The Journal of International Advanced Otology

J Int Adv Otol 2024; 20(3): 189–195 • DOI: 10.5152/iao.2024.231288

Original Article

The Impact of Oleuropein on Cisplatin-Induced Toxicity in Cochlear Cells in Relation to the Expression of Deoxyribonucleic Acid Damage-Associated Genes

Yüksel Olgun[1], Zekiye Altun[2], Merve Tütüncü[2], Selen Kum Özşengezer[2], Safiye Aktaş[2], Enis Alpin Güneri[1]

[1]Department of Otorhinolaryngology, Dokuz Eylül University School of Medicine, İzmir, Türkiye
[2]Department of Basic Oncology, Dokuz Eylül University Institute of Oncology, İzmir, Türkiye

ORCID iDs of the authors: Y.O. 0000-0003-1769-4224, Z.A. 0000-0002-1558-4534, M.T. 0000-0003-1573-9572, S.K.O. 0000-0002-7068-5979, S.A. 0000-0002-7658-5565, E.A.G. 0000-0003-2592-0463.

Cite this article as: Olgun Y, Altun Z, Tütüncü M, Kum Özşengezer S, Aktaş S, Güneri EA. The impact of oleuropein on cisplatin-induced toxicity in cochlear cells in relation to the expression of deoxyribonucleic acid damage-associated genes. *J Int Adv Otol.* 2024;20(3):189-195.

BACKGROUND: Different organs respond differently to cisplatin (CDDP)-induced toxicity. Oleuropein (OLE) is a natural phenolic antioxidant. The purpose of this study was to determine the potential protective effect of OLE against CDDP-induced ototoxicity by evaluating expression of genes associated with deoxyribonucleic acid (DNA) damage and repair in cochlear cells.

METHODS: House Ear Institute-Organ of Corti 1 (HEI-OC1) cells were treated using CDDP, OLE, and OLE–CDDP. The water-soluble tetrazolium salt assay was used for monitoring cell viability. Deoxyribonucleic acid damage in cells due to the CDDP, OLE, and combination treatments was determined using a flow-cytometric kit. The change in the expression of 84 genes associated with CCDP, OLE, and OLE–CDDP treatments that induced DNA damage was tested using the reverse transcription polymerase chain reaction array. Changes ≥3-fold were considered significant.

RESULTS: House Ear Institute-Organ of Corti 1 cell viability was significantly reduced by CDDP. The OLE–CDDP combination restored the cell viability. Cisplatin increased the H2AX ratio, while OLE–CDDP combination decreased it. Some of the DNA damage-associated genes whose expression was upregulated with CDDP were downregulated with OLE–CDDP, while the expression of genes such as Gadd45g and Rev1 was further downregulated. The expression of DNA repair-related Abl1, Dbd2, Rad52, and Trp53 genes was downregulated with CDDP, whereas their expression was upregulated with OLE–CDDP treatment.

CONCLUSION: In cochlear cells, the OLE–CDDP combination downregulated DNA damage-associated gene expression relative to that upregulated mainly by CDDP. The results revealed that OLE has a potential protective effect on CDDP-induced ototoxicity in cochlear cells by altering the expression of DNA damage-related genes.

KEYWORDS: Cisplatin, cochlear cells, oleuropein, DNA damage-related genes

INTRODUCTION

Cisplatin (CDDP) is the main chemotherapeutic agent that has been widely used in the treatment of many cancer types. The main important side effects of CDDP are ototoxicity, nephrotoxicity, neurotoxicity, and cardiotoxicity.[1-3] Cisplatin's acquired drug resistance and serious side effects limit its use despite its broad anticancer activity. Ototoxic effects of CDDP arise from oxidative damage of the cells, such as deoxyribonucleic acid (DNA) damage and lipid peroxidation.[4] Sensorineural hearing loss is one of the major irreversible side effects, occurring in 50%-70% of patients receiving CDDP.[5] Sensorineural hearing loss due to CDDP-induced ototoxicity causes problems in education and learning. Therefore, the mechanisms of CDDP-induced ototoxicity and sensorineural hearing loss should be understood, and possible protective agents should be evaluated.

Cisplatin treatment utilizes several repair pathways, including the nucleotide excision repair pathway, in cells to sense and repair CDDP-induced DNA adducts.[6] Through the assessment of tissue-specific transcriptomic and epigenomic profiling, a recent study revealed that CDDP shows a distinct spectrum of damage and repair in different organs, including kidney, liver, lung, and spleen.[6,7]

Corresponding author: Zekiye Altun, e-mail: zekiye.altun@deu.edu.tr, zekiyesaltun@gmail.com

Received: July 21, 2023 • **Revision requested:** August 31, 2023 • **Last revision received:** October 26, 2023 • **Accepted:** November 3, 2023 • **Publication Date:** May 23, 2024

Available online at www.advancedotology.org

On the other hand, in terms of *in-vivo* experimental organ damage, it is quite difficult to conduct studies that could reflect ototoxicity in the organ of corti cells. The House Ear Institute-Organ of Corti 1 (HEI-OC1) cell is very suitable for studying *in-vitro* ototoxicity models. From this point of view, studying the mechanisms behind DNA damage and repair could be interest in the future by increasing CDDP efficacy while reducing its side effects in important tissues such as the cochlea.

