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연세대학교 대학원

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이 논문을 석사 학위논문으로 제출함

2016년 1월

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김성균의 석사 학위논문을 인준함

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감사의 글

본 논문의 처음 연구 계획에서부터 완성에 이르기까지 학문적 기틀을 잡아주시고 소상한 가르침으로 지도해 주신 박상유 지도 교수님께 진심으로 감사드립니다. 또한 논문 작성 과정 및 심사에 귀중한 조언과 격려를 해주신 임기정 교수님, 김수기 교수님께도 깊은 감사를 드립니다. 바쁜 일정 속에서도 실험을 도와준 이세희 연구원에게도 감사의 마음을 전합니다.

이비인후과 전문의로서 출발한 이때에 본 논문이 좋은 밑거름이 될 수 있도록 쉬지 않고 노력하겠습니다.

지금까지 묵묵히 응원해주시고 기도해 주신 부모님과 든든한 지원군이 되어준 아내에게 진심으로 감사드립니다.

2016년 1월

김 성 균 드림

차 례

그림 차례

국 문 요 약

HEI-OC1 세포에서의 Cisplatin 이독성에 대한 alpha-tocopherol의 효과

목적: Alpha-tocopherol은 methylated phenol계열로 지용성 항산화물질로서 알려져 있으며 유리기를 줄이고 항산화 역할을 하는 비타민 E의 다른 형태이다. 저자는 alpha-tocopherol의 항상화 효과가 cisplatin에 의한 세포독성을 보호할 수 있음을 토대로 HEI-OC1 청각세포에서 cisplatin유발 이독성에 대한 효과를 알아보았다. **실험 재료 및 방법:** 10 μM 농도의 alpha-tocopherol을 24시간동안 HEI-OC1세포에 전처치 한 후, 15 μM 농도의 cisplatin을 추가하였다. 48시간 후 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)를 이용하여 세포 생존율을 측정하였으며 2′,7′-dichlorofluorescein diacetate (DCFH-DA) 형광 염료를 이용하여 세포내 활성산소 농도를 측정하였다. 이후 Annexin V-FITC and propidium iodide (PI) 염색으로 세포자멸사의 패턴을 분석하였고 caspase3/CPP32 형광분석 키트를 이용하여 caspase-3 활성도를 측정하였다. 또한 면역 블로팅을 통해 poly-ADP-ribose polymerase(PARP)를 분석하였다. **결과:** HEI-OC1 세포에 10 μM의 alpha-tocopherol을 전처치한 결과 cisplatin 유발 이독성에 대한 보호효과가 있음을 확인하였고 이독성으로 인하여 상승된 활성산소의 양도 유의하게 감소함을 알 수 있었다. Alpha-tocopherol을 전처치한 군은 cisplatin 단독군에 비해 활성산소를 15% 감소시켰고, 세포 괴사 및 후기 세포자멸사의 비율이

50% 감소하였다. 또한 alpha-tocopherol은 caspase-3 활성도 및 PARP 양을 감소시켰다. **결론:** 본 연구는 HEI-OC1 청각 세포에서 cisplatin 유발 이독성에 대한 alpha-tocopherol의 보호효과에 대한 것으로, alpha-tocopherol은 cisplatin유발 이독성으로 인하여 증가된 활성산소를 유의하게 감소시키며 세포자멸사를 줄이는 역할을 하는 것으로 판단된다.

핵심되는 말: Alpha-tocopherol; Cisplatin; 활성산소; 이독성; 항산화

1. Introduction

Cisplatin (cis-diamminedichloroplatinum II; CDDP) is a well-known chemotherapeutic agent that is widely used in the treatment of human solid tumors in head and neck, ovarian, cervical, and bladder cancers, among others. In spite of its efficacy, cisplatin has several severe adverse effects such as nephrotoxicity, neurotoxicity, and ototoxicity [1, 2]. Among them, ototoxicity, a medication-induced auditory or vestibular functional loss, can be progressive and permanent. Cisplatin has been reported to cause the death of outer hair cells in the organ of Corti [3, 4], and it is believed that cisplatin induces this ototoxicity by generating reactive oxygen species (ROS), resulting in the depletion of glutathione and antioxidant enzymes [5-7].

Tocopherols are a class of methylated phenols, known as fat-soluble antioxidants. Alpha-tocopherol is a form of vitamin E, which reduces free radicals and acts as an antioxidant. It has several different forms, all of which have a chromanol ring with a hydroxyl chain that donates a hydrogen atom to reduce ROS. Tocopherol is abundant in natural olive and sunflower oils and has been considered to be an effective antioxidant or protective drug without side effects when used clinically [8].

