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Original Article

Comparison of Cytotoxic and Ototoxic Effects of Lipoplatin and Cisplatin in Neuroblastoma In Vivo Tumor Model

Hande Evin Eskicioğlu[1,2,3], Yüksel Olgun[4], Tekincan Çağrı Aktaş[3], Safiye Aktas[3], Efsun Kolatan[5], Efe Serinan[3], Zekiye Altun[3], Günay Kırkım[1,2], Osman Yılmaz[5], Nur Olgun[6]

[1]Dokuz Eylül University, Vocational School of Health Services,İzmir, Turkey
[2]Department of Otorhinolaryngology Unit of Hearing Speech and Balance, Dokuz Eylül University Hospital, İzmir, Turkey
[3]Department of Basic Oncology, Dokuz Eylül University, Institute of Oncology, İzmir, Turkey
[4]Department of Otorhinolaryngology, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey
[5]Department of Laboratory Animals, Dokuz Eylül University, Institute of Health Sciences, İzmir, Turkey
[6]Department of Pediatric Oncology, Dokuz Eylül University, Institute of Oncology, İzmir, Turkey

ORCID IDs of the authors: H.E.E. 0000-0001-6614-2341, Y.O. 0000-0003-1769-4224, T.Ç.A. 0000-0002-4960-5225, S.A. 0000-0002-7658-5565, E.K. 0000-0003-4761-2779, E.S. 0000-0002-3682-7590, Z.A. 0000-0002-1558-4534, G.K. 0000-0003-4170-5317, O.Y. 0000-0001-7817-7576, N.O. 0000-0001-9591-0207.

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BACKGROUND: This study aimed to compare the cytotoxic, cytostatic, and ototoxic effects of lipoplatin compared to cisplatin application in the subcutaneous xenograft nude mouse neuroblastoma tumor model.

METHODS: In this study, C1300 neuroblastoma cells were administered subcutaneously to 21 male nude mice. When the tumor reached 150 mm^3 diameter, mice were randomized into 3 groups. Saline, cisplatin, and lipoplatin were given intraperitoneally. The auditory function tests were performed before administration and 72 hours after administration. Mice were sacrificed and the tumor and cochlea were removed after 72 hours. Histopathologic evaluation of necrosis and apoptosis was determined by the TdT-mediated dUTP-biotin nick end labeling (TUNEL) method. Cyclooxygenase 2, superoxide dismutase 2, and inducible nitric oxide synthase levels were determined by immunohistochemistry in tissue samples.

RESULTS: Apoptosis and necrosis rates were higher in lipoplatin group than in cisplatin group ($P = .035$ and $P = .010$, respectively) in tumor tissue. In the spiral ganglion, apoptosis and necrosis were lower in the lipoplatin group than in cisplatin group ($P = .002$ and $P = .002$, respectively). Cyclooxygenase 2 pattern in the cochlea was positive in both control and lipoplatin group and negative in cisplatin group ($P = .001$). Superoxide dismutase 2 and inducible nitric oxide synthase 2 protein expressions showed no difference between groups. The auditory functions were similar to baseline values and had a better threshold value in lipoplatin group than cisplatin group.

CONCLUSION: For the treatment of neuroblastoma, the use of lipoplatin seems to be beneficial in reducing side effects of cisplatin. We recommend that the mechanism of these properties of lipoplatin should be evaluated in further studies.

KEYWORDS: Neuroblastoma, lipoplatin, cisplatin, auditory brainstem response

INTRODUCTION

Neuroblastoma is the most common extracranial solid tumor derived from primordial neural crest cells in children. It causes 15% of all deaths in pediatric age malignancies. In neuroblastoma treatment, platinum-based agents, especially cisplatin (CDDP), play an important role.[1-4] However, CDDP has significant dose-limiting side effects such as ototoxicity, nephrotoxicity, and neurotoxicity.[5-7]

Lipoplatin (LIPO) is a polyethylene glycol (PEG)-coated, small, liposomal CDDP formulation held inside unilamellar vesicles. Liposomal CCDP is a nanoparticle of 110 nm size that consists of lipid and CDDP.[8] There are a few studies about LIPO that show significantly less toxicity when compared to CDDP.[8,9]

Corresponding author: Tekincan Çağrı Aktaş, e-mail: tekincanaktas@yahoo.com
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In our previous study (10), we checked up on hairy cells (HEIO-C1) in vitro at the same LC50 doses for neuroblastoma cells. HEI-OC1 cells treated with 20 mM CDDP and 750 mM LIPO had a 65% and 82% viability, respectively. Lipoplatin showed less toxic effects in the HEI-OC1 cells compared to CDDP at anti-tumoral doses. As a next step, we planned in vivo animal tumor model study.

This study aims to compare cytotoxic, cytostatic, and ototoxic effects of CDDP and LIPO in the subcutaneous xenograft nude mice model.

METHODS

This study was approved by the Dokuz Eylul University Animal Experiments Local Ethics Committee on December 27, 2016, with ethics committee approval number 15 given 75/2016 as protocol number. Written informed consent was obtained from all participants who participated in this study.