Oleuropein (OLE) is extracted from olive leaves and components of olive oil.[8] OLE has some antioxidant and anti-inflammatory activities against different kinds of cytotoxic drugs like CDDP, disorders, stroke, and radiation-induced cytotoxicity.[9,10] Oleuropein has shown protective effects against oxidative stress caused by hydrogen peroxide, which can occur in many conditions in the human body.[11,12] However, to date, the potential protective effect of OLE on CDDP-induced ototoxicity, with an emphasis on the changes in the expression of DNA damage- and repair-related genes, has not been studied. The aim of this study was to determine *in-vitro* whether OLE has a protective effect on CDDP-induced toxicity in relation to the expression of genes involved in DNA damage and repair in cochlear cells.

MATERIAL AND METHODS

Ethical approval of this study was obtained from Dokuz Eylul University Non-interventional Studies Ethics Committee (Approval number: 2019704-40, Date: February 20, 2019).

Cell Culture

The HEI-OC1 cells were kindly provided by Professor Kalinec. Cells were grown with 1% L-glutamine and 10% fetal bovine serum added to high-glucose DMEM (Gibco) at 33˚C incubator conditions.[13] The cells were grown in different quantities depending on the type of analysis. Oleuropein and CDDP were purchased from Sigma-Aldrich.

Assessment of Cell Viability

Cell viability was determined using the WST-1 (Roche, Germany) assay after treatment of cells with OLE, CDDP, and OLE–CDDP.[12] The HEI-OC1 cells were seeded at 1×10^4 cells/well. After overnight incubation, the cells were treated with OLE, CDDP, and OLE–CDDP for an additional 24 hours of incubation. The cells were then treated with 10 µL WST-1 solution and incubated for another 2 hours. After the incubation, the absorbances of the cells were read at 450/630 nm using an ELISA plate reader (Thermo Fisher Scientific, Boston, Mass, USA). Control cell absorbances were used as 100% viable cells. The viability of other OLE, CDDP, and OLE–CDDP-treated cells was calculated based on the absorbance of control cells. The cells were treated at least 3 times, and 6 replicates were used in cell viability experiments.

MAIN POINTS

- Prevention of cisplatin (CDDP)-induced ototoxicity remains controversial and needs further investigation.
- The expression of deoxyribonucleic acid (DNA) damage-related genes was found to be increased by CDDP, whi the oleuropein–cisplatin combination treatment decreased the expression of DNA-damage-related genes.
- Oleuropein has a potential protective effect against CDDP-induced ototoxicity by altering gene expressions associated with DNA damage and repair.

Evaluation of Deoxyribonucleic Acid Damage by H2AX

The histone H2AX is a member of the H2A protein family. The histone H2AX is phosphorylated at DNA strand breaks by ataxia telangiectasia mutated (ATM), ATM-Rad-3-associated (ATR), DNA protein kinase, and potentially other proteins.[7] DNA damage occurring in cells, particularly in relation to phosphorylated H2AX, was assessed using a flow-cytometric kit (BD, Roche Diagnostics GmbH, Mannheim, Germany). Cell samples were fixed and permeabilized. Following the use of BrdU, cells were treated with DNase. Deoxyribonuclease application aids in revealing BrdU epitopes. After the DNase treatment, the cells were simultaneously stained with fluorochrome-labeled anti-BrdU, cleaved PARP, and H2AX. Staining buffer was used for resuspension of cells. The cells were analyzed by flow cytometry (BD Accuri C6, Becton Dickinson, Franklin Lakes, NJ, USA).

Reverse Transcription Polymerase Chain Reaction Analysis

After a 24-hour incubation of HEI-OC1 cells with the agents, cells were harvested for reverse transcription polymerase chain reaction (RT-PCR) analysis. Gene expressions of DNA damage-related genes (n = 84 genes) were tested using a mouse RT-PCR array (Bio-Rad Laboratories,Hercules, California, USA) after RNA isolation and complementary DNA synthesis (Table 1).[13] Isolation of RNA was done using the RNA isolation kit (Qiagen). The amount and purity of RNA were both determined by absorbance measurement with a 260/A280 ratio of 1.8 : 2.1. Complementary DNA synthesis was carried out with the RT2 First Strand Kit (Qiagen) using a conventional thermocycler (NYX TECNIC Technical Cyclers, USA). Analysis of gene expression was performed in 96-well plates using the RT-PCR (LightCycler® 96, Roche, Germany). The PCR cycling conditions are as follows: one cycle at 95°C for 10 minutes, followed by 45 cycles at 95°C for 15 seconds and 65°C for 1 minute. Detection was performed using SYBR Green fluorescence. The DNA damage-related gene expressions were stated as fold changes relative to the control group. In this study, >3-fold changes were considered significant when evaluating gene expression.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 15.0 for Windows (SPSS Inc., Chicago, Ill, USA) and GraphPad Prism 9 program, and $P < .05$ were considered statistically significant. The nonparametric Mann–Whitney *U*-test and chi-square tests were also used. To obtain statistically applicable data, all experiments were repeated for a minimum of 3 times.

RESULTS

Viability of Cells

The viability of HEI-OC1 cells was significantly inhibited by the CDDP treatment, whereas it was slightly inhibited by the OLE treatment compared to the control group at 24-hour incubation ($P = .039$; $P = .059$) (Figure 1). The OLE–CDDP combination therapy significantly improved the reduced cell viability induced by CDDP, while the OLE–CDDP combination also slightly reduced cell viability in relation to the control group ($P = .045$, $P = .057$).

