treated to each well, and cells were incubated for 24 h and 48 h. MTT (2 mg/ml) was added to each well. After incubation for 3 h, MTT solution was removed, and 100 μl of DMSO was added to each well to dissolve the formazan crystals. The absorbance was determined at 540nm using a spectrophotometer (Beckman coulter).

Reactive Oxygen Species (ROS) Assay

Intracellular ROS levels were measured using 6-carboxy-2,7-

dichlorodihydrofluorescein diacetate (H_2DCFDA; Sigma Aldrich, St. Louis, MO) and dihydroethidium (DHE; Sigma Aldrich) fluorescent probe. Cells were haversted and then incuvated with 10 μM H_2DCFDA and 5 μM DHE for 30 min and 10min respectively. Cells (10,000 events) were determined by BD FACS Canto Ⅱ flow cytometer (BD Biosciences, NorthRyde, Australia).

Measurement of Apoptosis

Apoptosis was detected by flow cytometric analysis using the Annexin V-FITC apoptosis detection kit (BDPharmingen, CA). Cell's preparation was performed according to manufacturer's protocol. In Brief, CA-treated cells were collected by trypsinization (0.05% Trypsin-EDTA). Followed by centrifugation (890g, 4℃, 5 min). Cells were then suspended in Annexin V-binding buffer and treated with 1 μl of FITC Annexin V and/or 0.5 μl of PI (Propidium iodide). Cells (10,000 events) were analyzed by BD FACS Canto Ⅱ flow cytometer.

Acidic Vesicular Organelles (AVOs) Staining

Cells were seeded in 60 mm plate and incubated for 24 h. The medium was discarded and treated with CA or CDDP for 48 h in the presence or

absence of N-acetyl cysteine (NAC, 5 mM). Cells were stained by acridine orange (1 μg/ml, Sigma Aldrich) with phenol red free RPMI 1640(Life Technologies) at 37℃ in 15 min in the dark. The stained-cells were harvested and washed with PBS. Results were obtained using BD FACS Canto Ⅱ flow cytometer. The cells were cultured with same condition in confocal dish (SPL Life Sciences, Korea) and detected by confocal microscopy.

Western Blotting

Cells were harvested by trypsinization (0.05% Trypsin-EDTA) and centrifugation (890 x g, 4℃, 5 min). Then cell pellets were suspended in lysis buffer containing 3’ DW, 10% Triton X 100, EDTA, 0.1M Na_3VO_4, 0.1M PMSF and sodium deoxycolate on ice for 30 min. Following centrifugation at 14240g for 10 min, the supernatant was collected. BCA protein assay (Thermo scientific) was conducted to obtain protein concentration of lysed cells. To separate proteins by size, SDS-PAGE was conducted. Proteins were then transferred on nitrocellulose blotting membrane (GE healthcare life sciences, Germany) at 100 V for 1 h. Membranes were blocked with 5 % skim milk and incubated with appropriate primary antibodies overnight at 4 C. After membranes were rinsed 3 times with TBS-T, they were

incubated with horseradish peroxidase-conjugated anti-rabbit or anti-mouse immunoglobulin G antibodies (1:5,000) for 2 h at RT. West save up reagents (Abfrontier, Seoul, Korea), was utilized to detect protein bands (Agfa, Belgium).

Statistical analysis

All of the experiments were performed in triplicates and the data expressed as means ± SEM. Student's t-test and analysis of variance (ANOVA) with Bonferroni's test were performed for statistical comparison. GraphPad Prism 5 and statistical software SPSS 20.0 (SPSS Inc., Chicago, IL, USA) were used for the analysis. A P-value <0.05 was considered statistically significant.

RESULTS

CA inhibits cell growth of A2780/s and A2780/cis.

Human ovarian cancer cell lines, A2780/s and A2780/cis, were widely used to study chemoresistant phenotypes [21, 22]. Expectedly, our results from MTT assay demonstrates that the half-maximal inhibitory concentration (IC_{50}) of CDDP was markedly lower in A2780/s compared to A2780/cis Figure 1A. As suggested in Figure 1B, CA inhibited the proliferation of both cell lines in a dose-dependent manner. IC_{50} values of CA were computed as 43 ± 2.1 μM for A2780/s and 51 ± 2.1 μM for A2780/cis. To investigate cytotoxicity of CA in normal cells, human peripheral blood mononuclear cells (PBMCs), which were isolated from the blood of healthy individuals, were employed. CA did not show any cytotoxic effect on various concentration in PBMCs. Thus, CDDP appeared to be effective at inhibiting growth of the A2780/sensitive cells whereas A2780/cis cells were less sensitive to CDDP. However, CA exerted similar growth inhibitory effects on both cell lines.

CA synergizes the anti-cancer effect of CDDP in A2780/cis.

As shown in Figure 1A and B, CA appeared similar IC_{50} than IC_{50} of CDDP in two cell lines. To confirm the synergistic effect of CA and

CDDP, we treated both sensitive and resistant cells with different CDDP/CA ratio of concentrations. The concentration of CA was fixed at 40 μM, and CDDP was applied concomitantly with different concentrations Figure 2A and B. We incorporated Calcusyn software to analyze the synergistic effect of CDDP and CA. Our results show that the synergism was only present in the A2780/cis cells.

CDDP inhibit cell proliferation and induce apoptosis and autophagy by increasing ROS production in A2780/s.

