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Protective Effects of Melatonin and Alpha Lipoic Acid Against Cisplatin-Induced Ototoxicity

Cisplatinin İndüklediği Ototoksisite Üzerine Melatonin Ve Alfa Lipoik Asitin Etkileri

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ÖZET

Amaç: Bu çalışmada, sıçan modelinde cisplatine bağlı ototoksisiteye karşı melatonin ve alfa-lipoik asidin potansiyel koruyucu etkilerini beyin sapı işitsel uyarılmış potansiyelleri kullanarak araştırmak amaçlanmıştır.

Gereçler ve Yöntem: Altmış Sprague-Dawley sıçanı rastgele altı gruba ayrıldı: kontrol, cisplatin, melatonin, alfa-lipoik asit, cisplatin+melatonin ve cisplatin+alfa-lipoik asit. Cisplatin tek doz intraperitoneal enjeksiyon (10 mg/kg) şeklinde uygulandı. Melatonin (4 mg/kg) ve alfa-lipoik asit (100 mg/kg), cisplatin uygulamasından bir gün önce başlayarak sekiz gün boyunca günlük olarak verildi. Beyin Sapı İşitsel Uyarılmış Potansiyelleri başlangıçta ve 3., 7. ve 15. günlerde ölçüldü. Dalga latansları, interpik latanslar, işitme eşikleri ve dalga formu morfolojisi analiz edildi.

Bulgular: Cisplatin uygulaması, V. dalga latansında anlamlı uzama ve işitme eşiklerinde artışa neden oldu. Melatonin tedavisi, sadece cisplatin grubuna kıyasla V. dalga latansı ve işitme eşiklerindeki cisplatine bağlı değişiklikleri anlamlı ölçüde azalttı ($p<0.05$). Alfa-lipoik asit, cisplatine bağlı değişikliklere karşı anlamlı bir koruma göstermedi. Hem melatonin hem de alfa-lipoik asit grupları, tek başına uygulandıklarında dalga latanslarında değişiklikler gösterdi. Dalga formu bozulmaları en çok cisplatin grubunda görülürken, melatonin ve alfa-lipoik asit tedavi gruplarında daha az sıklıkta gözlendi.

Sonuç: Melatonin tedavisi, sıçanlarda cisplatine bağlı ototoksisiteyi anlamlı ölçüde azaltmaktadır ve bu muhtemelen güçlü antioksidan özelliklerinden kaynaklanmaktadır. Bu bulgu, cisplatin kemoterapisi alan hastalarda işitme kaybını önlemek veya en aza indirmek için melatoninin potansiyel bir terapötik strateji olabileceğini desteklemektedir. Bununla birlikte, alfa-lipoik asit bu çalışmada anlamlı bir koruma göstermemiştir ve bu konuda daha fazla araştırma yapılması gerekmektedir.

Anahtar Kelimeler: Cisplatin, ototoksisite, melatonin, alfa-lipoik asit, antioksidan

ABSTRACT

Aim: To investigate the potential protective effects of melatonin and alpha-lipoic acid against cisplatin-induced ototoxicity in a rat model using brainstem auditory evoked potentials.

Materials and Methods: Sixty Sprague-Dawley rats were randomly divided into six groups: control, cisplatin-only, melatonin-only, alpha-lipoic acid-only, cisplatin+melatonin, and cisplatin+alpha-lipoic acid. Cisplatin was administered as a single intraperitoneal injection (10 mg/kg). Melatonin (4 mg/kg) and alpha-lipoic acid (100 mg/kg) were administered daily for eight days, starting one day before cisplatin. Brainstem Auditory Evoked Potentials were measured at baseline and on days 3, 7, and 15. Wave latencies, interpeak latencies, hearing thresholds, and waveform morphology were analyzed.

Results: Cisplatin administration resulted in significant prolongation of wave V latency and increased hearing thresholds. Melatonin treatment significantly mitigated cisplatin-induced changes in wave V latency and hearing thresholds compared to cisplatin alone ($p<0.05$). Alpha-lipoic acid did not demonstrate significant protection against cisplatin-induced changes. Both melatonin and alpha-lipoic acid groups showed alterations in wave latencies when administered alone. Waveform distortions were most prevalent in the cisplatin group, with lower incidence in melatonin and alpha-lipoic acid treatment groups.

Conclusions: Treatment with melatonin significantly mitigates cisplatin-induced ototoxicity in rats, likely due to its potent antioxidant properties. This finding supports the potential of melatonin as a therapeutic strategy to prevent or minimize hearing loss in patients receiving cisplatin chemotherapy. However, alpha-lipoic acid did not exhibit significant protection in this study, warranting further investigation.