Table 1. The List of DNA Repair and DNA Damage-Related Genes

DNA Repair-Related Genes	DNA Damage-Related Genes
Abl1, Apex1, Atm, Blm, Brca1, Brca2, Chek1, Ddb2, Dclre1a, Ercc1, Ercc2 (Xpd), Exo1, Fancc, Fen1, H2afx, Hus1, Lig1, Mbd4, Mdc1, Mlh1, Mlh3, Mpg, Mre11a, Msh2, Msh3, Nbn (Nbs1), Nthl1, Ogg1, Parp1 (Adprt1), Parp2, Pcna, Pms2, Pole, Prkdc, Rad50, Rad51, Rad52, Rpa1, Trp53, Trp53bp1, Ung, Xpa, Xpc, Xrcc1, Xrcc2, Xrcc6 (G22p1), Wrn	Abl1, Atm, Atr, Atrx, Bax, Brca1, Brip1, Cdc25a, Cdc25c, Cdkn1a (p21Cip1/Waf1), Chek1, Chek2 (Rad53), Ddit3 (Gadd153/Chop), Fanca, Fancd2, Fancg, Gadd45a, Gadd45g, H2afx, Hus1, Mcph1, Mdc1, Mgmt (Agt), Mif, Parp1 (Adprt1), Parp2, Polh, Poli, Ppm1d, Ppp1r15a (Gadd34), Prkdc, Pttg1, Rad1, Rad9a, Rad17, Rad18, Rad21, Rad50, Rad51c, Rad51l1, Rev1, Rnf8, Smc1a, Smc3, Sumo1, Terf1, Topbp1, Trp53, Xrcc3

DNA Damage Results from H2AX Phosphorylation

Cisplatin increased the ratio of phosphorylated H2AX compared to the control cells ($P = .032$) (Figure 2). The OLE–CDDP combination treatment significantly reduced the increased H2AX levels compared to CCDP-treated cells ($P = .037$).

DNA Damage- and Repair-Related Gene Expressions

Cisplatin upregulated the expression of mainly H2afx, Mpg, Rad17, Rnf8, Xpa, and Xpc genes, while the expression of Abl1, Brca2, Ddb2, Ddit3, Gadd45g, Poli, Prkdc, Rad51b, Rev1, Smc1a, Terf1, Trp53, Trp53, and Trp5 genes was downregulated compared to the control group (Figure 3).

The expression of Chek1, Mpg, Prkdc, Rad1, Rad17, Rad18, Rad50, Rad51b, Rnf8, Xpa, and Xpc genes was found to increase in the OLE group. The expression of Bax, Ddit3, Hus1, Lig1, Mlh1, Nbn, Ogg1, Ppp1r15a, Rad52, Rev1, Smc1a, Ung, and Wrn genes was down-regulated in the OLE group compared with the control group cells (Figure 3).

The OLE–CDDP combination treatment upregulated the expression of Mpg, Rad17, and Xpa genes, while the expression of Atr, Cdc25c, Ercc2, Fen1, Gadd45a, Gadd45g, Mdc1, Mgmt, Mif, Nbn, Parp1, Pcna, Ppm1d, Pttg1, Rad18, Rad21, Rev1, Smc1a, Trp53, Trp53bp1, Ung, and Xrcc3 genes was downregulated compared to the control group (Figure 3).

Damage-associated genes of Cdc25c, Check1, Gadd45a, H2afx, Hus1, Mgmt, Mif, Ppm1d, Pttg1, Rad18, Rad21, Rev1, and Reno8 were up-regulated by cisplatin and down-regulated by OLE-CDDP induction. Gadd45g and Rev1 were further down-regulated.

The expression of Abl1, Dbd2, Rad52, and Trp53 genes associated with DNA repair was downregulated in the CDDP treatment group, while the expression of the same genes was upregulated in the OLE–CDDP combination treatment group. The upregulation of DNA repair-associated Fen1, Mlh1, Mpg, Nbn, Pcna, Trp53bp1, and Xpa genes induced by CDDP was reduced by the OLE–CDDP treatment (Figure 3).

DISCUSSION

Cisplatin is the main chemotherapeutic drug extensively used in several adult cancers, such as lung cancer, ovarian cancer, and childhood cancers, such as neuroblastoma.[4] Unfortunately, CDDP can cause dose-dependent cytotoxicity, especially in nerves, ears, and renal

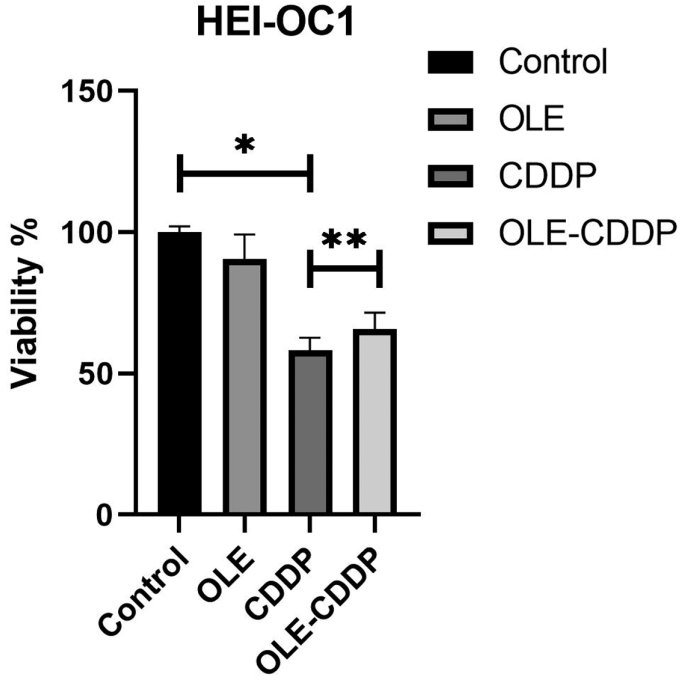

Figure 1. Cell viability assessment by WST-1 assay. CDDP significantly inhibited cell viability according to control (*$P = .039$). OLE–CDDP combination slightly increased cell viability when compared to CDDP treatment (**$P = .045$). OLE, oleuropein; CDDP, cisplatin; HEI-OC1, House Ear Institute-Organ of Corti 1.