Twenty-one male nude mice with an average weight of 20 g and age of 5-6 weeks were provided by the Dokuz Eylul University Faculty of Medicine Animal Experiments Research Lab. The number of mice was selected by prioritizing the 3R rule and using our previous studies to achieve statistical power greater than 80%. During the research, mice were kept at room temperature (20 ± 2 ºC), with 12-h day–night cycles, in air-conditioned cages with hepafilter, and had constant access to standard sterile mice pellets and water. All nude mice cages, water and feeder apparatus, and feeds were autoclaved before use. Before research was conducted, all mice were kept for a week to adapt to lab conditions. Nude mice without outer ear pathologies by sight and no existing eardrum or middle ear pathologies with otomicroscopy were included in the study.

Study Groups

Group 1 (Saline Solution) Control Group: The control group was established after basal hearing tests with subcutaneous injection of 10^6 cells/mL of C1300 neuroblastoma cells in order to form a tumor. When the tumor reached 150 mm^3 in size, 0.2 cc saline solution was given intraperitoneally (IP).

Group 2 (Cisplatin) CDDP Group: The CDDP group was established after basal hearing tests with subcutaneous injection of 10^6 cells/mL C1300 neuroblastoma cells in order to form a tumor. When the tumor reached 150 mm^3 in size, 0.2 cc saline solution containing 20 mg/kg CDDP was given IP.

Group 3 (Lipoplatin) LIPO Group: The LIPO group was established after basal hearing tests with a subcutaneous injection of 10^6 cells/mL of C1300 neuroblastoma cells in order to form a tumor. When the tumor reached 150 mm^3 in size, 0.2 cc saline solution containing 20 mg/kg LIPO was given IP.

Establishment of Neuroblastoma Tumor Model

Cell Culture

C1300 cells (DSMZ, ACC103®)) were cultured in Dulbecco's Modified Eagle's (DMEM) containing 10% fetal bovine serum and 1% L-glutamine, and 1% penicillin/streptomycin added. Culture solutions were renewed 2-3 times a week and left in a humidified 37°C, 5% CO_2 incubator for incubation. When the cultures reached 80% of the surface area, they were separated from the surface with trypsin/Ethylenediaminetetraacetic acid (EDTA) solution and redistributed in 1 : 4 cell ratio. For tumor establishment, 10^6 cells/mL of C1300 neuroblastoma cells were subcutaneously injected into nude mice. Tumor size was measured on the back daily (Figure 1A). When tumor size reached 150 mm^3 in size, mice were randomized into study groups with n = 7 in each group.

Drug Application

Cisplatin (1 mg/mL sterile solution cisplatin, EBEWE PHARMA®, Austria) was prepared in a 20 mg/kg dose and administered IP once when tumor size reached 150 mm^3 (smaller than 1 cm in diameter and approximately 10 days after injection) for group 2. Ototoxic dose for CDDP was determined by a pilot study with 4 nude mice (20 mg/kg). Lipoplatin (150 mg; 3 mg/mL, Regulon®, Athens, Greece & California) was given at the same dose.

Measurement of Auditory Functions

Ketamine and xylazine anesthetics were administered to nude mice before auditory brainstem response (ABR) tests. Intraperitoneal injection of 40 mg/kg of 10% ketamine (Ketalar flacon, Pfizer, USA) and 10 mg/kg of xylazine (Basilazin 2%, Bavet, İstanbul, Turkey) was used for anesthesia.

The ABR test was conducted before cell injection to establish basal auditory functions and on the third day of drug administration to

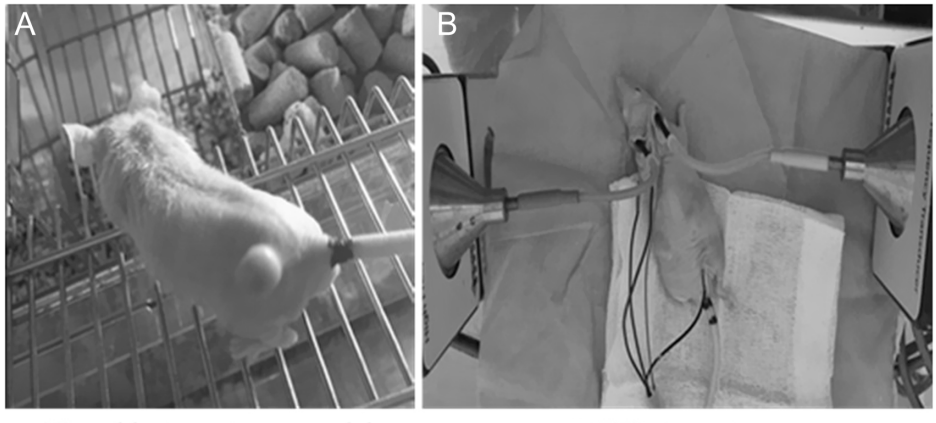

Figure 1. a,b. (a) Neuroblastoma tumor model. (b) ABR test design. ABR, auditory brainstem response.

determine auditory functions. Audiological tests were conducted in a room with a lower than 40 dB SPL(A) background sound while the mice were under anesthesia. Mice with an auditory evoked potential of 25 dB SPL or less were chosen for the study. To protect mice from hypothermia during the test, their eyes were covered with sterile gauze, and focused lights were used. To protect the eyes of mice from drying, sterile saline solution was dripped at regular intervals.

