Effect of Ecklonia Cava Polyphenol Extract in House Ear Institute-Organ of Corti 1 Cells Against Cisplatin Ototoxicity: A Preliminary Study

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Original Investigation

Abstract

Objective: Cisplatin is a widely used agent for the treatment of adult and childhood malignancies. Side effects such as nephrotoxicity, neurotoxicity, and ototoxicity lead to dose limitations. Ecklonia cava polyphenol extract (ECP) is a molecule obtained from algae that live in seawater in the Far East. ECP has recently been shown to have protective effects against oxidative stress. The aim of this study was to evaluate the possible protective effects of ECP on cisplatin ototoxicity.

Methods: In this study, we investigated the protective effects of ECP against cisplatin-induced cell death in mouse-derived House Ear Institute Organ of Corti (HEI-OC1) cochlear cells. Cisplatin (100 µM) and 1, 10, and 25 µM doses of ECP were administered to the cells, and the protective effects of ECP at 24 and

72 hours were investigated. Cell viability was evaluated by the WST-1 (water soluble tetrazolium salt).

Results: Cisplatin (100 µM) reduced cell viability in both the 24th and 72nd hour evaluation. Although the 25 µM dose of ECP showed otoprotective effects in the 24th hour, in the 72nd hour this effect disappeared. Other doses of ECP showed no otoprotective effects in the 24th and 72nd hours.

Conclusion: Although ECP showed some protective effects in the 24th hour against cisplatin ototoxicity, these effects disappeared by the 72nd hour. Further studies using recurrent and higher doses of ECP are required.

Keywords: Cisplatin, ototoxicity, ecklonia cava polyphenol extract (ECP), cochlear cell

Introduction

Cisplatin is an antineoplastic agent widely used in the treatment of childhood and adulthood cancers. This drug initiates apoptosis and cell death processes by inducing the formation of free oxygen and nitrogen radicals. Ototoxicity, nephrotoxicity, and neurotoxicity, which emerge as a result of oxidative stress-dependent mechanisms, are the most important side effects of cisplatin treatment (1, 2). Cisplatin ototoxicity emerges as symmetrical hearing loss in which particularly high frequencies are irreversibly affected. Many antioxidant agents such as sodium thiosulfate, N-acetylcysteine, acetyl-L-carnitine, Korean red ginseng, and resveratrol have been tested in vitro and in vivo in order to prevent this irreversible side effect (3-6).

Ecklonia cava polyphenol (ECP) extract is a polyphenol compound obtained from brown algae living in the seas of the Far East. It has been shown, in many studies, that this compound has antidia-

betic, antimicrobial, and anti-inflammatory effects in addition to antioxidant properties. It has been indicated that its antioxidant effects are induced by reducing particularly reactive oxygen radicals and by increasing the levels of enzymes such as catalase and glutathione peroxidase (7-9).

In this study, it was planned to investigate the possible protective effects of ECP against cisplatin ototoxicity in the cell culture of House Ear Institute Organ of Corti (HEI-OC1).

Methods

House Ear Institute Organ of Corti (House Ear Institute, Los Angeles, USA) is a cell culture obtained from immortal mouse cochlear cells. Cisplatin (Cisplatin-Ebewe® 50 mg/100 mL, Liba, Unterach, Austria) was diluted in a cell culture medium after the dose was determined. ECP (Seapolynol®, Botamedi Inc., Seoul, the Republic of Korea) was prepared fresh at the doses of 1, 10, and 25 µM.

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HEI-OC1 cells were used after being produced in a 33°C Dulbecco's modified Eagle's medium (DMEM) containing 10% CO₂, humidity, 10% fetal bovine serum, and 1% L-glutamine. HEI-OC1 cells were embedded in 96-well plates. For cell viability, cisplatin and ECP agents were added to the cell culture after 24 h when the cells became a mixture at the rate of 60–70%. The cisplatin dose was determined to be 100 µM.