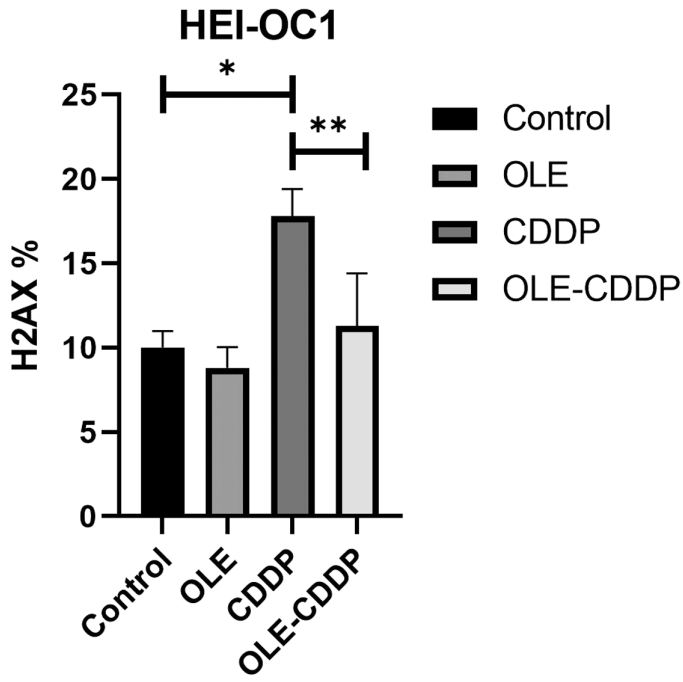

Figure 2. DNA damage assessment by H2AX phosphorylation ratio. CDDP increased the ratio of H2AX (*$P = .032$). OLE–CDDP combination treatment decreased the H2AX levels when compared to CCDP-treated cells (**$P = .037$). OLE, oleuropein; CDDP, cisplatin; HEI-OC1, House Ear Institute-Organ of Corti 1.

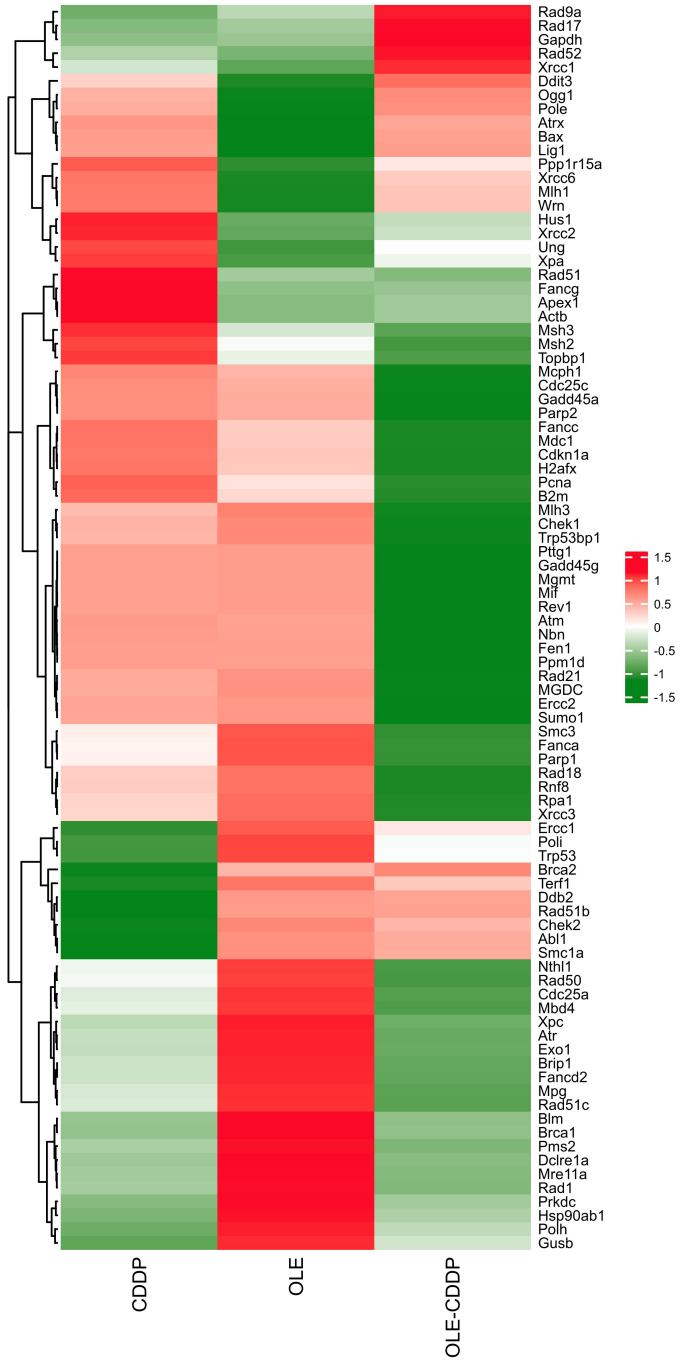

Figure 3. Heat map representation of differentially expressed genes belonging to DNA damage and repair processes induced by OLE, CDDP, and OLE-CCDP. The green color signifies lower expression, while the red color signifies higher expression. OLE, oleuropein; CDDP, cisplatin.

cells. The ototoxicity of CDDP may lead to permeant sensorineural hearing loss and dose limitations for cancer treatments in children.