The effects of α-tocopherol on cisplatin-induced ototoxicity were examined in guinea pigs, where it suppressed ototoxicity and nephrotoxicity by suppressing increased ROS production [9]. Alpha-tocopherol and tiopronin co-therapy was demonstrated to significantly slow the progressive high-frequency hearing loss induced by cisplatin [10]. Alpha-tocopherol also protects against cisplatin-induced ototoxicity without interfering with cisplatin's antitumor efficacy [11].

Our study investigated the antioxidative effect of α-tocopherol on cisplatin-induced ototoxicity in HEI-OC1 auditory cells.

2. Material and methods

2.1. Chemicals

Cisplatin (CAS no. 15663-27-1) and α-tocopherol (CAS no. 4345-03-3) were purchased from Sigma Chemical Co. (St Louis, MO, USA).

2.2. Cell culture

The HEI-OC1 cell line was kindly provided by F. Kalinec (House Ear Institute, Los Angeles, CA, USA). HEI-OC1 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Gibco BRL, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS; genDEPOT, Texas, U.S.A) and 50 U/ml interferon-γ without antibiotics at 33°C under an atmosphere of 10% CO_2. HEI-OC1 cells express several molecular markers that are characteristic of sensory cells in the organ of Corti, and are extremely sensitive to ototoxic drugs [12]. Cisplatin was prepared as a 5 mM stock solution in the cell culture media and added directly to the culture well.

2.3. Cell viability assay

Cell viability was measured by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) assay. The auditory cells (2×10^4 cells/well of a 48-well plate) were incubated with 15 μM of cisplatin (IC_{50}; the concentration required for 50% inhibition) for 48 h. In order to examine the protective effect of α-tocopherol on cisplatin-induced cytotoxicity in HEI-OC1

cells, the cells were pretreated with α-tocopherol at a concentration of 10 μM for 24 h, before exposure to cisplatin (15 μM, 48 h). For the MTT assay, 100 μL of the MTT solution (5 mg/mL) was added to the cells, and then the plates were incubated for 3 h at 33°C in an atmosphere of 10% CO_2 and 90% air. The MTT cell assay is designed to measure cell viability and proliferation in the presence of a combination of cytotoxic or protective agents and is based on the quantitative measurement of the extracellular reduction of the yellow-colored, water-soluble Tetrazolium dye to insoluble formazan crystals by metabolically active cells. This reduction is mediated by the mitochondrial enzyme lactate dehydrogenase (LDH). LDH is found in most body tissues, including blood cells and cardiac myocytes. Since LDH is released at the time of tissue damage, it is used as a marker of common cellular damage and apoptotic/necrotic disease. When dissolved in an appropriate solvent, these formazan crystals exhibit a purple color, the intensity of which is proportional to the number of viable cells and can be measured by using a spectrophotometer at 570 nm. The optical density was measured by using a microplate reader at 570 nm (Spectra Max, Molecular Devices, Sunnyvale, CA, USA).

2.4. Measurement of intracellular ROS production

The intracellular ROS level was measured by using a fluorescent dye, 2′,7′-dichlorofluorescein diacetate (DCFH-DA, Calbiochem, USA). In the presence of an oxidant, DCFH is converted to the highly fluorescent 2′, 7′-dichlorofluorescein (DCF). For the assay, HEI-OC1 cells were incubated in the dark for 30 min at 37°C with 50 μM DCFH-DA. Fluorescence was analyzed by using a FACScan flow cytometer (BD Biosciences, Heidelberg, Germany) at an excitation wavelength of 495 nm and an emission wavelength of 530 nm (FL-1) with gating at 10,000 cells/sample.

2.5. Fluorescent microscope

Cells were grown on the Cell Culture Slides (SPL, Gyeonggido, Korea). Cells were washed twice with serum-free medium without phenol red and incubated with 50 mM DCFH-DA in serum-free medium without phenol red for 30 min at 33℃. After three washings with serum free medium without phenol red, cells were fixed with 3.7% glutaraldehyde for 10 min at room temperature. Cells were incubated with 10 mg/mL Hoechst 33258 (Sigma, St Louis, MO, USA) for 20 min at room temperature in the dark. After washing twice with PBS and mounting, the fluorescence images from multiple fields of view were obtained using an Olympus ix71 microscopy with a long-term real-time live cell image system (LAMBDA DG-4, Sutter Int., Novato, CA, USA).

2.6. Flow cytometric assay of apoptosis

The rate of apoptosis among HEI-OC1 auditory cells was determined by using Annexin V-FITC (Ezway Annexin V-FITC Apoptosis Detection Kit, Komabiotech, Seoul, Korea). The specific binding of Annexin V-FITC occurred during incubation of the cells for 15 min at room temperature in binding buffer containing a saturating concentration of Annexin V-FITC and PI (propidium iodide). Afterwards, the cells were analyzed by flow cytometry (BD Biosciences, Heidelberg, Germany) for a cell count of 10000.