Auditory Brainstem Response Test

The ABR test was recorded with Intelligent Hearing Systems (IHS, Miami, Fla, USA) Smart-EP 10 version. Calibration was made by IHS. During recording, subdermal needle electrodes were used. The active electrode was implanted to the vertex, reference electrode was implanted in the tested ear, and ground electrode was implanted ventrolateral of the other ear (under ear). Electrode impedance was kept below 1 kΩ. The bioelectrical response was collected by the electrodes and analog signals were transformed into digital signals with a 31.3 μs sampling time. To reduce the spectrum of the signal at 4, 8, 12, 16, 20, and 32 kHz, up-to-down time of 1000 ms tone burst was used with a Blackman envelope. The lowest amplitude level to get wave III was the hearing threshold for that frequency. Starting from 70 dB SPL, wave III was recorded and reduced by 10 dB intervals for above threshold, 5 dB intervals near and below the threshold, and increased by 5 dB intervals when wave III was not recorded[7] (Figure 1B).

Sacrifice and Dissection of Organ and Tissues of Nude Mice

Sacrifice was done 72 hours after CDDP, LIPO, and saline injection. Halothane inhalation anesthesia was used before sacrifice. Exsanguination from the vena cava inferior was used for sacrifice. The heart was removed. All organs and tissues were dissected. Mice were decapitated and cochleae and brain stem were dissected for histopathology. All samples were put in 10% formalin for tissue fixation before light microscope and immunohistochemical analysis.

Histopathologic Examination of Tissues

Nude mice were incised on the back under sterile conditions and tumor tissue was removed. Tumor tissue was split for microscopic preparation and fresh tumor cell suspension. The section that was reserved for microscopic preparation was paraffinized after fixation. After tissue preparation was completed, neuroblastoma tumor tissue and cochlea were examined histopathologically. The periauricular zone including cochlea and nearby tissue was decalcified using 5% glacial acetic acid after formalin fixation. Brain, kidney, liver, heart, and lung were examined histopathologically for metastasis and drug-related damage. Polylysine-coated slides with 5 μm thickness were stained for apoptosis using the TUNEL method. Necrosis was evaluated by microscopic examination and counted under a light microscope. All areas were examined under 400× magnification. Necrotic areas were separated by views of ghost cell areas in pink color without prominent nuclei and cellular details.

Apoptosis Evaluation by TUNEL Method

Apoptosis was evaluated by a TUNEL(Roche-11684795910, Roche Diagnostics, Basel Switzerland) in situ cell death detection kit. Slides were left at 60°C overnight. After heating, deparaffinization by xylol and rehydration with decreasing alcohol concentration series were completed. Slides were treated with 20 μg/mL of proteinase K for 15 minutes before washing with distilled water for 5 minutes. Afterward, slides were treated with 3% H_2O_2 for 5 minutes and washed with phosphate buffer solution for 5 minutes. Slides were incubated at 37°C with terminal deoxynucleotidyl transferase (TdT) enzyme overnight according to the manufacturer's instructions. After incubation, slides were treated for 30 minutes with anti-streptavidin peroxidase and washed for 10 minutes at room temperature. The brown color in apoptotic cells was provided by diaminobenzidine peroxidation. After counterstaining with Mayer's hematoxylin, dehydration in increasing alcohol series and clearing in xylol were completed. Apoptosis evaluation was done by counting 1000 cells in 5 zones and recording the average count in tumor sections. Cochlear and spiral ganglion cells were counted, and positive brown cell percentages of total cells were reported as apoptosis percentage.[7]

Histochemical Analysis of Superoxide Dismutase 2, Cyclooxygenase 2, and Inducible Nitric Oxide Synthase

Immunohistochemical analysis was conducted by Ventana Discovery® automated staining system. Cochlear tissues were put on 3 μm-thick positively charged slides. After overnight heating at 60°C, slides were loaded into the system for deparaffinization, fixation, permeabilization, and antigen expression. After preparation steps, blocker + primary antibody was applied. Following that, staining with 3,3'-Diaminobenzidine (DAB) chromogen was done using the streptavidin-biotin peroxidation method. This step was followed by hematoxylin staining and cleaning before being covered under entellan. Counting was done under a light microscope and the results were recorded as percentage.

Statistical Analysis

Cisplatin, lipoplatin, and saline groups were compared with one another for baseline and post-treatment audiologic tests. Data are presented with 95% percentile and/or mean ± standard variation. All statistical analysis was done with Statistical Package for the Social Sciences v.22 (IBM SPSS Corp.; Armonk, NY, USA). After definitive statistical data, Kruskal–Wallis and Friedman variance analysis was used for intergroup differences. To determine the group that caused the statistical difference, the Mann–Whitney *U* test with Bonferroni correction was used. For intragroup measurements, the Wilcoxon signed-rank test with Bonferroni correction was used.