Previous researches have shown that CDDP triggered ROS production in various types of cancer including ovarian cancer [17, 23]. To examine changes in ROS level, in A2780/sensitive cells, the cells were treated with CDDP in the absence or presence of NAC for 48 h. Cells were stained with either H_2DCFDA and DHE to detect relative levels of hydrogen peroxide and superoxide anion respectively. After treated with CDDP (1 μM) for 48 h, mean fluorescent strength of H_2DCFDA and DHE probe were significantly increased by 1.9 fold and 3.0 fold change. Next, to investigate whether ROS are important drivers of cell death in the drug-sensitive cell line, we incorporated N-acetyl cysteine (NAC) to scavenge ROS generated by the drug treatment Figure 3A. In this condition, A2780/s cells were viewed by phase contrast microscope

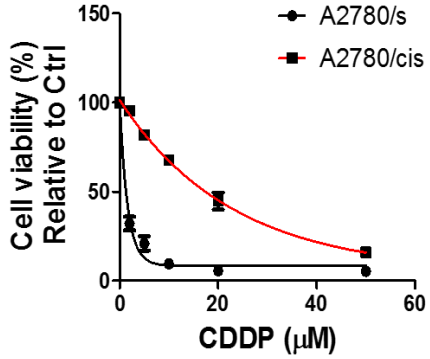
and performed by MTT assay Figure 3B, C. We observed significant reduction of cytotoxicity when NAC was treated 1 h prior to the drug treatments in A2780/s cells. To confirm the cell death mechanism by CDDP, we detected apoptosis Figure 3D, E and autophagy Figure 3F-H. As a result, both apoptosis and autophagy was significantly induced by CDDP. In this condition, apoptotic and autophagic effect was reversed by NAC. Our data suggest, elevated production of ROS by CDDP plays a key role in promoting cell death in A2780/s.

Combination of CA and CDDP induces ROS-mediated apoptotic and autophagic cell death in A2780/cis.

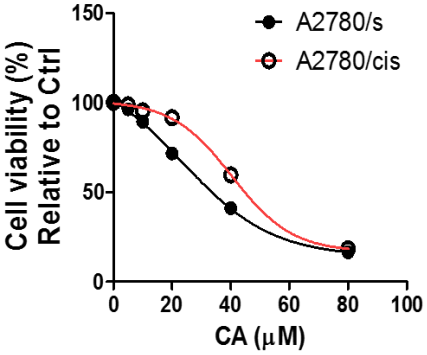
Likewise, treatment of CDDP (10 μM) alone in A2780/cis could elevate ROS level Figure 4A, B, but cytotoxicity was minimal with 75.1% cell viability after 48 h exposure figure 4D. However co-treatment of CA and CDDP dramatically heightened ROS generation Figure 4A, B and significantly induced cell death in A2780/cis Figure 4C, D. Interestingly, the effect of combinational treatment was reversed by NAC (5mM). Taken together, these results suggest that ROS might be key mediators of cell death induced by co-treatment of CA and CDDP. Moreover, apoptotic populations and PARP cleavage were substantially increased when the CA and CDDP were treated

simultaneously then treated with CA or CDDP alone Figure E, F. These results indicated that the co-treatment is highly potent at inducing apoptosis in A2780/cis cells. To evaluate the effect of excessive production of ROS other than apoptosis, we confirmed whether the autophagy was induced by CA and CDDP in A2780/cis cells. Autophagy is characterized by acidic vesicular organelle (AVO) formation (autolysosome). As shown in Figure 5A, C, AVO-positive cells were increased by CA and CDDP respectively. Consistent with AVO staining, expression of autophagic marker proteins were increased Figure 5B, D. Combining the two treatments induced the autophagy than either of the drugs alone but upregulated-autophagy was negated by NAC Figure 5E-G. Our results show that addition of CA sensitized CDDP-resistant ovarian cancer cells than treatment of CDDP sole.

(A)


A2780/s
A2780/cis
Cell viability (%)
Relative to Ctrl
150
100
50
0
0
20
40
60
CDDP (μM)

(B)


A2780/s
A2780/cis
Cell viability (%)
Relative to Ctrl
150
100
50
0
0
20
40
60
80
100
CA (μM)

(C)

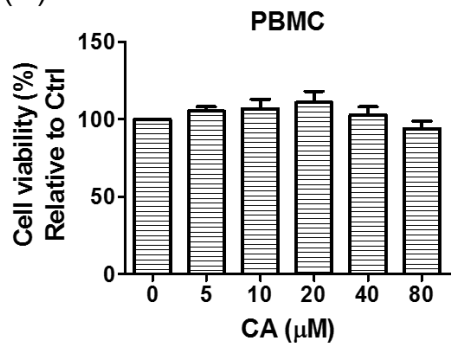
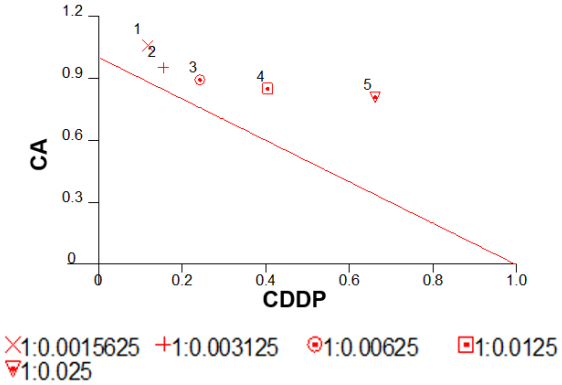

PBMC
Cell viability (%)
Relative to Ctrl
150
100
50
0
0
5
10
20
40
80
CA (μM)

Figure 1. CDDP and CA inhibit viability of ovarian cancer cells.