2.7. Measurement of caspase-3 activity

The enzymatic activity of caspase-3 was assayed with a caspase3/CPP32 fluorometric assay kit (Biovision, Milpitas, California, USA) according to the manufacturer's protocol. Auditory cell line lysate was prepared in a lysis buffer on ice for 10 min and centrifuged for 5 min at 14,000 rpm. The protein concentration in each lysate was measured. The catalytic activity of caspase-3 in the cell lysate was measured by proteolytic cleavage of 50 mM DEVD-pNA and fluorometric substrate for 2 h at 37°C. The mixture incubated with no DEVD-pNA substrate was used as a negative control. The plates were read by microplate reader (Spectra Max, Molecular Devices, Sunnyvale, CA, USA) at a 400 nm excitation filter and a 505 nm emission filter.

2.8. Immunoblotting of PARP

Following primary antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA): poly-ADP-ribose polymerase(PARP). For the assay, HEI-OC1 cells were cultured and treated with 15 uM cisplatin for 48 h in the presence or absence of alpha tocopherol (10uM, 24 h pretreatment). Cell lysates were used in RIPA buffer (Cell Signaling Technology, Danvers, MA, USA). The proteins (20 ug/sample) were immediately heated for 5 min at 100℃ and were subjected to SDS-PAGE on gels. Separated proteins were transferred to nitrocellulose membranes, and western blotting was performed using a gel loading kit and protein transfer system kit (BioRad, Hercules, CA, USA). Membranes were blocked by treatment with 5% skim milk in Tris buffer solution containing 0.1% Tween (TBST) and subsequently incubated with the primary polyclonal antibodies at a final dilution of 1: 1000. After three washes in TBST, membranes were incubated with peroxidase conjugated secondary antibodies (final dilution, 1: 2000) in blocking buffer for 1 h and subsequently washed. Detection was performed by chemiluminescence using an ECL solution

(Gendepot, Barker TX, USA). Beta-actin was used as a loading control. For the calculation of band density, Image J was used (Imagej.nih.gov/ij/index.html).

2.9. Statistical analysis

All values are represented as mean ± standard deviation (S.D.). For data analysis, we used the SPSS 22.0 statistical program. The paired *t* test was used for pairs of data, while multiple data were analyzed by ANOVA. A *p* value of less than 0.05 was considered statistically significant.

3. Results

3.1. Tocopherol protects against cisplatin-induced cytotoxicity in auditory cells

To establish an appropriate dose of cisplatin, cell viability was examined in the HEI-OC1 cell line. As shown in Figure 1-A, HEI-OC1 cells were treated with various concentrations of α-tocopherol (1–20 μM) for 48 h. Alpha-tocopherol at concentrations of more than 20 μM significantly induced cytotoxicity as compared with concentrations of less than 10 μM in the HEI-OC1 cells (***$p < 0.001$). Thus, 10 μM was chosen as the ideal α-tocopherol concentration to demonstrate a protective effect against cisplatin-induced ototoxicity. Compared with the negative control, 10 μM α-tocopherol did not promote cellular growth, showing similar cell viability (99.7 ± 8%, not significant as compared to the control group; $p > 0.05$).

Alpha-tocopherol at a concentration of 10 μM provided significant protection (50.3 ± 2.7% for the cisplatin group vs. 69.1 ± 6.4% for the cisplatin-plus-tocopherol group) against the toxic effect

of 15 μM cisplatin applied for 48 h (***$p < 0.001$). Compared with the cisplatin group, treatment with 5 μM α-tocopherol also showed significant protective effect against cytotoxicity (***$p < 0.001$), but treatment with 1 μM α-tocopherol did not show any beneficial effect ($p > 0.05$).

Cisplatin-induced ototoxicity and the protective effect of α-tocopherol were also verified by microscopic morphological examination. Cell lines incubated with cisplatin or α-tocopherol were stained with Hoechst 33342 and DCFH-DA. In the control group, normal auditory cells were well-cultured and spindle-shaped (Fig. 2-A). In the group treated with 10 μM α-tocopherol, auditory cells had an appearance similar to the cells in the control group (Fig. 2-B). Under treatment with 15 μM cisplatin, the cell population was decreased, necrotic cell bodies were increased, and ROS were increased (Fig. 2-C). In the cisplatin-plus-tocopherol group, α-tocopherol protected HEI-OC1 cells against cisplatin-induced cytotoxicity. Compared with the cisplatin group, the cell population was increased, and both necrotic bodies and ROS were decreased (Fig. 2-D). Thus, the protective effect of α-tocopherol on cisplatin-induced cytotoxicity was seen microscopically in auditory cells, reflecting similar results as obtained by the cell viability assay.