The study groups were formed as follows:

Group 1: Control group

Group 2: The group to which 1 µM ECP was given

Group 3: The group to which 10 µM ECP was given

Group 4: The group to which 25 µM ECP was given

Group 5: The group to which 100 µM cisplatin was given

Group 6: The group to which a combination of 100 µM cisplatin and 1 µM ECP was given

Group 7: The group to which a combination of 100 µM cisplatin and 10 µM ECP was given

Group 8: The group to which a combination of 100 µM cisplatin and 25 µM ECP was given

Here, 24 h and 72 h after the administration of the agents, 10 µl of WST-1 (water-soluble tetrazolium salt) (Roche, Mannheim, Germany) solution was applied to each well, and they were kept in an incubator with 10% CO₂ at 33°C for 2 h. Subsequently, the absorbance of the removed cells was read at the 450 nm/630 nm wavelengths in an ELISA plate reader (Thermo), and the cell viability was determined. Control cell viability was taken as 100%, and the cell viability changes produced by the agents were expressed as a percentage.

The obtained data were evaluated using Statistical Package for the Social Sciences (SPSS Inc.; version 16.0, Chicago, USA), the Mann-Whitney U-test was used for statistical evaluation, and $p < 0.05$ was considered significant.

This study was carried out in accordance with the Declaration of Helsinki in the Basic Oncology Laboratories of the Dokuz Eylul University, Institute of Oncology.

Results

In the 24th hour of the analysis of the agents applied to the HEI-OC1 cell culture, it was observed that the cell viabilities reduced in Groups 2 and 3 (89.4% and 84.2%, respectively), and the cell viability was seen as 100% in Group 4 (Figure 1). With regard to Group 5, the cell viability decreased significantly (68.4%) as compared to Group 1 ($p < 0.05$). When ECP was administered together with cisplatin, the cell viability changed depending on the ECP dose; the dose in which the cell viability was the best preserved was determined as 25 µM (89.5%), and this value was found to be statistically significant ($p < 0.05$).

Cell viability with regard to cisplatin administration was found to be about 48.3% at the end of 72 h. Although the cell viability was higher in Group 8 than Group 5 (52.7%), this difference was not statistically significant ($p > 0.05$) (Figure 2)

Figure 1. Effect of cisplatin and ECP administration on cell viability in HEI-OC1 cell cultures (24th hour)

Figure 2. Effect of cisplatin and ECP administration on cell viability in HEI-OC1 cell cultures (72nd hour)

Discussion

In addition to nephrotoxic and neurotoxic side effects that are included in the treatment protocol of many adult and childhood malignancies, cisplatin is an antineoplastic agent that is known to cause ototoxicity. The lack of hearing due to ototoxicity deteriorates the life quality by negatively affecting the expressive language, particularly in young children. Caspase-dependent and caspase-independent apoptosis processes begin as this agent causes the overexpression of free oxygen radicals in the inner ear, which is because the cochlear antioxidant enzymes cannot neutralize these radicals. This process mainly affects the inner hair cells, stria vascularis, and spiral ganglion neurons. Although many active agents such as N-acetyl cys-

teine, acetyl-L-carnitine, resveratrol, and Korean red ginseng can prevent the ototoxic effect of cisplatin, there is still no agent accepted by the American Food and Drug Association (3-6, 10, 11).

It is reported that 8.8'-bieckol, 6.6'-bieckol, 7-phloroeckol, phlorofurofueckol A, eckol, and 2-phloroeckoli, which offer the antioxidant, anti-inflammatory, and anti-allergic properties of ECP extracts, carry many different phlorotannin molecules such as phlorotannin A (12-14). Polyphenols are electron-rich compounds and can inhibit the formation of oxidative radicals by facilitating electron donor reactions (15). These phlorotannin compounds have been shown to increase myeloperoxidase and glutathione levels in in vitro experiments (13).

Considering the ototoxicity mechanisms created by cisplatin, ECP was used at different doses in this study carried out in order to demonstrate the possible protective effects of ECP. While 1 and 10 μM ECP administered along with cisplatin did not increase the cell viability at a statistically significant level at the 24th hour, it was observed to increase the cell viability at a dose of 25 μM ECP. However, no protective effect on the cell viability against cisplatin toxicity was observed at the 72nd hour even at the dose of 25 μM ECP.