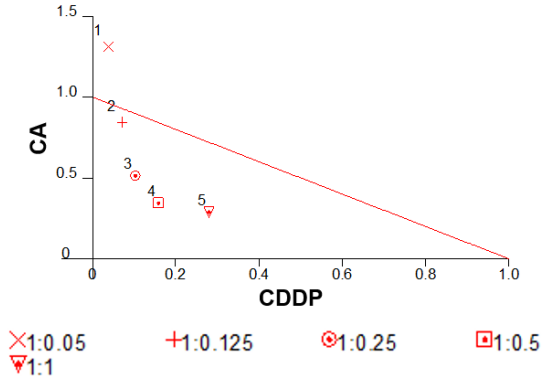
(A) CDDP and (B) CA suppressed A2780/s and A2780/cis cell viability in a concentration-dependent manner for 48 h. Peripheral blood mononuclear cells (PBMCs) were treated with either (C) CA for 48 h. Inhibition of cell viability was determined by MTT assay. Data represent the mean ± SEM values of three independent experiments.

(A)



CA (μM)	CDDP (μM)	CI
40	0.0625	1.177
40	0.125	1.106
40	0.25	1.135
40	0.5	1.255
40	1	1.469

(B)



CA(μM)	CDDP(μM)	CI
40	2	1.352
40	5	0.917
40	10	0.614
40	20	0.503
40	40	0.566

Figure 2. Combination of CA and CDDP appears synergistic effects on only A2780/cis.

(A, B) Synergism of CA and CDDP was analyzed Calcusyn software in A2780/s and A2780/cis, respectively. Combination Index (CI) indicates that < 1 is synergism, = 1 is additive effect, and > 1 is antagonism**.**

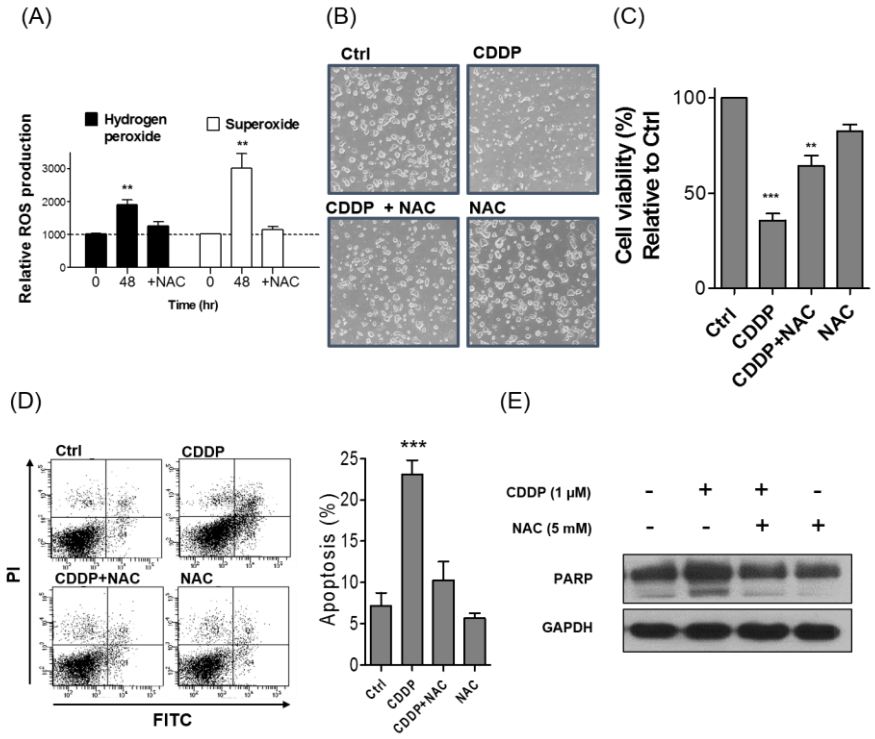

(A)
Hydrogen peroxide
Superoxide
Relative ROS production
3000
2000
1000
0
0
48
+NAC
0
48
+NAC
Time (hr)
(B)
Ctrl
CDDP
CDDP + NAC
NAC
(C)
Cell viability (%) Relative to Ctrl
100
50
0
Ctrl
CDDP
CDDP+NAC
NAC
(D)
Ctrl
CDDP
CDDP+NAC
NAC
PI
FITC
Apoptosis (%)
25
20
15
10
5
0
Ctrl
CDDP
CDDP+NAC
NAC
(E)
CDDP (1 μM)
NAC (5 mM)
PARP
GAPDH
(F)
Ctrl
CDDP
0.8%
12.95%
CDDP+NAC
NAC
1.7%
1.7%
PE
FITC
(G)
CDDP (1 μM)
NAC (5 mM)
LC3B-Ⅰ
LC3B-Ⅱ
GAPDH
(H)
Ctrl
CDDP
CDDP + NAC
NAC
AVOs
Cytosol & Nuclei
Merge

Figure 3. CDDP inhibit growth and induce apoptosis and autophagy of ovarian cancer cells through ROS generation in A2780/s.

(A-H) A2780/s was treated with DMSO or CDDP (1 μM) in the absence or presence of NAC (5 mM) for 48 h. (A) Intracellular ROS levels were measured by staining with H_2DCFDA (10 μM) and DHE (5 μM) dye, and (B) cells were observed under a phase contrast microscope to detect their morphological changes after CDDP treatment. (C) Cytotoxic effect was determined by MTT assay. Apoptotic cell death was detected by (D) flow cytometry and (E) Western blot. Autophagic cell death was analyzed by (F, H) AVO staining and (G) Western blot. GAPDH was used as a loading control. (H) AVOs were detected by confocal microscopy and shown as red color. Data represent the mean ± SEM values of three independent experiments. **indicates $p < 0.01$ and ***indicates $p < 0.001$ compared with control.