3.2. Alpha-tocopherol significantly reduced the cisplatin-induced increase in ROS

To investigate the effects of α-tocopherol on intracellular ROS generation induced by cisplatin, auditory cells were treated with 15 μM cisplatin in the presence or absence of 10 μM α-tocopherol for 48 h. Measurement of ROS was completed by using a FACScan flow cytometer (Fig. 3-A). Compared to the control group (100%), the cisplatin group showed significant ROS generation (178.8 ± 12%, ***$p < 0.001$). However, ROS production in the cisplatin-plus-tocopherol group (151.5 ± 17%) was significantly lower than that in the cisplatin group (**$p < 0.01$). There was no

significant difference in ROS production between the control and α-tocopherol (85.5 ± 6%, $p > 0.05$).

3.3. Alpha-tocopherol significantly reduced both cisplatin-induced necrosis and late apoptosis

Flow cytometry analysis showed patterns of cell death such as necrosis or late apoptosis (Fig. 4-A). In both the control and 10 μM α-tocopherol groups, the percentages of necrosis and late apoptosis were similar (3 ± 2.7% and 2.7 ± 1.2% in control, 3.3 ± 2.6% and 2.9 ± 1.7% in α-tocopherol). In the 15 μM cisplatin group, a significant increase in density was observed in both necrosis and late apoptosis area (14.4 ± 6.7% and 37.7 ± 7.2%, respectively), as compared with the control and tocopherol groups (**$p < 0.01$ and ***$p < 0.001$). Alpha-tocopherol treatment significantly reduced both necrosis and late apoptosis (Fig. 4-B). Necrosis was reduced from 14.4% to 7.1 ± 6% (*$p < 0.05$), and late apoptosis was reduced from 37.7% to 18.2 ± 6.8% (***$p < 0.001$).

3.4. Alpha-tocopherol inhibited the expression of caspase-3 activity

Caspase-3 activity is involved in cisplatin-induced toxicity and related to apoptotic changes in cisplatin ototoxicity. The administration of 15 mM cisplatin increased the activity of caspase-3 (7.61 ± 1.31 fold over the normal control) (Fig. 5). Pretreatment of HEI-OC1 cells with α -tocopherol, however, significantly reduced caspase-3 activity (6.83 ± 1.28-fold over the normal control) compared with cells treated with cisplatin alone (*$p < 0.05$).

3.5. Alpha-tocopherol inhibited the expression of PARP related to cisplatin-induced apoptosis

The cleavages of PARP facilitate cellular disassembly and serve as a marker of cells undergoing apoptosis. In the cisplatin-treated group, increased cleavage of PARP was observed (2.42 ± 1.84 fold over the normal control) (Fig. 6). The pretreatment of HEI-OC1 cells with 10 μM α-tocopherol decreased cleaved PARP (1.89 ± 1.38 fold over the normal control) as compared with cells treated with cisplatin alone (*$p < 0.05$). Thus, this result also supports the existence of an anti-apoptotic effect of α-tocopherol.

4. Discussion

Many antioxidants have been used to prevent cisplatin-induced ototoxicity, and the protective mechanism of antioxidants was thought to be due to inhibition of ROS production, reduction of apoptosis, and improvement in cell viability. Glutathione and metformin especially were proven to be effective antioxidants in reducing ROS and cellular apoptosis [1, 6, 15]

In this study, we investigated the protective mechanisms of α-tocopherol on cisplatin-induced otototoxicity using ROS and sequential apoptotic cascade. Alpha-tocopherol can reduce ROS production by 15% and decrease necrosis and late apoptosis by about 50% in cisplatin-induced cytotoxicity. α-tocopherol significantly decreased caspase-3 activity and reduced the cleaved PARP. Caspase-3 is well known for factor that activates endonuclease and lead to cell death, and it is responsible for the proteolytic reaction of protein, such as PARP, which is important role of cell viability. We also demonstrated that α-tocopherol protects cells from cisplatin-induced ototoxicity, although it did not promote cellular growth. Thus, tocopherol showed a 36.2% increase in cell viability as compared with cisplatin, which is comparable to an 18.9% increase of outer hair cell

viability in an *in vivo* study of guinea pigs [9].

Alpha-tocopherol protects hair cells from oxidative damage by trapping peroxide radicals, interrupting and suppressing the lipid peroxidation chain reaction, and directly clearing superoxide anion radicals [10]. Antioxidant compounds may also function as free radical scavengers and can ensure upstream protection of the cochlea before cellular activation of the apoptotic pathway [13]. Because cisplatin induces oxidative stress, it can cause irreversible cell damage and apoptosis [14] by interacting with nucleophilic sites on the DNA chain [15].