In a study conducted in vivo and in vitro that investigated the interaction of cisplatin with ECP with regard to anticancer and toxic effects, it was found that ECP improved cisplatin-induced nephrotoxicity and cisplatin enhanced the tumor inhibitory effect. While the formation of free radicals in healthy cells decreased, it was contrarily seen that free oxygen radicals increased and apoptosis was triggered in cancerous cells (16). Similarly, the antiproliferative effect of ECP components in breast cancer cell culture has been demonstrated (17).

It has been shown in a study of Chang et al. (18) that dieckol an ECP component, plays a protective role against gentamicin-induced cell damage in cochlear cell culture and continues its antimicrobial action at the same time. Dieckol has been shown to prevent the formation of oxygen radicals by inhibiting proinflammatory enzymes such as nitric oxide synthase and cyclooxygenase-2 while inducing antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (19, 20). In another study, Chang et al. (21) demonstrated audiotologically and histologically that the application of ECP intraperitoneally protected the inner ear against acoustic trauma. In this study, 5 consecutive ECP applications were performed before high-volume exposure. Although PGF2a induced by acoustic trauma leads to ischemia, it causes glutamate release in the inner hair cells and, consequently, loss of function in neurons. In this study, it has been suggested that the polyphenol compound inhibits this effect and prevents neuronal loss and, therefore, hearing loss (21). In our study, different from the others, we found that while HEI-OC1 increased the cell viability in vitro in cochlear cells at a 25 μM dose of ECP at the 24th hour, it did not show this

possible protective effect at the 72nd hour. This data suggests that repeated high doses of ECP, rather than single doses, may be beneficial to reduce the detrimental effects of cisplatin on the cell viability.

In an in vitro study of murine hippocampus cell culture, it has been suggested that ECP protects neuronal structures against the toxic effect of hydrogen peroxide (22). Free oxygen radicals formed by the effect of hydrogen peroxidase disrupt the structure of the cell membrane by rapidly inducing lipid peroxidation in the cells of the brain and provide benefits of cell stabilization by preventing the lipid peroxidation of phlorotannins. However, by ensuring intracellular Ca^{2+} remains at its normal levels, these compounds also inhibit the initiation of proapoptotic processes increasing with the effect of free oxygen radicals (22). The formation of intracellular mitochondrial dysfunction after ischemia or oxidative stress disrupts intracellular Ca^{2+} regulation, which increases protease activity that initiates cell destruction. In an in vivo and in vitro study, it was reported that ECP compensated the cell Ca^{2+} levels against ischemia or hydrogen peroxide damage and, hence, prevented cell damage (23). High energy requirement, particularly for neurons, makes these cells more susceptible to oxidative stress. ECP provides a neuroprotective effect by activating the antioxidant systems and contributing to homeostasis (22, 23). Dieckol, an ECP component, has been suggested to provide neuroprotective effect by providing microglial suppression (24).

Many of the studies that demonstrate the antioxidant effect of ECP extract compounds have been carried out in order to present their protective effect against radioactivity (25-29). In the prevention of cell damage caused by radiotherapy-induced oxidative stress, polyphenol compounds have been shown to exert antioxidant effects directly by reacting with oxygen radicals or indirectly by activating enzyme systems such as catalase, superoxide dismutase, and myeloperoxidase (15, 28-30). It has been suggested that eckol, an ECP component, inhibits apoptosis in the cells against radiation damage by suppressing the proapoptotic p53 and Bax genes and by inducing the antiapoptotic Bcl-2 gene (30).

This study is the first in vitro study to investigate the possible protective effects of ECP against cisplatin ototoxicity. The fact that ECP was not tested in higher and repeated doses and not evaluated in terms of possible protective mechanisms are the weaknesses of our study. Different design studies to be carried out in the future can answer these questions.

Conclusion

In this study, which was performed in HEI-OC1 cell cultures, although the cell viability decreased due to cisplatin administration, there was no auto-protective effect with ECP administration at the doses studied. The possible auto-protective effects of ECP against cisplatin ototoxicity should be investigated in other in vivo and in vitro studies with higher and repeated doses.

Ethics Committee Approval: Authors declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", (amended in October 2013).

Informed Consent: Not required in this study.

Peer-review: Externally peer-reviewed.