RESULTS

Tumoral Apoptosis and Necrosis Percentage Comparison in All Groups

Mean apoptosis was 23.21% (±5.72) (12.7%-30.3%) in the control group; 37.57% (±11.25) (26.1%-59.5%) in the CDDP group; and 61.16% (±24.58) (11.2%-83.6%) in the LIPO group. Mean necrosis was 3.99% (±1.7) (1.8%-6.4%) in the control group; 6.21% (±5.94) (2.2%-19.5%) in the CDDP group; and 1.39% (±1.46) (0.1%-4.3%) in the LIPO group (Figure 2).

The Mann–Whitney *U* non-parametric test was used to determine significant differences between control and CDDP groups. There was a significant difference between apoptosis levels ($P = .006$), whereas there was no significant difference between necrosis levels ($P = .655$).

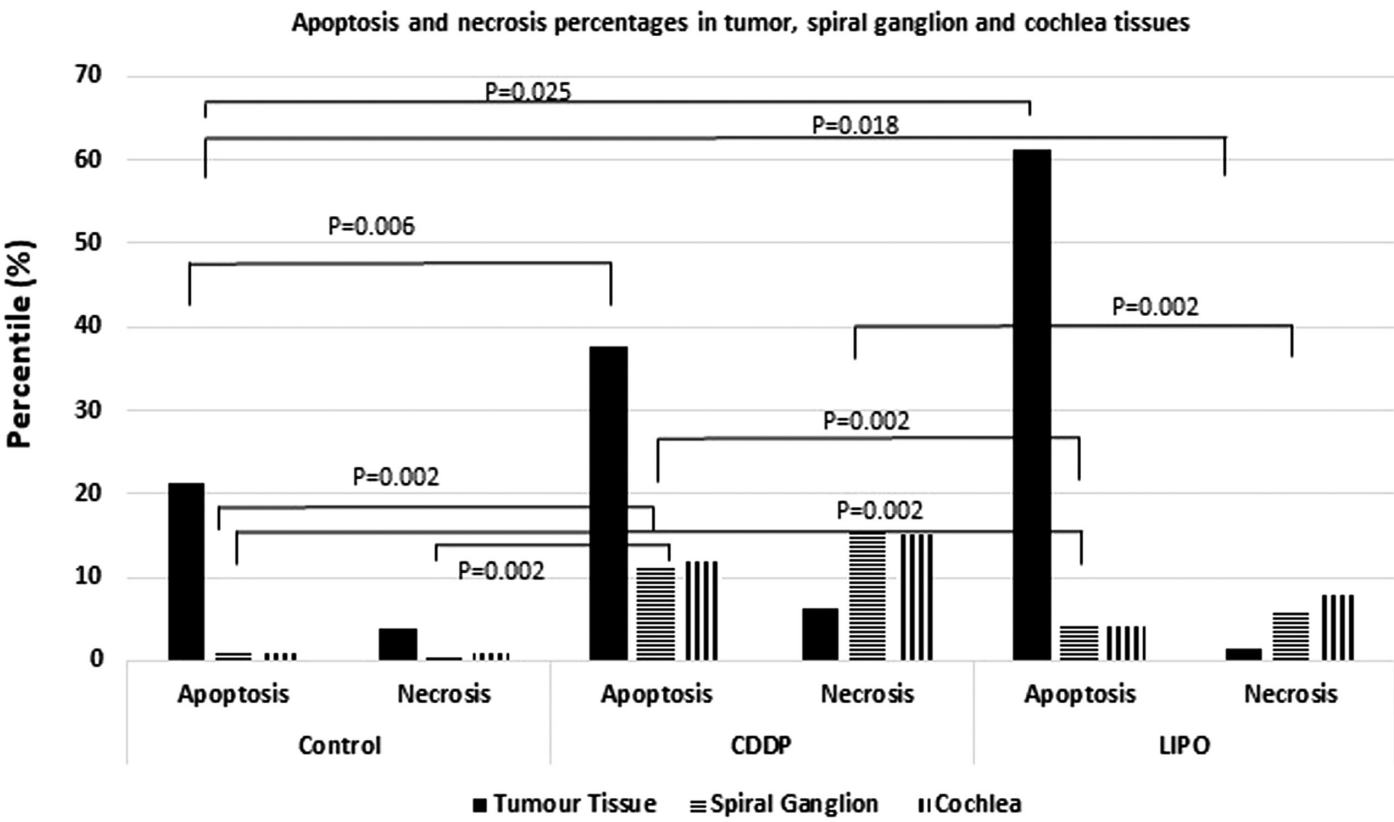

Figure 2. Mean values of apoptosis and necrosis in tumor, spiral ganglion, and cochlea of all groups ($P = .002$ for both apoptosis and necrosis).