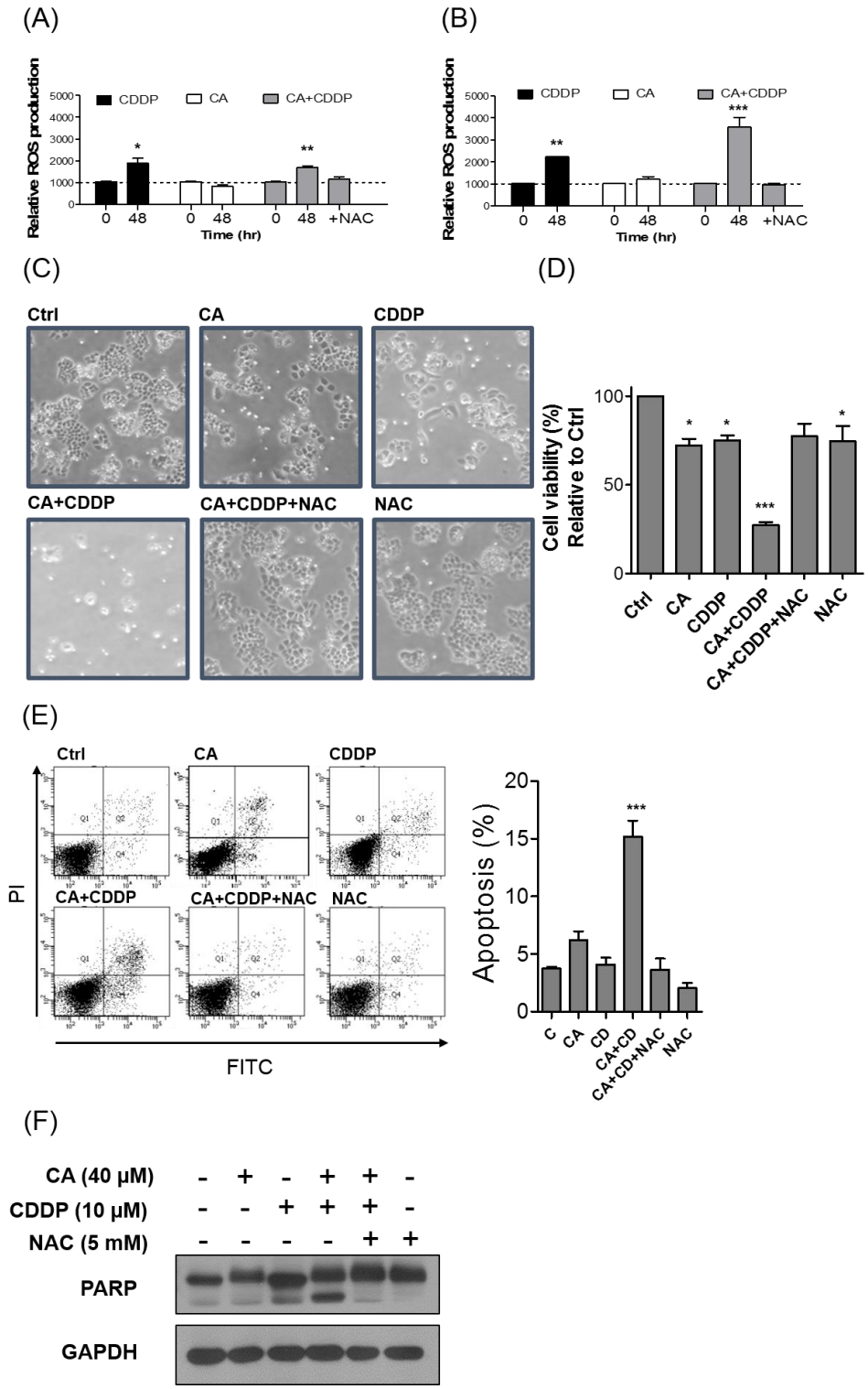

(A)
Relative ROS production
CDDP
CA
CA+CDDP
Time (hr)
(B)
Relative ROS production
CDDP
CA
CA+CDDP
Time (hr)
+NAC
(C)
Ctrl
CA
CDDP
CA+CDDP
CA+CDDP+NAC
NAC
(D)
Cell viability (%) Relative to Ctrl
Ctrl
CA
CDDP
CA+CDDP
CA+CDDP+NAC
NAC
(E)
Ctrl
CA
CDDP
CA+CDDP
CA+CDDP+NAC
NAC
PI
FITC
Apoptosis (%)
C
CA
CD
CA+CD
CA+CD+NAC
NAC
(F)
CA (40 μM)
CDDP (10 μM)
NAC (5 mM)
PARP
GAPDH

Figure 4. Combination of CA and CDDP generates ROS and induces apoptosis in A2780/cis.

(A-F) A2780/cis was treated with DMSO control or CA (40 μM) or CDDP (10 μM) alone, or CA and CDDP in the absence or presence of NAC (5 mM) for 48 h. (A) Intracellular ROS levels were determined by staining with H_2DCFDA (10 μM) and DHE (5 μM) dye, and (B) cells were viewed under a phase contrast microscope to detect their growth inhibition after CDDP and/or CA treatment. Effect of combination 40 μM CA and 10 μM CDDP on A2780/cis was determined by (D) MTT assay and apoptosis was detected by (E) flow cytometry and (F) Western blot. All results represent the mean ± SEM values of three independent experiments. *indicates $p < 0.1$, **indicates $p < 0.01$ and ***indicates $p < 0.001$ compared with control.