To date, some possible protective agents, including sodium thiosulfate, acetyl-L-carnitine, resveratrol, Korean red ginseng, and *N*-acetylcysteine, have been tested and mechanisms determined against CDDP-induced sensorineural hearing loss *in-vitro* and *in-vivo*.[14-17] However, there is still a need to find new agents against CDDP-induced ototoxicity. In addition, knowledge about CDDP-induced ototoxicity should be reevaluated by researchers. Therefore, in this study, we examined the mechanism of CDDP use on DNA damage by analyzing changes in gene expression. Furthermore, OLE was examined against CDDP-induced cochlear DNA damage and related gene expressions for the first time.

The mechanisms of CDDP-associated hearing loss have been described to include oxidative stress, DNA damage, inflammation, and induction of cell death pathways such as apoptosis, necroptosis, and ferroptosis.[14,15,18,19] Therefore, there are many possible protective targets in the cochlear cells.[16] First, CDDP enters cochlear cells using some channels in the membrane, such as mechanoelectrical transduction, transient receptor potential of membrane, and organic cation transporters. After entry of CDDP into cells, CDDP induces apoptosis by upregulation of ATM and p53 activation due to DNA damage of cochlear cells. This leads to increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS), lipid peroxidation, and decrease in antioxidant enzymes. Furthermore, CDDP can increase calcium influx into cells and the expression of NADPH oxidase isoform 3. Cisplatin-mediated increase in RNS and ROS leads to induction of STAT1 and JNK pathways and increased nitric oxide production.[16] It has also been recently reported that decreased mechanistic target of rapamycin complex 2/AKT(PKB, protein kinase B) signaling pathway activation is associated with CDDP-induced ototoxicity and acoustic trauma.[20] Depending on the mechanisms of CDDP-induced ototoxicity, possible protective agents that can overcome these mechanisms are still being identified.

Oleuropein has been partially protected from noise-induced hearing damage in experimental noise-induced hearing loss in rats.[23] One study examined the hematological damage of CDDP and the role of OLE in Sprague–Dawley rats and revealed that the raised total oxidant stress and 8-hydroxy-deoxyguanine adducts were relieved by OLE, like the findings of this study. Furthermore, another study has shown that OLE has a potential preventive cytoprotective effect on CDDP-associated renal toxicity.[24] In this study, it has been shown for the first time that OLE alone contributes to DNA damage-related gene expression changes in corti cells such as Chek1, Mpg, Prkdc, Rad1, Rad17, Rad18, Rad50, Rad51b, Xpa, and Xpc associated with double-strand break (DSB) repair response and ATM/ATR signaling. On the other hand, the expression of most of the DNA damage-related genes such as Bax, Ddit3, Hus1, Lig1, Mlh1, Nbn, Ogg1, Ppp1r15a, Rad52, Rev1, Rnf8, Smc1a, Ung, and Wrn was downregulated with OLE in cochlear cells.

In this study, CDDP inhibited cell viability, while the OLE combination relieved this inhibition in HEI-OC1 cells. In addition, DNA damage was induced by the CDDP treatment by increasing H2AX phosphorylation ratios.[7] The CDDP–OLE combination reduced the increased DNA damage caused by the CDDP treatment in HEI-OC1 cells. In a study of comparing the possible protective effects of OLE and hydroxytyrosol on H_2O_2-induced damage in human lymphocytes, the results, which were compatible with this study, showed that hydroxytyrosol had a better protective effect.[24] In trophoblast cells, OLE also attenuated oxidative stress induced by hydrogen peroxide exposure.[8]

Few studies examined some DNA damage- or repair-related genes to understand the ototoxic effect of CDDP both *in-vitro* and *in-vivo*.[24,25] Turan et al[24] did not find any association between CDDP-induced ototoxicity and Ercc1, Ercc2, and Xrcc1 gene polymorphisms in

children. In this study, Ercc1 gene expression was downregulated with all agents, but Ercc2 gene expression was more downregulated with OLE–CDDP treatment. Msh3 gene polymorphism related to the mismatch repair pathway has been proposed as a chemoradiation therapy toxicity response-related gene polymorphism in head and neck squamous cell carcinoma patients.[26] In this study, Msh3 gene expression was reduced in both OLE and OLE–CDDP treatments in HEI-OC1 cells. Oleuropein may act as an anti-toxicity agent associated with CDDP by regulating the mismatch repair gene expression.

In this study, the expression of DNA damage- and DNA repair-related genes in relation to CDDP, OLE, and their combinations was studied for the first time in the organ of Corti cells. The upregulation of DNA repair-associated Fen1, Mlh1, Mpg, Nbn, Trp53bp1, Xpa, and Xpc genes induced by CDDP was reduced by the OLE–CDDP treatment. CDDP-upregulated damage-associated genes Gadd45a, Gadd45g, H2afx, Mgmt, Ppm1d, Pttg1, Rad21, Rev1, and Reno8 downregulated induction by OLE–CDDP, while Prkdc, Rad17, and Smc1 was further induced.