The Mann–Whitney *U* non-parametric test was used to determine significant differences between the control and LIPO groups. There was a significant difference between apoptosis levels ($P = .025$) and between necrosis levels ($P = .018$). In the LIPO group, apoptosis was 61.15% compared to 23.21% in the control group. In the control group, necrosis was 3.99% compared to 1.39% in the LIPO group.

Comparison of Apoptosis and Necrosis Percentages in Spiral Ganglion in All Groups

Mean apoptosis ratios in cochlea tissues were 1%, 12%, and 4% in control, CDDP, and LIPO groups, respectively. Mean necrosis ratios in cochlea tissues were 0%, 16%, and 8% in control, CDDP, and LIPO groups, respectively (Figure 2). Figure 2 also shows apoptosis and necrosis ratios in spiral ganglia and tumor tissues in the 3 groups.

Significant statistical difference was observed between pairs of control, LIPO, and CDDP groups for apoptosis and necrosis ($P = .002$). The CDDP group had significantly higher apoptosis and necrosis rates than the control and LIPO groups ($P = .002$, Mann–Whitney *U* test).

Comparison of Cochlear and Tumoral SOD2, COX2, and INOS Expression Levels Between All Groups

Between all groups, cochlear expression levels of superoxide dismutase 2 (SOD2) and inducible nitric oxide synthase (INOS) (– for negative, + for minimum expression, ++ for average expression) were compared by the chi-Square test and no significant difference was detected between groups. No further tests were performed between 2 groups. For all groups, cochlear expression levels of cyclooxygenase 2 (COX2) were compared by the chi-Square test and a significant difference was found ($P = .001$). There was no expression of COX2 in the CDDP group, whereas all animals in the other 2 groups had (+) staining pattern for COX2 expression (Table 1). In tumor tissues, COX2 had the same (+) staining pattern in all 3 groups (Figure 3).

Auditory Examination Results and Comparisons of All Groups

There was no significant difference between right and left ears in the control group on both baseline and 72-hour ABR tests conducted at 8, 12, 16, 20, and 32 kHz. There was no significant difference between right and left ears in the CDDP group at both baseline and 72-hour ABR tests conducted at 8 kHz, but there were significant differences between baseline and 72-hour ABR for 12, 16, 20, and 32 kHz. There was no significant difference between right and left ears in the LIPO

Table 1. SOD2, INOS2, and COX2 Expression Levels in Cochlea in All Groups

	Group	- n	+n	++n	*P*
SOD2	Control	0	2	5	.466
	CDDP	0	4	3	
	LIPO	0	4	3	
INOS2	Control	0	7	0	.350
	CDDP	0	7	0	
	LIPO	0	6	1	
COX2	Control	0	7	0	<.001
	CDDP	7	0	0	
	LIPO	0	7	0	

COX-2, cyclooxygenase 2; INOS, inducible nitric oxide synthase; SOD-2, superoxide dismutase 2; LIPO, lipoplatin; CDDP, cisplatin.

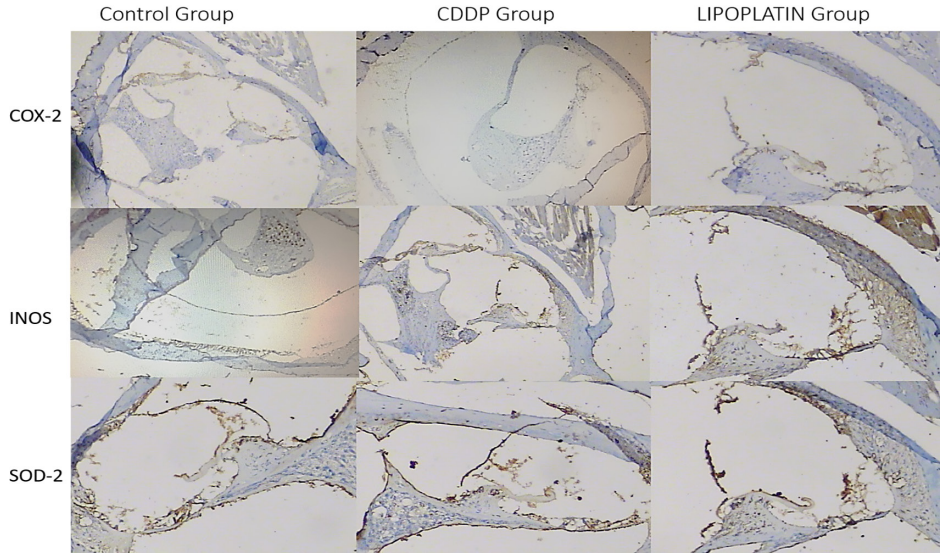

Figure 3. Immunohistochemical expression patterns of pro-inflammatory in cochlear corti organ. INOS and SOD-2 expression did not differ among groups. COX-2 expression is positive in the control and LIPO group, while it is negative in CDDP group. COX-2, cyclooxygenase 2; INOS, inducible nitric oxide synthase; SOD-2, superoxide dismutase 2; LIPO, lipoplatin; CDDP, cisplatin.

group at both baseline and 72-hour ABR tests conducted at 8, 12, and 16 kHz, but there was a significant difference between baseline and 72-hour ABR for 20 and 32 kHz. There was hearing damage at 12, 16, 20, and 32 kHz in the CDDP group and at 20 and 32 kHz in the LIPO group.