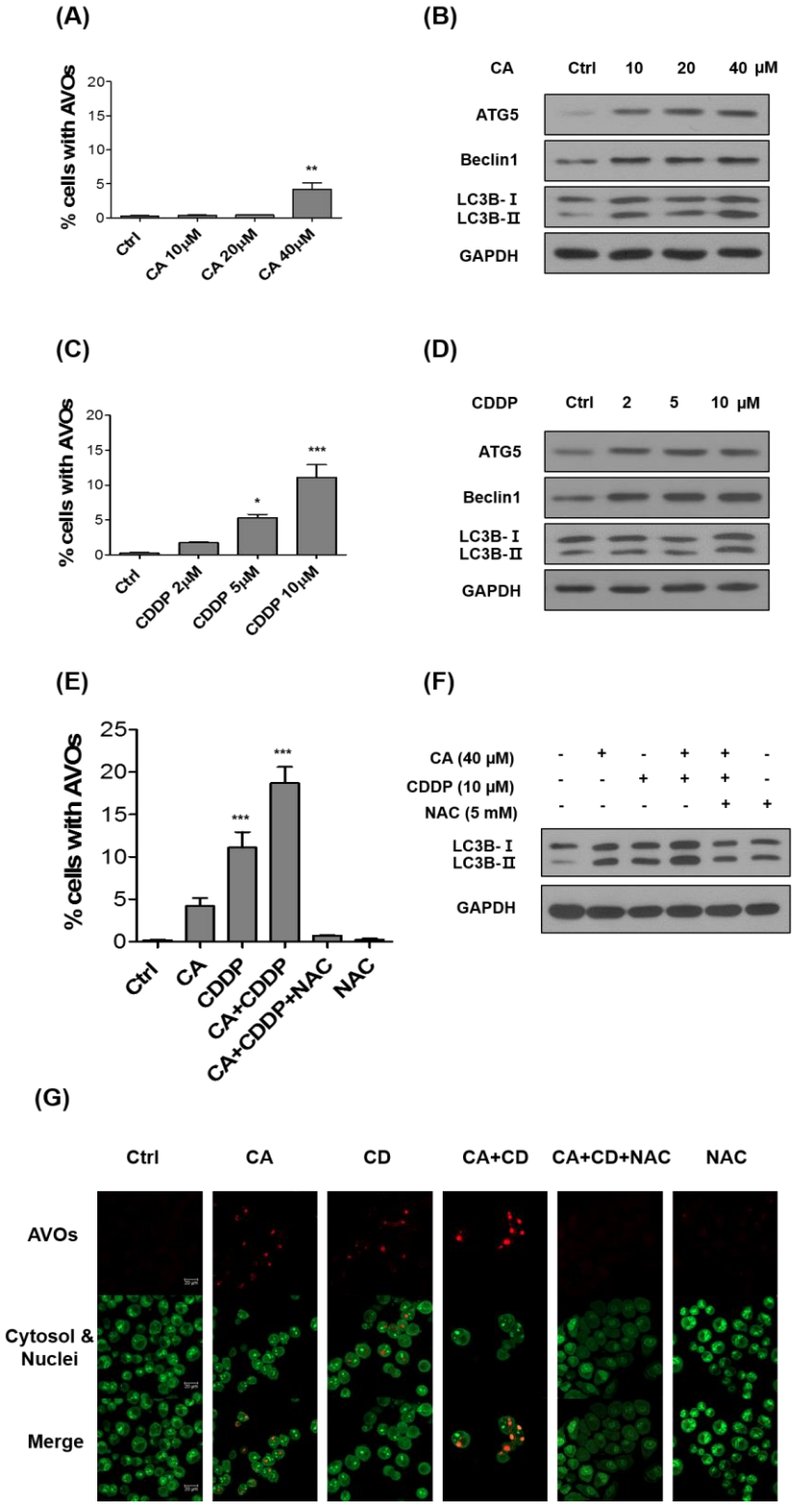

(A)
% cells with AVOs
20
15
10
5
0
**
Ctrl
CA 10μM
CA 20μM
CA 40μM
(B)
CA
Ctrl
10
20
40 μM
ATG5
Beclin1
LC3B- I
LC3B-II
GAPDH
(C)
% cells with AVOs
20
15
10
5
0
*
***
Ctrl
CDDP 2μM
CDDP 5μM
CDDP 10μM
(D)
CDDP
Ctrl
2
5
10 μM
ATG5
Beclin1
LC3B- I
LC3B-II
GAPDH
(E)
% cells with AVOs
25
20
15
10
5
0
***
***
Ctrl
CA
CDDP
CA+CDDP
CA+CDDP+NAC
NAC
(F)
CA (40 μM)
CDDP (10 μM)
NAC (5 mM)
LC3B- I
LC3B-II
GAPDH
(G)
Ctrl
CA
CD
CA+CD
CA+CD+NAC
NAC
AVOs
Cytosol &
Nuclei
Merge

Figure 5. Co-treatment of CA and CDDP induces autophagy formation in A2780/cis.

(A, C, E, G) Autophagy was analyzed in A2780/cis treated with CA (40 μM) and CDDP (10 μM) for 48 h by AVO staining. *indicates $p < 0.1$, **indicates $p < 0.01$ and ***indicates $p < 0.001$. (B, D, F) Changes in protein levels of autophagy markers (ATG5, Beclin1, and LC3B) by CA and CDDP were detected by western blotting. Protein expression was normalized to GAPDH expression. (G) AVOs were detected by confocal microscopy and shown as red color.