In particular, H2afx, Mpg, Rad17, Rnf8, Xpa and Xpc gene expression was upregulated by cisplatin, while Abl1, Abl1, Brca2, Ddb2, Ddit3, Gadd45g, Poly, Prkdc, Rad51b, Rev1, Smc1a, Terf1, Trp53, Trp53 and Trp53bp1 gene expression was downregulated compared to the control group. Mpg is the base-excision repair-related gene and upregulated by OLE more than CDDP and OLE–CDDP treatments in this study. The Rad17 gene is also related to Brca1 expression in DNA damage, especially in cancer development.[21,27] The Rad17 and Brca genes encode 2 DNA repair proteins that play major roles in proper cell cycle regulation and the regulation of genomic stability. In cochlear cells, Rad17 expression was most increased in the OLE–CDDP-treated group, while it was less in the CDDP and OLE groups. On the other hand, Brca1 gene expression in the CDDP and OLE–CDDP groups decreased at the same level. Brca2 gene expression was also negatively affected by both CDDP and OLE–CDDP treatments. The fact that Brca1 gene expression, which contributes to genomic integrity and is a vital gene for DNA repair, is downregulated by CDDP and the addition of OLE does not change this downregulation suggests that OLE may not have a regulatory role on the expression of this major DNA repair gene.[22,28]

E3 ubiquitin ligase of RNF8 promotes a DNA damage response. In one study, Rnf8 deletion caused aging of the cochlea, which was associated with apoptosis.[29] In this study, the expression of Rns8 gene was upregulated by CCDP and was reversed by the OLE–CDDP combination treatment. The Rnf8 gene has multiple roles related to the response of DNA damage, such as cell cycle control, telomere protection, and transcriptional regulation. The CDDP-upregulated expression of Rnf8 in HEIOC cells may be associated with the genomic instability response of cells with DSB damage. A combination of CDDP and OLE may aid in the recovery against DNA damage in the cochlear cell. While the combination of OLE and Rev1 was effective in inducing Rev1 expression in corti cells, Rev1 is involved in the error-free bypass of CDDP DNA interstrand cross-links, which allows CDDP to up-regulate the expression of the Rev1 gene.[30] Nucleotide excision repair-related Xpa and Xpc genes may be increased in HEI-OC1 cells due to CDDP sensitivity.[31] On the other hand, the fact that the combination of CDDP and OLE reduced the expression of both Xpa and Xpc genes to some extent in this study may be related to the protective role of OLE in CDDP toxicity.

The CDDP treatment upregulated the expression of DNA damage-associated genes Gadd45a, Gadd45g, H2afx, Ppm1d, Rad21, and Reno8, while the expression of the same genes was downregulated by the OLE–CDDP treatment, while Prkdc and Rad17 were further induced. Gadd45g and Gad45a are DNA repair-related genes, and Gadd45g increased expression due to CDDP and was reduced by the combination of OLE in corti cells. Stimulated gene expression of H2afx also indicates that CDDP upregulated the expression of DNA damage-related genes, resulting in DSBs, while the expression of the same genes was down-regulated by the OLE–CDDP combination.[32] Yimit et al[6] reported that different types of CDDP-induced DNA damage and repair were associated with multiple factors, including transcription and chromatin status in different organ types. Furthermore, in addition to nuclear damage, mitochondria are another target for CDDP. It has been suggested that studying the effect of OLE on mitochondrial damage and repair mechanism may be of interest in the future in the direction of increasing CDDP activity while reducing its side effects in normal tissues.

Mgmt is the O-6-methylguanine DNA methyltransferase that eliminates the alkyl groups from alkylated DNA strands and acts as a DNA repair function against alkylating agents such as CDDP.[33] The combination of CDDP and OLE in HEI-OC1 cells significantly reduced the expression of Mgmt gene compared to CDDP. Ppm1d is one of the p53 target genes, and the expression of oncogenic phosphatases induced by CDDP due to DNA damage was reduced by the OLE–CDDP combination.[34] The Ppm1 gene expression changes in this study suggest that the alteration of Ppm1 associated with nucleotide excision repair and stimulation of platinum drug resistance. Changes in Pttg1 gene expression could also be related to CDDP chemosensitivity and revised by the repair of DNA by the OLE–CDDP combination.[28]

Consistent with the findings of Zhou et al,[35] the up-regulation of Prkdc gene was found to be a DNA damage response to CDDP treatment. In this study, Prkdc gene expression was downregulated in HEI-OC1 cells, which might be related to CDDP sensitivity. Moreover, reducing the expression of this gene through the combination of OLE indicates that it may be effective in the modulation of cochlear toxicity. The expression of Smc1 gene was downregulated in the CDDP treatment group but only to a lesser extent in the OLE–CDDP combination group. This may be related to the coordination of both homologous and nonhomologous end-joining repair processes and interaction with the Rad52 pathway.[36]

Different forms of DNA damage and repair due to CDDP use are linked to many factors, including transcription and chromatin status in various organ types, according to one study.[6] Furthermore, in addition to nuclear damage, mitochondria are another target for CDDP. It has been suggested that studying the mechanism of mitochondrial damage and repair may be of future interest by increasing CDDP efficacy while reducing its side effects in normal tissues.

Antioxidants such as OLE, NAC, ALC, inhibitors of membrane transporter proteins, and inhibitors of different cellular pathways activated by CDDP may have beneficial effects in preventing the toxic effects of CDDP.[14-16] However, it is important that the ideal protective

agents against CDDP-induced toxicity have properties or mechanisms of action that may preserve or not interfere with the antitumoral effect of CDDP.