The hearing thresholds in 2 groups were compared with the Mann–Whitney *U* test. When hearing thresholds in control and CDDP groups were compared, there was no significant difference at 8 kHz, but there was a significant change at 12, 16, 20, and 32 kHz showing bilaterally increased hearing threshold in the CDDP group. When hearing thresholds of control and LIPO groups were compared, there was no significant difference at 8 and 12 kHz, but there was a significant change at 16, 20, and 32 kHz showing bilaterally increased hearing threshold in the LIPO group (Figure 4).

DISCUSSION

Neuroblastoma is the second most common solid tumor in children. The best chemotherapeutic agent for neuroblastoma treatment is CDDP. Cisplatin has dose-limiting side effects such as ototoxicity, nephrotoxicity, and neurotoxicity. Ototoxicity in children may advance to total hearing loss and significantly limit development. Because of

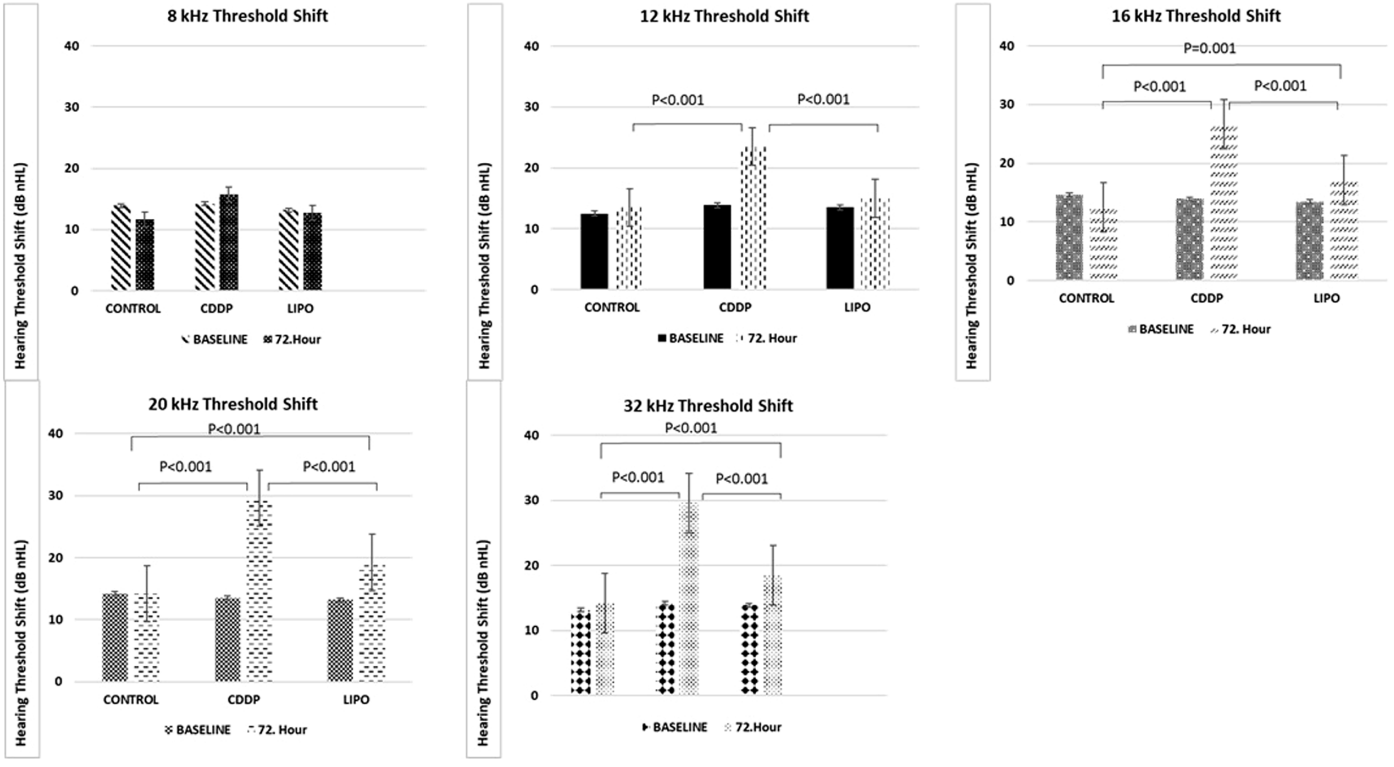

Figure 4. Comparison of baseline and 72-hour auditory tests in all groups.

this, there are studies aiming to replace CDDP treatment with similarly effective treatment. Liposomal formulations aim to reduce the side effects of existing drugs without compromising effectiveness. Based on this, it is possible that liposomal CDDP (LIPO) may reduce serious side effects such as ototoxicity during neuroblastoma treatment.[11,12]

In this study, we established an *in* vivo neuroblastoma tumor model to determine the effects of CDDP and LIPO in neuroblastoma treatment and side effects like ototoxicity. Tumor development and ototoxicity were examined at both tissue level and functional level.[13]

Casagrande et al[14] found in 2014 that LIPO caused less systemic toxicity and tumor death with higher apoptosis rates when compared with CDDP in the ovary cancer model. Both CDDP and LIPO significantly triggered apoptotic cell death in our neuroblastoma tumor model. At the same time, LIPO caused significantly higher apoptosis when compared to CDDP.