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References

- Gunes D, Kırkım G, Demiral P, Mutafoğlu K, Serbetcioğlu B, Olgun N. Platinum-induced ototoxicity in children and adolescents with cancer. *J Int Adv Otol* 2009; 5: 345-55.
- Rybak LP. Mechanisms of cisplatin ototoxicity and progress in otoprotection. *Curr Opin Otolaryngol Head Neck Surg* 2007; 15: 364-9. [CrossRef]
- Olgun Y. Cisplatin Ototoxicity: Where we are? *J Int Adv Otol* 2013; 9: 403-16.
- Altun Z, Olgun Y, Ercetin P, Aktaş S, Kırkım G, Şerbetcioğlu B, et al. Protective effect of acetyl-L carnitine against cisplatin ototoxicity: role of apoptosis-related genes and pro-inflammatory cytokines. *Cell Prolif* 2014; 47: 72-80. [CrossRef]
- Olgun Y, Kırkım G, Altun Z, Aktaş S, Kolatan E, Kiray M, et al. Protective effect of Korean red ginseng on cisplatin ototoxicity: Is it effective enough? *J Int Adv Otol* 2016; 12: 177-83. [CrossRef]
- Im GJ, Chang JW, Choi J, Chae SW, Ko EJ, Jung HH. Protective effect of Korean red ginseng extract on cisplatin ototoxicity in HEI-OC1 auditory cells. *Phytother Res* 2010; 24: 614-21.
- Kang K, Park Y, Hwang HJ, Kim SH, Lee JG, Shin HC. Antioxidative properties of brown algae polyphenolics and their perspectives as chemopreventive agents against vascular risk factors. *Arch Pharm Res* 2003; 26: 286-93. [CrossRef]
- Li Y, Qian ZJ, Ryu BM, Lee SH, Kim MM, Kim SK. Chemical components and its antioxidant properties in vitro: An edible marine brown alga, *Ecklonia cava*. *Bioorg Med Chem* 2009; 17: 1963-73. [CrossRef]
- Kim SK, Lee DY, Jung WK, Kim JH, Choi I, Park SG, et al. Effects of *Ecklonia cava* ethanolic extracts on airway hyperresponsiveness and inflammation in a murine asthma model: role of suppressor of cytokine signaling. *Biomed Pharmacother* 2008; 62: 289-96. [CrossRef]
- Mukherjee D, Rybak LP. Pharmacogenomics of cisplatin-induced ototoxicity. *Pharmacogenomics* 2011; 12: 1039-50. [CrossRef]
- Olgun Y, Kırkım G, Kolatan E, Kiray M, Bağrıyanık A, Olgun A, et al. Friend or Foe? Oral resveratrol on cisplatin ototoxicity. *Laryngoscope* 2014; 124: 760-6. [CrossRef]
- Kang MC, Cha SH, Wijesinghe WA, Kang SM, Lee SH, Kim EA, et al. Protective effect of marine algae phlorotannins against AAPH-induced oxidative stress in zebrafish embryo. *Food Chem* 2013; 138: 950-5. [CrossRef]
- Li Y, Lee SH, Le QT, Kim MM, Kim SK. Anti-allergic effects of phlorotannins on histamine release via binding inhibition between IgE and Fc epsilonRI. *J Agric Food Chem* 2008; 56: 12073-80. [CrossRef]
- Singh IP, Bharate SB. Phloroglucinol compounds of natural origin. *Nat Prod Rep* 2006; 23: 558-91. [CrossRef]
- Kang KA, Lee KH, Chae S, Zhang R, Jung MS, Lee Y, et al. Eckol isolated from *Ecklonia cava* attenuates oxidative stress induced cell damage in lung fibroblast cells. *FEBS Lett* 2005; 579: 6295-304. [CrossRef]
- Yang YI, Ahn JH, Choi YS, Choi JH. Brown algae phlorotannins enhance the tumoricidal effect of cisplatin and ameliorate cisplatin nephrotoxicity. *Gynecol Oncol* 2015; 136: 355-64. [CrossRef]