An animal study demonstrated the protective effect of tocopherol on hearing damage. Alpha-tocopherol suppressed cisplatin-induced hearing loss at high frequencies, substantial outer hair cell (OHC) losses in the basal and second turns of the cochlea, increases in lipid peroxidation with apoptotic changes of the cochlea, increases in creatinine (Cr) and blood urea nitrogen (BUN) levels in the serum, and body weight loss [9].

Furthermore, tocopherol does not interfere with the antitumor efficacy of cisplatin while protecting from cisplatin-induced systemic and neurologic toxicities [11]. Sarna et al. found enhanced tumor growth inhibition in mice with lymphoma when the animals received both vitamin E and cisplatin [16]. A diet rich in several antioxidants, including vitamin E, was able to slow the growth rate of lung carcinoma in mice receiving cisplatin [17]. Enhanced cisplatin antitumor activity against neuroblastoma in mice receiving vitamin E was also reported [18]. The mechanism of the enhanced antineoplastic activity of cisplatin in animals receiving vitamin E is unclear, but some authors think that it may be due to increased cell membrane permeability to cisplatin [16-18].

Additionally, α-tocopherol has been demonstrated to be effective against damage induced by other exogenous factors such as noise and gentamicin-induced oto-vestibulotoxicity in guinea pigs [19, 20].

Although our experiment was conducted with an HEI-OC1 cell line designed to evaluate drug-induced ototoxicity, clinical availability of tocopherol in patients treated with cisplatin also remains uncertain. Further studies will be focused on animal experiments to confirm the underlying protective mechanism and to establish the appropriate dose of α-tocopherol applicable in the clinical setting.

5. Conclusion

Alpha-tocopherol significantly reduced the cisplatin-induced increase of ROS and decreased cellular necrosis and late apoptosis, thereby inhibiting cisplatin-induced cytotoxicity in HEI-OC1 cells. Our study suggests that α-tocopherol might be useful as a preventive supplement for patients receiving cisplatin who have concerns about potential ototoxicity.

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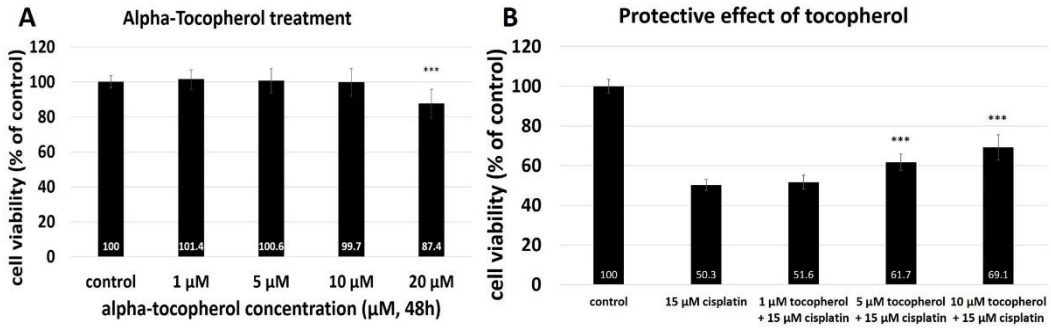

Figure 1. Effect of α-tocopherol in the auditory cell line culture. (A) HEI-OC1 cells were treated with various concentration of α-tocopherol (1–20 µM) for 48 h. Results show that α-tocopherol at concentrations of more than 20 µM significantly induced cytotoxicity as compared with concentrations of less than 10 µM (***$p < 0.001$). Thus, 10 µM was chosen as the ideal α-tocopherol concentration to demonstrate a protective effect against cisplatin-induced ototoxicity. (B) Alpha-tocopherol provided significant protection (50.3% ± 2.7% for the cisplatin group vs. 69.1% ± 6.4% for the cisplatin-plus-tocopherol group) against the toxic effect of 15 µM cisplatin applied for 48 h (***$p < 0.001$, as compared with the cisplatin group, results from 7 separate experiments in triplicate).