DISCUSSION

The main result of our study is that in CDDP-resistant ovarian cancer cells, the combination of CDDP with CA synergistically enhanced the cytotoxic effect by inducing ROS-mediated autophagy and apoptosis against chemoresistant ovarian cancer cells.

CDDP has been widely used to treat a large panel of solid neoplasms for decades. However, chemoresistance currently represents the major impediment to the clinical use of CDDP [24]. Combination chemotherapy, to overcome the CDDP-resistance, is a common strategy to treat cancer due to the rapid development of drug resistance and heterogeneity of cancer cells [25, 26]. We selected a phytochemical as a candidate compound which could act synergistically with CDDP. One of the advantages of harnessing naturally-occurring phytochemicals in cancer therapy would be that the phytochemicals show no cytotoxic effect on normal cells in most cases [27]. Furthermore, considerable anti-cancer effects of phytochemicals with various mechanisms of action have been reported in ovarian cancer [28-30]. Among many other types of plant-derived compounds, we incorporated CA. CDDP and CA share the mechanism of cytotoxic effect that they both stimulate ROS production [14, 31, 32]. So, we hypothesized that CA and CDDP may have synergistic effect by

boosting generation of ROS. The lowest CA and CDDP concentrations in combination at which the synergism observed were 40 μM and 10 μM respectively. So, further experiments were conducted with the concentrations listed above. In accordance with our expectation, compared to the sole treatment of either CA or CDDP, co-treatment of CA and CDDP dramatically elevated intracellular ROS production.

Intracellular ROS could be produced by various sources such as the mitochondria, NADPH oxidases, or even from exogenous triggers such as natural products and other pharmacological agents [10]. Depending on the ROS level, cancer cells could encounter survival or death. Cancer cells need the high energy for aberrant proliferation, but ROS as byproduct of energy metabolism were excessively accumulated [33]. To adapt to severe circumstances, ROS scavenging systems are up-regulated such as glutathione or tumor suppressors (BRCA1, p53, etc.) [10]. We propose to target the tumor adaptation by an anticancer therapy that forces the accumulation of excessive ROS and the induction of cell death. When CA was treated on A2780/cis, ROS level was not changed while CDDP treatment triggered ROS production. Interestingly, co-treatment of CA and CDDP markedly increased ROS level. The exact mechanisms of how CA synergistically increases ROS production when treated with CDDP are still unknown. The

underpinning mechanism of the synergistic effect of CA and CDDP might be associated with suppression of antioxidant system.

Autophagy is critical for maintaining cellular homeostasis by mediating lysosomal degradation pathway that eliminates damaged cells or organelles under the extreme conditions to protect cells. [4] Dysregulation of autophagy can lead to various disease including cancer. The role of autophagy in cancer is controversy, because autophagy can be involved in promotion or inhibition of tumorigenesis. [5, 34] Recent studies have shown that cytotoxic autophagy activated by ROS in tumor cells treated with chemotherapy. [35, 36] We quantified the accumulation of acridine orange stained AVOs after treatment using flow cytometry and confocal microscopy. Treatment of A2780/cis cells with CA and CDDP significantly increased AVOs and cleaved LC3B compared to control and sole treatment cells. Also, induction of autophagy detected by confocal microscopy was consistent with the protein expression and the results from FACS.

In conclusion, we first describe that combination treatment with CA and CDDP exert synergistic effect. We found that the CDDP-sensitive cells were prone to the increased ROS production. Subsequently, apoptosis and autophagic cell death were induced when treated with CDDP alone. In comparison to the CDDP-sensitive cells,

the resistant cells were less responsive to CDDP. We demonstrated that CA significantly sensitizes the resistant cells through an increase of ROS generation thereby inducing apoptosis and autophagic cell death. We anticipate that a novel combination of CA and CDDP contributes to development of new therapeutic approaches for CDDP-resistant ovarian cancer. Therefore, we propose CA as a potent phytochemical that could be used with conventional therapeutic agents to overcome chemoresistance.

REFERENCES

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2016, C A: a cancer journal for clinicians, 66 (2016) 7-30.

[2] G.C. Jayson, E.C. Kohn, H.C. Kitchener, J.A. Ledermann, Ova rian cancer, Lancet (London, England), 384 (2014) 1376-1388.

[3] S. Vaughan, J.I. Coward, R.C. Bast, Jr., A. Berchuck, J.S. Be rek, J.D. Brenton, G. Coukos, C.C. Crum, R. Drapkin, D. Etemad moghadam, M. Friedlander, H. Gabra, S.B. Kaye, C.J. Lord, E. L engyel, D.A. Levine, I.A. McNeish, U. Menon, G.B. Mills, K.P. Nephew, A.M. Oza, A.K. Sood, E.A. Stronach, H. Walczak, D.D. Bowtell, F.R. Balkwill, Rethinking ovarian cancer: recommendatio ns for improving outcomes, Nature reviews. Cancer, 11 (2011) 71 9-725.

[4] G. Marino, M. Niso-Santano, E.H. Baehrecke, G. Kroemer, Sel f-consumption: the interplay of autophagy and apoptosis, Nature r eviews. Molecular cell biology, 15 (2014) 81-94.

[5] V.W. Rebecca, R.K. Amaravadi, Emerging strategies to effecti vely target autophagy in cancer, Oncogene, 35 (2016) 1-11.

[6] X. Zhang, H. Wei, Z. Liu, Q. Yuan, A. Wei, D. Shi, X. Yan g, J. Ruan, A novel protoapigenone analog RY10-4 induces breast cancer MCF-7 cell death through autophagy via the Akt/mTOR pathway, Toxicology and applied pharmacology, 270 (2013) 122-1

28.