This study demonstrated for the first time that oleuropein, a major component of olives and olive tree leaves, has a potential protective effect against CDDP-induced ototoxicity in HEI-OC1 cells by altering the expression of genes associated with DNA damage and repair response. The OLE–CDDP combination generally decreased DNA damage-related gene expression in cochlear cells. Further *in-vitro* and *in-vivo* studies should be performed in the future to reveal possible OLE-mediated protective mechanisms of CDDP-induced ototoxicity.

Limitations

It is a deficiency that DNA damage could not be further evaluated in cells by a method such as immunofluorescence microscopic analysis. Deoxyribonucleic acid damage and repair gene expressions could not be confirmed by protein analysis methods such as western blot. Deoxyribonucleic acid damage signaling pathway could not be analyzed in this study.

Ethics Committee Approval: This study was approved by Ethics Committee of Dokuz Eylul University (Approval number: 2019704-40, Date: February 20,2019).

Informed Consent: Informed consent was obtained from the patients who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – Y.O., Z.A., E.A.G.; Design – Y.O., Z.A.; Supervision – Y.O., Z.A., E.A.G., S.A.; Resources – Y.O., Z.A., S.A.; Materials – Y.O., Z.A., S.K.Ö., M.T.; Data Collection and/or Processing – Y.O., Z.A., S.K.Ö., M.T.; Analysis and/or Interpretation – Y.O., Z.A.; Literature Search – Y.O., Z.A.; Writing – Y.O., Z.A., S.A., S.K.Ö., M.T.; Critical Review – Y.O., Z.A., S.A.

Declaration of Interests: The authors have no conflict of interest to declare.

Funding: This study was financially supported by Dokuz Eylül University Scientific Research Projects (2019.KB.SAG.061).

REFERENCES

1. Gunes D, Kirkim G, Kolatan E, et al. Evaluation of the effect of acetyl L-carnitine on experimental cisplatin ototoxicity and neurotoxicity. *Chemotherapy*. 2011;57(3):186-194. [CrossRef]
2. Tufekci O, Gunes D, Ozoğul C, et al. Evaluation of the effect of acetyl L-carnitine on experimental cisplatin nephrotoxicity. *Chemotherapy*. 2009;55(6):451-459. [CrossRef]
3. Bayrak S, Aktaş S, Altun Z, et al. Antioxidant effect of acetyl-l-carnitine against cisplatin-induced cardiotoxicity. *J Int Med Res*. 2020;48(8): 300060520951393. [CrossRef]
4. Altun ZS, Güneş D, Aktaş S, Erbayraktar Z, Olgun N. Protective effects of acetyl-L-carnitine on cisplatin cytotoxicity and oxidative stress in neuroblastoma. *Neurochem Res*. 2010;35(3):437-443 [published correction appears in Erbayrktar, Zübeyde [corrected to Erbayraktar, Zübeyde]. *Neurochem Res*. 2010;35(4):679. [CrossRef]
5. Teitz T, Fang J, Goktug AN, et al. CDK2 inhibitors as candidate therapeutics for cisplatin- and noise-induced hearing loss. *J Exp Med*. 2018;215(4):1187-1203. [CrossRef]
6. Yimit A, Adebali O, Sancar A, Jiang Y. Differential damage and repair of DNA-adducts induced by anti-cancer drug cisplatin across mouse organs. *Nat Commun*. 2019;10(1):309. [CrossRef]
7. Olive PL, Banáth JP. Kinetics of H2AX phosphorylation after exposure to cisplatin. *Cytom B*. 2009;76(2):79-90. [CrossRef]
8. Pirković A, Vilotić A, Borozan S, et al. Oleuropein attenuates oxidative stress in human trophoblast cells. *Antioxidants (Basel)*. 2023;12(1):197. [CrossRef]
9. Geyikoğlu F, Çolak S, Türkez H, et al. Oleuropein ameliorates cisplatin-induced hematological damages via restraining oxidative stress and DNA injury. *Indian J Hematol Blood Transfus*. 2017;33(3):348-354. [CrossRef]
10. Amani F, Allahbakhshian Farsani M, Gholami M, Aghamiri SMR, Bakhshandeh M, Hossein Mohammadi M. The protective effect of oleuropein against radiation-induced cytotoxicity, apoptosis, and genetic damage in cultured human lymphocytes. *Int J Radiat Biol*. 2021;97(2):179-193. [CrossRef]
11. Nousis L, Doulias PT, Aligiannis N, et al. DNA protecting and genotoxic effects of olive oil related components in cells exposed to hydrogen peroxide. *Free Radic Res*. 2005;39(7):787-795. [CrossRef]
12. Zorić N, Kopjar N, Rodriguez JV, Tomić S, Kosalec I. Protective effects of olive oil phenolics oleuropein and hydroxytyrosol against hydrogen peroxide-induced DNA damage in human peripheral lymphocytes. *Acta Pharm*. 2021;71(1):131-141. [CrossRef]
13. Serinan E, Altun Z, Aktaş S, Çeçen E, Olgun N. Comparison of cisplatin with lipoplatin in terms of ototoxicity. *J Int Adv Otol*. 2018;14(2):211-215. [CrossRef]
14. Altun Z, Olgun Y, Ercetin P, et al. Protective effect of acetyl-l-carnitine against cisplatin ototoxicity: role of apoptosis-related genes and pro-inflammatory cytokines. *Cell Prolif*. 2014;47(1):72-80. [CrossRef]
15. Düzenli U, Altun Z, Olgun Y, et al. Role of N-acetyl cysteine and acetyl-l-carnitine combination treatment on DNA-damage-related genes induced by radiation in HEI-OC1 cells. *Int J Radiat Biol*. 2019;95(3):298-306. [CrossRef]
16. Ramkumar V, Mukherjea D, Dhukhwa A, Rybak LP. Oxidative stress and inflammation caused by cisplatin ototoxicity. *Antioxidants (Basel)*. 2021;10(12):1919. [CrossRef]
17. Freyer DR, Brock PR, Chang KW, et al. Prevention of cisplatin-induced ototoxicity in children and adolescents with cancer: A clinical practice guideline. *Lancet Child Adolesc Health*. 2020;4(2):141-150. [CrossRef]
18. Degterev A, Huang Z, Boyce M, et al. Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nat Chem Biol*. 2005;1(2):112-119. [CrossRef]
19. Mei H, Zhao L, Li W, et al. Inhibition of ferroptosis protects House Ear Institute-organ of Corti 1 cells and cochlear hair cells from cisplatin-induced ototoxicity. *J Cell Mol Med*. 2020;24(20):12065-12081. [CrossRef]
20. Fu X, Li P, Zhang L, et al. Activation of Rictor/mTORC2 signaling acts as a pivotal strategy to protect against sensorineural hearing loss. *Proc Natl Acad Sci U S A*. 2022;119(10):e2107357119. [CrossRef] [published correction appears in *Proc Natl Acad Sci U S A*. 2022;119(20):e2204956119. (https://doi.org/10.1073/pnas.2204956119)]
21. Olgun Y, Kırkım G, Kolatan E, et al. Friend or foe? Effect of oral resveratrol on cisplatin ototoxicity. *Laryngoscope*. 2014;124(3):760-766. [CrossRef]
22. Olgun Y, Kırkım G, Altun Z, et al. Protective effect of Korean red ginseng on cisplatin ototoxicity: is it effective enough? *J Int Adv Otol*. 2016;12(2):177-183. [CrossRef]
23. Kümüş Ö, Olgun Y, Mungan Durankaya S, Aktaş S, Kirkim G, Sütay S. Oleuropein effect on noise-induced hearing loss. *J Int Adv Otol*. 2022;18(2):118-124. [CrossRef]
24. Geyikoglu F, Emir M, Colak S, et al. Effect of oleuropein against chemotherapy drug-induced histological changes, oxidative stress, and DNA damages in rat kidney injury. *J Food Drug Anal*. 2017;25(2):447-459. [CrossRef]