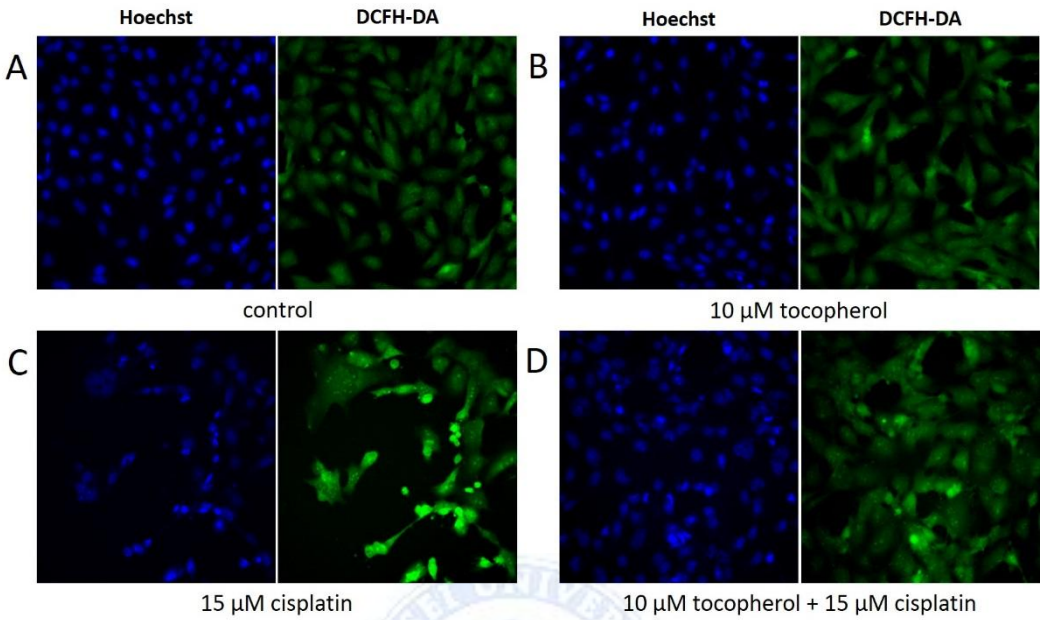

Figure 2. Representative microscopic appearance of HEI-OC1 cells stained with Hoechst 33342 (left) and 2′,7′-dichlorofluorescein diacetate (DCFH-DA, right): Protective effect of α-tocopherol on cisplatin-induced cytotoxicity in the auditory cell culture. (A) Control. (B) 10 μM α-tocopherol, which has a similar appearance to the control group. (C) 15 μM cisplatin. This figure shows a decreased cell population and necrotic cell bodies, and increased reactive oxygen species (ROS). (D) Cisplatin-plus-tocopherol group. Alpha-tocopherol protected against cisplatin-induced cytotoxicity. Compared with the cisplatin group, the cell size was within the normal range. Necrotic bodies and ROS were decreased. Left picture: Hoechst stains, Right picture: DCFDA fluorescent confocal microscope (×200).

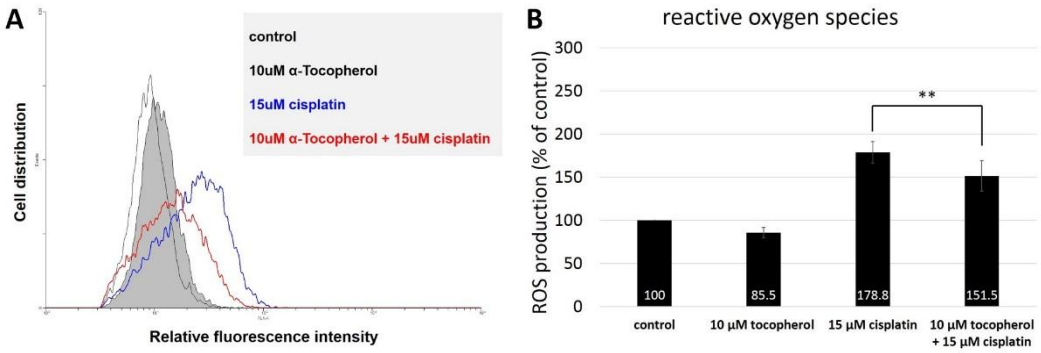

Figure 3. Measurement of intracellular reactive oxygen species (ROS) production. (A) Representative figure measured ROS by using a FACScan flow cytometer: Protective effect of α-tocopherol on cisplatin-induced cytotoxicity in the auditory cell culture. ROS production in the cisplatin-plus-tocopherol group (red) was significantly lower than that in the cisplatin group (blue). Control (black) and α-tocopherol treated group (gray) have similar ROS productions, which are significantly lower than the cisplatin group or the cisplatin-plus-tocopherol group. (B) Summary of the anti-ROS effect of α-tocopherol on cisplatin-induced cytotoxicity in auditory cells. In the cisplatin group, ROS was increased by 178.8% as compared with control, but α-tocopherol treatment significantly reduced ROS production to 151.5% (**$p < 0.01$, as compared with cisplatin-treated group, results from 5 separate experiments in triplicate).