Spiral ganglia in the cochlear region were also examined for cell death in order to determine neural-based ototoxicity besides sensorial cochlear tissue. More apoptosis and necrosis than the control group were observed in CDDP and LIPO groups. But in the CDDP group, both cell death markers were higher than in the LIPO group. This supports the hypothesis of LIPO having less ototoxic effect than CDDP. Brainstem Auditory Evoked Potentials (BAEP) test results show the relatively better hearing threshold of the LIPO group compared to the CDDP group, indicating LIPO's advantage over CDDP in terms of better cytotoxic effect and less ototoxic effect.[15]

Expression patterns of important antioxidant and pro-inflammatory pathway molecules, COX2, SOD2, and INOS, were examined in our study. Cyclooxygenase 2 was not expressed in the CDDP group in cochlear tissue, whereas there was a slight positive expression pattern in LIPO and control group. There was no pattern difference for SOD2 and INOS in cochlear tissue. Cyclooxygenase 2 affects prostaglandin production in cancer cells and reduces levels of nitric oxide (NO), a pro-apoptotic agent.[16] There is no comparison targeting this in known studies, but Santos et al[17] suggested that mitochondrial oxygen radicals may explain CDDP's cytotoxic effect on kidneys causing nephrotoxicity side effects. Single-dose CDDP injection of 10 mg/kg caused depletion of NADPH and glutathione and raised levels of pro-apoptotic caspase-3.[17] The similar cochlear expression patterns in the LIPO and control groups for COX2 suggest that LIPO may reduce pro-apoptotic factors and inhibit apoptosis in cochlear cells.

Our study is the first study comparing the effects of LIPO and CDDP across multiple mechanisms in the neuroblastoma in vivo model. This is our study's distinctive trait. For study limitations, there were non-equal tumor growth rates due to the live animal model, sudden unexplained animal deaths post-treatment was prevented by a pilot study, and the different timings of the sacrifice of mice are limiting factors in our study.

This study shows advantages of Lipo over CDDP like inhibition of pro-apoptotic factors via COX2 expression, lower apoptosis and necrosis compared to CDDP and better hearing thresholds compared to CDDP. In addition to the COX2 expression being slightly positive in tumor tissues from all 3 groups. All of these findings support the literature knowledge about LIPO's reduction of the dose-limiting side effects of CDDP while inducing a strong apoptotic effect on the tumor, making LIPO a safer and more effective alternative chemotherapeutic agent to CDDP. Additionally, Boulikas18 reported that lipoplatin substantially reduced the renal toxicity, peripheral neuropathy, ototoxicity, and myelotoxicity, as well as nausea/vomiting and asthenia of cisplatin in phase I, II, and III clinical studies with enhanced or similar efficacy to cisplatin. In literature, it is suggested that the advantage of LIPO over CDDP is caused by its ability to enter tumoral vasculature due to increased permeability of neo-angiogenic vasculature. This causes increased drug delivery to the primary tumor and metastatic sites. Nanoparticles are thought to be taken in either by phagocytosis or by direct fusion with cell membrane.[18-21] Future research is needed to determine which molecular pathways play a role in LIPO's effect on tumor cells, tumor niche, and healthy tissues.

The limitation of our study is that we used a single dose of CDDP and LİPO. In the treatment of human cancer, multidose, multicycle protocols are generally used. In animal model studies, especially with nude mice, it is not always possible to mimic multidose, multicycle protocols as in human. Because, these protocols take time over several months, which is not suitable for mice life period. Besides exitus might occur in multicycles over time due to diarrhea, vomiting, infections, or organ failure in these cellular immunodeficient mice. In many animal model studies, single toxic dose is used for toxicity studies. One of the administration dose of CDDP in neuroblastoma patients is 10 mg/m^2 single dose with 28 days intervals. Ototoxicity is a major problem in childhood cancers because, after cure, they will have a longer life span than adults.

CONCLUSION

The hypothesis of our research is that LIPO treatment has a significantly less-lethal effect on healthy tissue compared to CDDP and our findings of the spiral ganglion and cochlea support this hypothesis. We also managed to answer some of the following questions: LIPO has different and better apoptotic and necrotic effects, causes less cell death in spiral ganglion, and more expression of COX2 when compared to CDDP.

All these results show the need for advanced studies aiming to explain tumoral effects, microenvironmental effects, molecular mechanisms, and pathways that reduce side effects and increase the effectiveness of LIPO. In conclusion, mice in the LIPO group had better hearing thresholds than mice in the CDDP group.