[7] R. Mathew, V. Karantza-Wadsworth, E. White, Role of autoph agy in cancer, Nature reviews. Cancer, 7 (2007) 961-967.

[8] M.Y. Fong, S. Jin, M. Rane, R.K. Singh, R. Gupta, S.S. Kak ar, Withaferin A synergizes the therapeutic effect of doxorubicin t hrough ROS-mediated autophagy in ovarian cancer, PloS one, 7 (2012) e42265.

[9] W. Hao, X. Zhang, W. Zhao, X. Chen, Psoralidin induces aut ophagy through ROS generation which inhibits the proliferation of human lung cancer A549 cells, PeerJ, 2 (2014) e555.

[10] C. Gorrini, I.S. Harris, T.W. Mak, Modulation of oxidative st ress as an anticancer strategy, Nature reviews. Drug discovery, 12 (2013) 931-947.

[11] R. Wang, R. Wang, B. Yang, Extraction of essential oils fro m five cinnamon leaves and identification of their volatile compo und compositions, Innovative Food Science & Emerging Technolo gies, 10 (2009) 289-292.

[12] H.F. Yeh, C.Y. Luo, C.Y. Lin, S.S. Cheng, Y.R. Hsu, S.T. C hang, Methods for thermal stability enhancement of leaf essential oils and their main constituents from indigenous cinnamon (Cinna momum osmophloeum), Journal of agricultural and food chemistry,

61 (2013) 6293-6298.

[13] F. Roth-Walter, A. Moskovskich, C. Gomez-Casado, A. Diaz-Perales, K. Oida, J. Singer, T. Kinaciyan, H.C. Fuchs, E. Jensen-J arolim, Immune suppressive effect of cinnamaldehyde due to inhib ition of proliferation and induction of apoptosis in immune cells: implications in cancer, PloS one, 9 (2014) e108402.

[14] C. Yu, S.L. Liu, M.H. Qi, X. Zou, Cinnamaldehyde/chemoth erapeutic agents interaction and drug-metabolizing genes in colore ctal cancer, Molecular medicine reports, 9 (2014) 669-676.

[15] J.S. Huang, Y.H. Lee, L.Y. Chuang, J.Y. Guh, J.Y. Hwang, Cinnamaldehyde and nitric oxide attenuate advanced glycation end products-induced the Jak/STAT signaling in human renal tubular cells, Journal of cellular biochemistry, 116 (2015) 1028-1038.

[16] S.H. Hong, I.A. Ismail, S.M. Kang, D.C. Han, B.M. Kwon, Cinnamaldehydes in Cancer Chemotherapy, Phytotherapy research : PTR, 30 (2016) 754-767.

[17] R. Marullo, E. Werner, N. Degtyareva, B. Moore, G. Altavill a, S.S. Ramalingam, P.W. Doetsch, Cisplatin induces a mitochondr ial-ROS response that contributes to cytotoxicity depending on mit ochondrial redox status and bioenergetic functions, PloS one, 8 (2 013) e81162.

[18] M.S. Tsai, S.H. Weng, Y.H. Kuo, Y.F. Chiu, Y.W. Lin, Synergistic effect of curcumin and cisplatin via down-regulation of thymidine phosphorylase and excision repair cross-complementary 1 (ERCC1), Molecular pharmacology, 80 (2011) 136-146.

[19] R.F. Araujo, Jr., L.A. Soares, C.R. da Costa Porto, R.G. de Aquino, H.G. Guedes, P.R. Petrovick, T.P. de Souza, A.A. Araujo, G.C. Guerra, Growth inhibitory effects of Phyllanthus niruri extracts in combination with cisplatin on cancer cell lines, World journal of gastroenterology, 18 (2012) 4162-6168.

[20] K. Selvendiran, S. Ahmed, A. Dayton, M.L. Kuppusamy, B. K. Rivera, T. Kalai, K. Hideg, P. Kuppusamy, HO-3867, a curcumin analog, sensitizes cisplatin-resistant ovarian carcinoma, leading to therapeutic synergy through STAT3 inhibition, Cancer biology & therapy, 12 (2011) 837-845.

[21] N. Mohell, J. Alfredsson, A. Fransson, M. Uustalu, S. Bystrom, J. Gullbo, A. Hallberg, V.J. Bykov, U. Bjorklund, K.G. Wiman, APR-246 overcomes resistance to cisplatin and doxorubicin in ovarian cancer cells, Cell death & disease, 6 (2015) e1794.

[22] J.C. Meier, B. Haendler, H. Seidel, P. Groth, R. Adams, K. Ziegelbauer, B. Kreft, G. Beckmann, A. Sommer, C. Kopitz, Knockdown of platinum-induced growth differentiation factor 15 abrogates p27-mediated tumor growth delay in the chemoresistant ovarian cancer model A2780cis, Cancer medicine, 4 (2015) 253-267.

[23] Z. Ai, Y. Lu, S. Qiu, Z. Fan, Overcoming cisplatin resistanc e of ovarian cancer cells by targeting HIF-1-regulated cancer meta bolism, Cancer letters, 373 (2016) 36-44.

[24] A. Davis, A.V. Tinker, M. Friedlander, "Platinum resistant" o varian cancer: what is it, who to treat and how to measure benef it?, Gynecologic oncology, 133 (2014) 624-631.