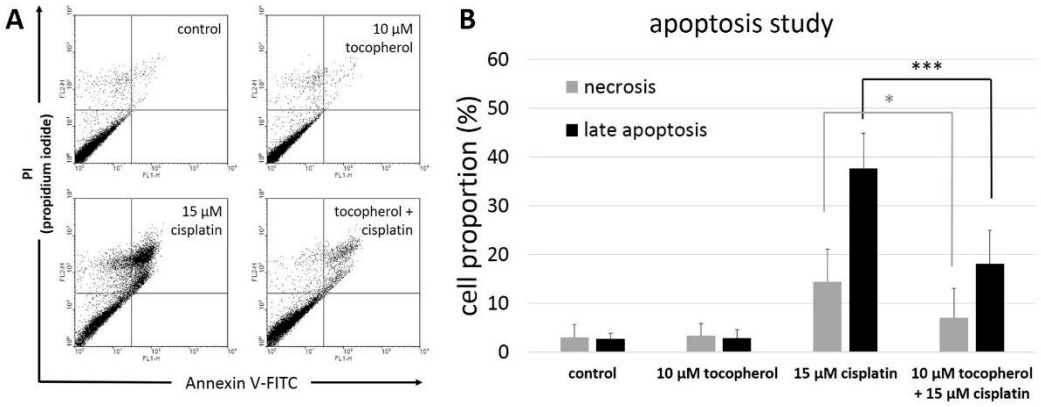

Figure 4. Apoptosis study of α-tocopherol. (A) Representative data of flow cytometry analysis showed cellular apoptosis patterns such as necrosis (left-upper panel) or late apoptosis (right-upper panel). In the 15 µM cisplatin group, increased density was observed in late apoptosis area. However, tocopherol treatment reduced the density in both upper panels. (B) Application of 15 µM cisplatin induced a significant increase of both necrosis and late apoptosis, as compared with control and tocopherol groups (**$p < 0.01$). Alpha-tocopherol treatment significantly reduced both necrosis and late apoptosis. Necrosis was reduced from 14.4% to 7.1% (*$p < 0.05$), and late apoptosis was reduced from 37.7% to 18.2% (***$p < 0.001$). Results from 7 separate experiments.

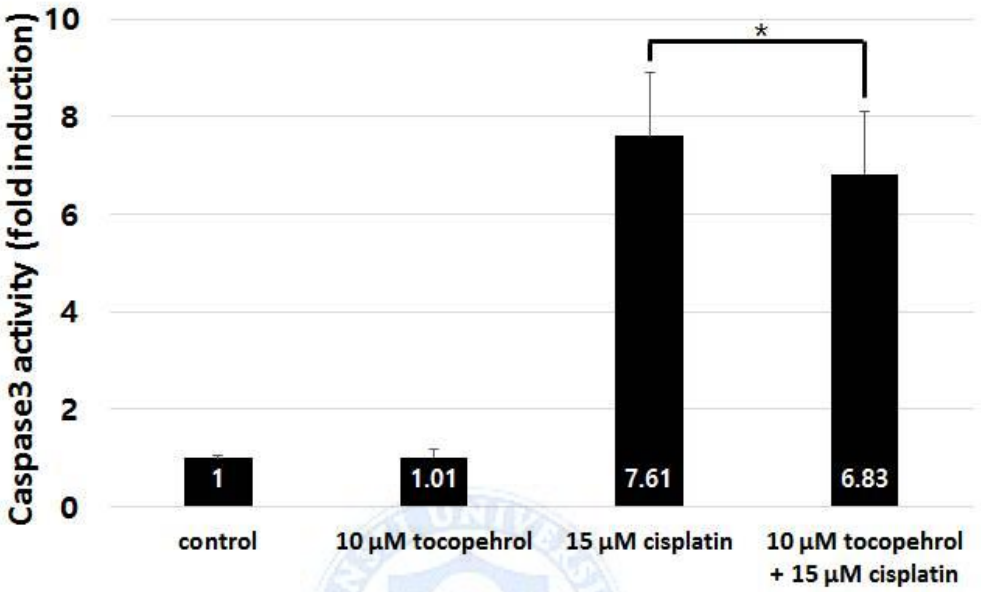

Figure 5. Measurement of caspase-3 activity. Data shows that pretreatment α-tocopherol, compared with cisplatin alone, inhibited the expression of caspase-3 (7.61 ± 1.31 fold over the normal control for the cisplatin group vs. 6.83 ± 1.28 fold over the normal control for the cisplatin-plus-tocopherol group). (*$p < 0.05$, compared with the cisplatin-treated group.)