25. Turan C, Kantar M, Aktan Ç, et al. Cisplatin ototoxicity in children: risk factors and its relationship with polymorphisms of DNA repair genes ERCC1, ERCC2, and XRCC1. *Cancer Chemother Pharmacol*. 2019;84(6):1333-1338. [CrossRef]
26. Nogueira GAS, Costa EFD, Lopes-Aguiar L, et al. Polymorphisms in DNA mismatch repair pathway genes predict toxicity and response to cisplatin chemoradiation in head and neck squamous cell carcinoma patients. *Oncotarget*. 2018;9(51):29538-29547. [CrossRef]
27. Negrini S, Gorgoulis VG, Halazonetis TD. Genomic instability--an evolving hallmark of cancer. *Nat Rev Mol Cell Biol*. 2010;11(3):220-228. [CrossRef]
28. Hong H, Jin Z, Qian T, et al. Falcarindiol enhances cisplatin chemosensitivity of hepatocellular carcinoma via down-regulating the STAT3-modulated PTTG1 pathway. *Front Pharmacol*. 2021;12:656697. [CrossRef]
29. Li TT, Xu P, Bai ZG, Cui ZT, Cai XG, Zhou B. Expression of RNF8 on cochlear apoptosis and aging in mice of different ages. *J Biol Regul Homeost Agents*. 2019;33(2):543-550.
30. Bezalel-Buch R, Cheun YK, Roy U, Schärer OD, Burgers PM. Bypass of DNA interstrand crosslinks by a Rev1-DNA polymerase ζ complex. *Nucleic Acids Res*. 2020;48(15):8461-8473. [CrossRef]
31. Damia G, Guidi G, D'Incalci M. Expression of genes involved in nucleotide excision repair and sensitivity to cisplatin and melphalan in human cancer cell lines. *Eur J Cancer*. 1998;34(11):1783-1788. [CrossRef]
32. Chen JH, Zhang P, Chen WD, et al. ATM-mediated PTEN phosphorylation promotes PTEN nuclear translocation and autophagy in response to DNA-damaging agents in cancer cells. *Autophagy*. 2015;11(2):239-252. [CrossRef]
33. Kokkinakis DM, Brickner AG, Kirkwood JM, et al. Mitotic arrest, apoptosis, and sensitization to chemotherapy of melanomas by methionine deprivation stress. *Mol Cancer Res*. 2006;4(8):575-589. [CrossRef]
34. Cole AJ, Dickson KA, Liddle C, et al. Ubiquitin chromatin remodelling after DNA damage is associated with the expression of key cancer genes and pathways. *Cell Mol Life Sci*. 2021;78(3):1011-1027. [CrossRef]
35. Zhou Y, Liu F, Xu Q, et al. Inhibiting Importin 4-mediated nuclear import of CEBPD enhances chemosensitivity by repression of PRKDC-driven DNA damage repair in cervical cancer. *Oncogene*. 2020;39(34):5633-5648. [CrossRef]
36. Schär P, Fäsi M, Jessberger R. SMC1 coordinates DNA double-strand break repair pathways. *Nucleic Acids Res*. 2004;32(13):3921-3929. [CrossRef]