[25] J. Michels, I. Vitale, L. Senovilla, D.P. Enot, P. Garcia, D. Lissa, K.A. Olaussen, C. Brenner, J.C. Soria, M. Castedo, G. Kro emer, Synergistic interaction between cisplatin and PARP inhibitor s in non-small cell lung cancer, Cell cycle (Georgetown, Tex.), 12 (2013) 877-883.

[26] R. Mir, A. Tortosa, F. Martinez-Soler, A. Vidal, E. Condom, A. Perez-Perarnau, T. Ruiz-Larroya, J. Gil, P. Gimenez-Bonafe, Mdm2 antagonists induce apoptosis and synergize with cisplatin o vercoming chemoresistance in TP53 wild-type ovarian cancer cells, International journal of cancer, 132 (2013) 1525-1536.

[27] J.H. Lee, T.O. Khor, L. Shu, Z.Y. Su, F. Fuentes, A.N. Kon g, Dietary phytochemicals and cancer prevention: Nrf2 signaling, epigenetics, and cell death mechanisms in blocking cancer initiatio n and progression, Pharmacology & therapeutics, 137 (2013) 153-171.

[28] H. Gwak, S. Kim, D.N. Dhanasekaran, Y.S. Song, Resveratro

l triggers ER stress-mediated apoptosis by disrupting N-linked glycosylation of proteins in ovarian cancer cells, Cancer letters, 371 (2016) 347-353.

[29] B. Kim, H.S. Kim, E.J. Jung, J.Y. Lee, K.T. B, J.M. Lim, Y. S. Song, Curcumin induces ER stress-mediated apoptosis through selective generation of reactive oxygen species in cervical cancer cells, Molecular carcinogenesis, 55 (2016) 918-928.

[30] H. Gwak, Y. Kim, H. An, D.N. Dhanasekaran, Y.S. Song, Metformin induces degradation of cyclin D1 via AMPK/GSK3beta axis in ovarian cancer, Molecular carcinogenesis, (2016).

[31] J. Noh, B. Kwon, E. Han, M. Park, W. Yang, W. Cho, W. Yoo, G. Khang, D. Lee, Amplification of oxidative stress by a dual stimuli-responsive hybrid drug enhances cancer cell death, Nature communications, 6 (2015) 6907.

[32] M. Wangpaichitr, C. Wu, M. You, J.C. Maher, V. Dinh, L.G. Feun, N. Savaraj, N',N'-Dimethyl-N',N'-bis(phenylcarbonothioyl) Propanedihydrazide (Elesclomol) Selectively Kills Cisplatin Resistant Lung Cancer Cells through Reactive Oxygen Species (ROS), Cancers, 1 (2009) 23-38.

[33] S. Krishna, I.C. Low, S. Pervaiz, Regulation of mitochondrial metabolism: yet another facet in the biology of the oncoprotein Bcl-2, The Biochemical journal, 435 (2011) 545-551.

[34] D.A. Gewirtz, The Challenge of Developing Autophagy Inhibition as a Therapeutic Strategy, Cancer research, 76 (2016) 5610-5614.

[35] K. Wang, C. Zhang, J. Bao, X. Jia, Y. Liang, X. Wang, M. Chen, H. Su, P. Li, J.B. Wan, C. He, Synergistic chemopreventive effects of curcumin and berberine on human breast cancer cells through induction of apoptosis and autophagic cell death, Scientific reports, 6 (2016) 26064.

[36] Z.F. Shang, Q. Wei, L. Yu, F. Huang, B.B. Xiao, H. Wang, M. Song, L. Wang, J. Zhou, J. Wang, S. Li, Suppression of PC-1/PrLZ sensitizes prostate cancer cells to ionizing radiation by attenuating DNA damage repair and inducing autophagic cell death, Oncotarget, 7 (2016) 62340-62351.

국문초록

난소암은 가장 치명적인 부인과 암입니다. 현재 난소암을 치료하는데 주요한 장애물은 백금 저항성이다. Cisplatin (CDDP)은 대표적인 백금계 약물이며 여러 암세포에서 활성산소종(Reactive oxygen species, ROS)을 유발하여 항암효과를 나타낸다. *Cinnamomum cassia* 줄기 껍질에서 추출한 cinnamaldehyde (CA)는 다양한 암 종에서 항암효과를 가지며 ROS 생성에 의한 세포사멸을 유도하는 것으로 알려져 있다. 이전 연구에 따르면, 세포 스트레스 요인에 의한 ROS의 발생은 많은 암에서 세포사멸뿐만 아니라 자가 포식을 촉진시킨다. 따라서 우리는 난소암세포에서 CA와 CDDP가 ROS가 매개된 세포사멸과 자가 포식을 증가시키는지, 두 물질의 시너지가 있는지에 대한 여부를 확인해보았다. 우리는 두 가지 유형의 난소암 세포주를 선택했다. (CDDP에 대해 민감한 A2780/s 및 CDDP에 대해 내성을 지닌 A2780/cis). A2780/s 세포에서 낮은 농도 (1 μM)의 CDDP를 처리하면 세포독성을 나타내며 ROS가 매개된 세포사멸과 자가 포식이 유도되었다. 그러나 A2780/cis 세포에서 높은 농도 (10 μM)의 CDDP를 처리했을 때 큰 영향이 없었다. 이 조건에서

A2780/cis 세포에 CA를 함께 처리하였을 때 시너지를 보이는 성장-억제 효과가 눈에 띄게 증가하였고 ROS 매개된 세포사멸과 자가 포식 작용이 유도되었다. 따라서 CA와 CDDP의 조합에 의한 과도한 ROS는 난소암의 항암제내성을 극복하는 방법으로 제안될 수 있을 것이다.