Keywords: Cisplatin, ototoxicity, melatonin, alpha-lipoic acid, antioxidants

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INTRODUCTION

Cisplatin, a cornerstone of cancer chemotherapy, frequently induces ototoxicity, characterized by irreversible inner ear damage and subsequent hearing loss. This debilitating side effect necessitates strategies to mitigate ototoxicity without compromising cisplatin's anti-cancer efficacy. Oxidative stress, driven by free radical generation, is a key mechanism underlying cisplatin-induced cochlear damage. Antioxidants, capable of neutralizing free radicals, present a promising therapeutic avenue (1). This study investigates the otoprotective potential of two such antioxidants: melatonin and alpha lipoic acid (ALA). Melatonin, a hormone with established antioxidant and anti-inflammatory properties (2-4), scavenges reactive oxygen and nitrogen species and modulates cytokine production. ALA, a potent antioxidant and mitochondrial cofactor (5), directly neutralizes free radicals and regenerates endogenous antioxidants like vitamins C and E, glutathione, and coenzyme Q10 (6). Furthermore, ALA modulates inflammatory signaling pathways (5). We hypothesize that melatonin and ALA will attenuate cisplatin-induced ototoxicity. This research aims to elucidate their protective mechanisms and inform the development of therapeutic strategies to improve the quality of life for patients receiving cisplatin.

MATERIALS AND METHODS

This study was conducted at the Department of Otorhinolaryngology, Selçuk University Meram Faculty of Medicine, utilizing rats obtained from the Selçuk University Experimental Medicine Research and Application Center. A total of 60 healthy adult female Sprague-Dawley rats, approximately three months old, were used. The weight of the rats ranged from 180 to 220 grams, with an average weight of 200 grams. Throughout the study, the rats were maintained in a controlled environment with a 12-hour light/dark cycle, temperature maintained at 20±2°C, and relative humidity at 50±10%, with air changed 15 times per hour. Food and water were provided ad libitum. The rats were housed in groups of five in polycarbonate cages with a floor area of 1820 cm2 (Tecniplast Company, Italy). Approval from the Ethics Committee of the Faculty of Medicine at Selçuk University was obtained prior to the commencement of the study (number: 2007/18).

The rats were divided into six groups:

- Group 1 (Control Group -C-; n = 10): This group received intraperitoneal (i.p.) injections of 1 mg/kg physiological serum for 8 days to counteract the stress caused by injections in other groups.
- Group 2 (Cisplatin Group -CP-; n = 10): A single i.p. injection of 10 mg/kg cisplatin (Cis-Diammineplatinum II chloride) was administered.
- Group 3 (Melatonin Group -Mel-; n = 10): Melatonin (Melatonine for synthesis C13H15N2O2, M: 232.28 g/mol, Merck, Germany) was administered i.p. at a dose of 4 mg/kg for 8 days.
- Group 4 (Alpha-Lipoic Acid Group -ALA-; n = 10): Alpha-lipoic acid (DL-α-lipoic acid > 98.0% HPLC, Fluka) was administered i.p. at a dose of 100 mg/kg for 8 days.
- Group 5 (Cisplatin + Melatonin Group -CP+Mel-; n = 10): A single i.p. injection of 10 mg/kg cisplatin was administered, followed by i.p. injections of 4 mg/kg melatonin starting one day before the cisplatin injection and continuing for 8 days.
- Group 6 (Cisplatin + Alpha-Lipoic Acid Group -CP+ALA-; n = 10): A single i.p. injection of 10 mg/kg cisplatin was administered, followed by i.p. injections of 100 mg/kg alpha-lipoic acid starting one day before the cisplatin injection and continuing for 8 days.

In all groups, brainstem auditory evoked potentials (BAEPs) were measured 30 minutes before the initial injections. The day of the first cisplatin injections was designated as day 0. For groups other than the control group, BAEPs were measured 4 times in total, on days 0, 3, 7, and 15. For the control group, BAEPs were measured 3 times, on days 0, 7 and 15.

Brainstem Auditory Evoked Potential (BAEP) Measurements

Prior to recording evoked potentials, animals were anesthetized with intramuscular injections of ketamine hydrochloride (50 mg/kg) and xylazine (10 mg/kg). The animals were then placed in a sound-attenuated and electrically isolated environment. BAEP measurements were performed using Oxford Instruments MedelecSynergy EMG and EP Systems. TDH-49p headphones and Viasys Healthcare TECA Needles subdermal needle electrodes were used. Active electrodes were placed in the retroauricular region, the reference electrode at the vertex along the midline, and the neutral electrode between the eyes on the median line (7,8).

Auditory stimuli were delivered alternately to each ear using click stimuli, with the non-stimulated ear masked with white noise. Stimuli were delivered at a frequency of 15 Hz, with an analysis time of 10 ms (9). To determine the threshold, stimulus intensity was reduced in increments of 10 dB from above-threshold levels and in 5 dB steps near the threshold. Each test presented 1000-2000 click stimuli. I., III., and V. wave formations were observed on the monitor. Latencies of I., III., and V. waves, as well as I-III, III-V, and I-V interpeak latencies (IpLs), were recorded. BAEP recordings were assessed based on the wave latency-intensity functions. Threshold values were determined and any variations in wave formation were documented. The single dose cisplatin injection (10 mg/kg) method was utilized as previously described in studies demonstrating ototoxicity (10,11).