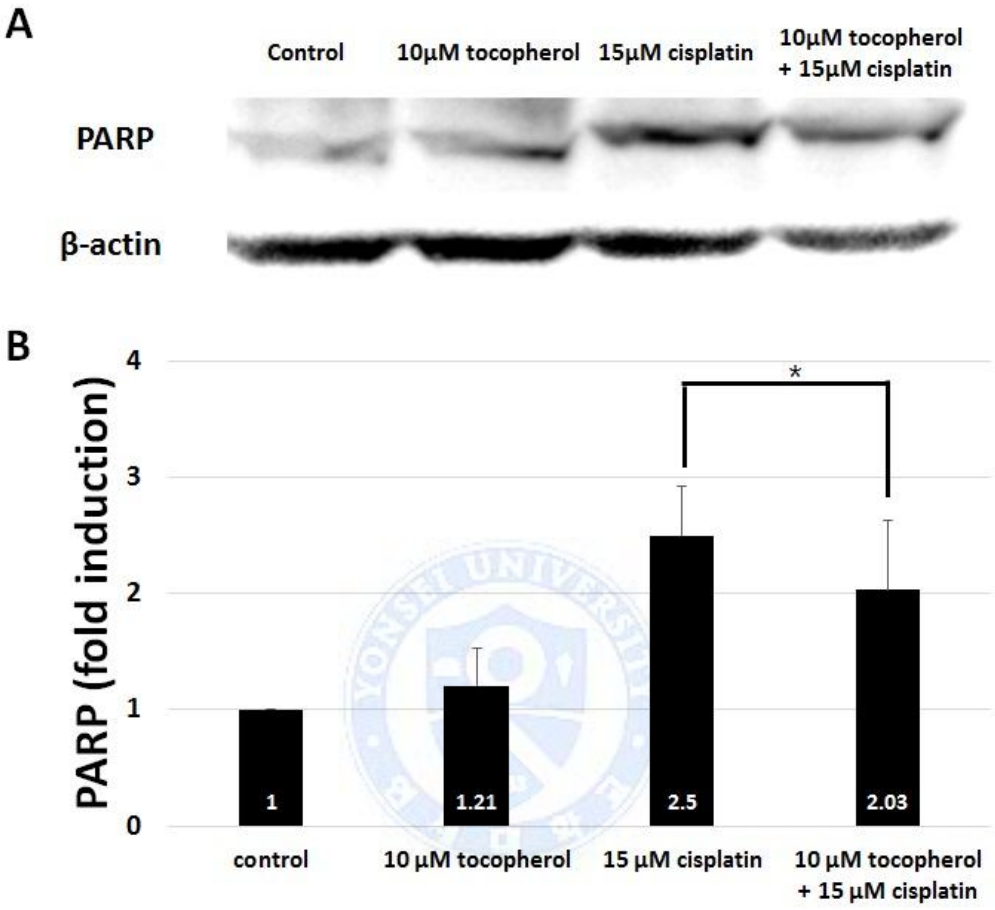

Figure 6. Immunoblotting of PARP. In the cisplatin treated group, increased cleavage of PARP was observed (2.42 ± 1.84 fold over the normal control). The pretreatment of HEI-OC1 cells with 10μM tocopherol significantly decreased cleaved PARP (1.89 ± 1.38 fold over the normal control) as compared with cells treated with cisplatin alone (*$p < 0.05$) (A, B).

Abstract

The Effects of the Antioxidant α-Tocopherol on Cisplatin-Induced Ototoxicity in HEI-OC1 Auditory Cells

Objectives: Alpha-tocopherol is a class of methylated phenols, known as fat-soluble antioxidants, and is a different form of vitamin E, which reduces free radicals and acts as an antioxidant. We hypothesized that the antioxidative effect of α-tocopherol could protect against cisplastin-induced cytotoxicity, and thus evaluated its effects on cisplatin-induced ototoxicity in HEI-OC1 auditory cells. **Methods:** HEI-OC1 cells were pretreated with α-tocopherol at a concentration of 10 μM for 24 h, and then exposed to 15 μM cisplatin for 48 h. The cellular viability was measured by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. The intracellular reactive oxygen species (ROS) level was measured by using a fluorescent dye, 2′,7′-dichlorofluorescein diacetate (DCFH-DA). Both Annexin V-FITC and propidium iodide (PI) staining were performed to analyze the pattern of apoptosis. The enzymatic activity of caspase-3 was assayed with caspase3/CPP32 fluorometric assay kit. Also, it was assessed by immunoblotting technique of poly-ADP-ribose polymerase (PARP). **Results:** Pretreatment with 10 μM α-tocopherol protected HEI-OC1 auditory cells against cisplatin-induced cytotoxicity. Alpha-tocopherol significantly reduced the cisplatin-induced increase in ROS. Alpha-tocopherol treatment induced a 15% reduction of ROS and 50% decrease in necrosis and late apoptosis as compared to cisplatin treatment. Alpha-tocopherol also decreased the activation of caspase-3 and reduced levels of PARP. **Conclusion:** This study was investigated the protective effects of α-tocopherol on cisplatin-induced ototoxicity in an auditory cell line. Tocopherol significantly reduced a cisplatin-induced increase in

ROS, thereby inhibiting cisplatin-induced cytotoxicity.

Keywords: Alpha-tocopherol; Cisplatin; Reactive oxygen species; Ototoxicity; Antioxidants.