Ethics Committee Approval: Ethical committee approval was received from Dokuz Eylül University Animal Experiments Local Ethics Committee (December 27, 2016, 15 - 75/2016).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.

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

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Declaration of Interests: The authors declare that they have no competing interest.

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REFERENCES

1. Olgun Y, Aktaş S, Altun Z, et al. Analysis of genetic and non genetic risk factors for cisplatin ototoxicity in pediatric patients. *Int J Pediatr Otorhinolaryngol*. 2016;90:64-69. [CrossRef]
2. Bertolini P, Lassalle M, Mercier G, et al. Platinum compound-related ototoxicity in children: long-term follow-up reveals continuous worsening of hearing loss. *J Pediatr Hematol Oncol*. 2004;26(10):649-655. [CrossRef]
3. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. *Eur J Pharmacol*. 2014;740:364-378. [CrossRef]
4. Knight KR, Kraemer DF, Neuwelt EA. Ototoxicity in children receiving platinum chemotherapy: underestimating a commonly occurring toxicity that may influence academic and social development. *J Clin Oncol*. 2005;23(34):8588-8596. [CrossRef]
5. Grewal S, Merchant T, Reymond R, McInerney M, Hodge C, Shearer P. Auditory late effects of childhood cancer therapy: a report from the Children's Oncology Group. *Pediatrics*. 2010;125(4):e938-e950. [CrossRef]
6. 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]
7. Kirkim G, Olgun Y, Aktas S, et al. Is there a gender-related susceptibility for cisplatin ototoxicity? *Eur Arch Otorhinolaryngol*. 2015;272(10):2755-2763. [CrossRef]
8. Zahednezhad F, Zakeri-Milani P, Shahbazi Mojarrad J, Valizadeh H. The latest advances of cisplatin liposomal formulations: essentials for preparation and analysis. *Expert Opin Drug Deliv*. 2020;17(4):523-541. [CrossRef]
9. Rybak LP, Husain K, Morris C, Whitworth C, Somani S. Effect of protective agents against cisplatin ototoxicity. *Am J Otol*. 2000;21(4):513-520.
10. 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]
11. Kushner BH, Budnick A, Kramer K, Modak S, Cheung NK. Ototoxicity from high-dose use of platinum compounds in patients with neuroblastoma. *Cancer*. 2006;107(2):417-422. [CrossRef]
12. Landier W, Knight K, Wong FL, et al. Ototoxicity in children with high-risk neuroblastoma: prevalence, risk factors, and concordance of grading scales—A report from the Children's Oncology Group. *J Clin Oncol*. 2014;32(6):527-534. [CrossRef]. Li Y, Womer RB, Silber JH. Predicting cisplatin ototoxicity in children: the influence of age and the cumulative dose. *Eur J Cancer*. 2004;40(16):2445-2451. [CrossRef]
13. Park MS, De Leon M, Devarajan P. Cisplatin induces apoptosis in LLC-PK1 cells via activation of mitochondrial pathways. *J Am Soc Nephrol*. 2002;13(4):858-865. [CrossRef]
14. Casagrande N, Celegato M, Borghese C, Mongiat M, Colombatti A, Aldinucci D. Preclinical activity of the liposomal cisplatin lipoplatin in ovarian cancer. *Clin Cancer Res*. 2014;20(21):5496-5506. [CrossRef]
15. Devarajan P, Tarabishi R, Mishra J, et al. Low renal toxicity of lipoplatin compared to cisplatin in animals. *Anticancer Res*. 2004;24(4):2193-2200.
16. Cao BJ, Reith ME. Nitric oxide inhibits uptake of dopamine and N-methyl-4-phenylpyridinium (MPP+) but not release of MPP+ in rat C6 glioma cells expressing human dopamine transporter. *Br J Pharmacol*. 2002;137(8):1155-1162. [CrossRef]
17. Santos NA, Bezerra CS, Martins NM, Curti C, Bianchi ML, Santos AC. Hydroxyl radical scavenger ameliorates cisplatin-induced nephrotoxicity by preventing oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. *Cancer Chemother Pharmacol*. 2008;61(1):145-155. [CrossRef]
18. Boulikas T. Clinical overview on Lipoplatin: a successful liposomal formulation of cisplatin. *Expert Opin Investig Drugs*. 2009;18(8):1197-1218. [CrossRef]
19. Tippayamontri T, Kotb R, Paquette B, Sanche L. Efficacy of cisplatin andLipoplatin in combined treatment with radiation of a colorectal tumor in nude mouse. *Anticancer Res*. 2013;33(8):3005-3014.
20. Bragado P, Armesilla A, Silva A, Porras A. Apoptosis by cisplatin requires p53 mediated p38alpha MAPK activation through ROS generation. *Apoptosis*. 2007;12(9):1733-1742. [CrossRef]
21. Kurioka T, Mogi S, Tanaka M, Yamashita T. Activity-dependent neurodegeneration and neuroplasticity of auditory neurons following conductive hearing loss in adult mice. *Cell Mol Neurobiol*. 2021;41(1):31-42. [CrossRef]