- Kong CS, Kim JA, Yoon NY, Kim SK. Induction of apoptosis by phloroglucinol derivative from *Ecklonia Cava* in MCF-7 human breast cancer cells. *Food Chem Toxicol* 2009; 47: 1653-8. [CrossRef]
- Chang MY, Byon SH, Shin HC, Han SE, Kim JY, Byun JY, et al. Protective effects of the seaweed phlorotannin polyphenolic compound dieckol on gentamicin-induced damage in auditory hair cells. *Int J Pediatr Otorhinolaryngol* 2016; 83: 31-6. [CrossRef]
- Kang MC, Kang SM, Ahn G, Kim KN, Kang N, Samarakoon KW, et al. Protective effect of a marine polyphenol, dieckol against carbon tetrachloride induced acute liver damage in mouse. *Environ Toxicol Pharmacol* 2013; 35: 517-23. [CrossRef]
- Lee SH, Han JS, Heo SJ, Hwang JY, Jeon YJ. Protective effects of dieckol isolated from *Ecklonia cava* against high glucose-induced oxidative stress in human umbilical vein endothelial cells. *Toxicol In Vitro* 2010; 24: 375-81. [CrossRef]
- Chang MY, Han SY, Shin HC, Byun JY, Rah YC, Park MK. Protective effect of a purified polyphenolic extract from *Ecklonia cava* against noise-induced hearing loss: Prevention of temporary threshold shift. *Int J Pediatr Otorhinolaryngol* 2016; 87: 178-84. [CrossRef]
- Kang SM, Cha SH, Ko JY, Kang MC, Kim D, Heo SJ, et al. Neuroprotective effects of phlorotannins isolated from a brown alga, *Ecklonia cava*, against H₂O₂-induced oxidative stress in murine hippocampal HT22 cells. *Environ Toxicol Pharmacol* 2012; 34: 96-105. [CrossRef]
- Kim JH, Lee NS, Jeong YG, Lee JH, Kim EJ, Han SY. Protective efficacy of an *Ecklonia cava* extract used to treat transient focal ischemia of the rat brain. *Anat Cell Biol* 2012; 45: 103-13. [CrossRef]
- Cui Y, Park JY, Wu J, Lee JH, Yang YS, Kang MS, et al. Dieckol attenuates microglia-mediated neuronal cell death via ERK, Akt and NADPH Oxidase-mediated pathways. *Korean J Physiol Pharmacol* 2015; 19: 219-28. [CrossRef]
- Park SJ, Ahn G, Lee NH, Park JW, Jeon YJ, Jee Y. Phloroglucinol (PG) purified from *Ecklonia cava* attenuates radiation-induced apoptosis in blood lymphocytes and splenocytes. *Food Chem Toxicol* 2011; 49: 2236-42. [CrossRef]
- Zhang R, Kang KA, Piao MJ, Ko DO, Wang ZH, Lee IK, et al. Eckol protects V79-4 lung fibroblast cells against γ-ray radiation-induced apoptosis via the scavenging of reactive oxygen species and inhibiting of the c-Jun NH(2)-terminal kinase pathway. *Eur J Pharmacol* 2008; 591: 114-23. [CrossRef]

27. Moon C, Kim SH, Kim JC, Hyun JW, Lee NH, Park JW, et al. Protective effect of phlorotannin components phloroglucinol and eckol on radiation-induced intestinal injury in mice. *Phytother Res* 2008; 22: 238-42. [\[CrossRef\]](#)
28. Park E, Ahn GN, Lee NH, Kim JM, Yun JS, Hyun JW, et al. Radioprotective properties of eckol against ionizing radiation in mice. *FEBS Lett* 2008; 582: 925-30. [\[CrossRef\]](#)
29. Jang J, Ye BR, Heo SJ, Oh C, Kang DH, Kim JH, et al. Photo-oxidative stress by ultraviolet-B radiation and antioxidative defense of eckstolonol in human keratinocytes. *Environ Toxicol Pharmacol* 2012; 34: 926-34. [\[CrossRef\]](#)
30. Park E, Lee NH, Joo HG, Jee Y. Modulation of apoptosis of eckol against ionizing radiation in mice. *Biochem Biophys Res Commun* 2008; 372: 792-7. [\[CrossRef\]](#)