Statistical Analysis:

Data were analyzed using SPSS 13.0 software. Descriptive statistics were reported as mean ± standard deviation. Variance analysis was conducted for group comparisons across repeated measurements, with post-hoc Tukey tests identifying specific group differences. Within-group repeated measurements were analyzed using the Wilcoxon signed-rank test, while between-group comparisons were performed using the Mann-Whitney U test. Categorical variables were compared using the chi-square test. Statistical significance was set at $p<0.05$. Results were visualized through graphs and tables.

Table 1. Latencies of waves I, III, and V of Brainstem Auditory Evoked Potentials (BAEPs) in all experimental and control groups at days 0, 3, 7, and 15.

	Day 0. (Mean±sd)	Day 3. (Mean±sd)	Day 7. (Mean±sd)	Day 15. (Mean±sd)	p
Wave I					
Control	1.75±0.17		1.80±1.12	1.78±0.13	
CP	1.75±0.18	1.79±0.13	1.72±0.14	1.72±0.11	
ALA	1.73±0.15	1.71±0.14*	1.71±0.09	1.71±0.12*	0.013 / 0.047
Mel	1.83±0.13	1.79±0.13	1.84±0.10	1.79±0.15	
CP+ALA	1.74±0.17	1.79±0.12*	1.82±0.07*	1.71±0.12	0.001 / <0.001
CP+Mel	1.67±0.18	1.76±0.10*	1.76±0.14*	1.72±0.12*	<0.001 /<0.001/ 0.005
Wave III					
Control	3.78±0.18		3.70±0.17	3.65±0.17	
CP	3.78±0.27	3.75±0.12	3.80±0.21	3.73±0.12	
ALA	3.84±0.36	3.83±0.10	3.84±0.09	3.79±0.10	
Mel	3.71±0.18	3.79±0.09*	3.84±0.11*	3.71±0.09	<0.001 / 0.002
CP+ALA	3.90±0.29	3.74±0.15*	3.79±0.12*	3.69±0.15*	<0.001 /<0.001/ <0.001
CP+Mel	3.90±0.21	3.83±0.17*	3.76±0.13*	3.75±0.11*	0.005 /<0.001/ <0.001
Wave V					
Control	5.76±0.23		5.82±0.10	5.70±0.14	
CP	5.71±0.14	5.73±0.42*	5.71±0.26	5.73±0.10	0.020
ALA	5.74±0.16	5.75±0.09	5.79±0.12	5.82±0.28	
Mel	5.76±0.14	5.79±0.14	5.76±0.28	5.82±0.05	
CP+ALA	5.71±0.13	5.75±0.11*	5.85±0.07*	5.76±0.08*	0.009 /<0.001/ 0.003
CP+Mel	5.81±0.15	5.81±0.11	5.86±0.05*	5.74±0.10*	<0.001 / <0.001

CP: Cisplatin ALA: Alpha-Lipoic Acid Mel: Melatonin *: Indicates a statistically significant difference compared to Day 0 (P < .05).

RESULTS

Three rats in the melatonin group and two rats in the ALA group were lost due to peritonitis on days 6 and 8, respectively.

Wave Latencies

Table 1 presents the latencies of waves I, III, and V of BAEPs in all experimental and control groups at different time points (days 0, 3, 7, and 15).

Cisplatin administration significantly prolonged wave V latency at day 3 compared to baseline ($p<0.05$). Alpha-lipoic acid (ALA) alone shortened wave I latency at days 3 and 15 ($p<0.05$), while melatonin alone prolonged wave III latency at days 3 and 7 ($p<0.05$). In the combined treatment groups, both cisplatin + ALA and cisplatin + melatonin induced significant changes in wave I, III, and V latencies across various time points compared to baseline ($p<0.05$). Crucially, cisplatin + melatonin mitigated the cisplatin-induced prolongation of wave V, demonstrating a significant difference between these groups ($p<0.05$). Similarly, the cisplatin + melatonin group exhibited significantly shorter wave V latencies compared to the cisplatin + ALA group ($p<0.05$). ALA and cisplatin + melatonin groups showed altered wave III latencies compared to the control ($p<0.05$).

Interpeak Latencies (IPLs)

Table 2 illustrates the IPLs for I-III, I-V, and III-V in all experimental and control groups at different time points (days 0, 3, 7, and 15). Analysis of IPLs revealed that cisplatin significantly increased III-V IPL at days 3 and 7 ($p<0.05$). While both ALA and melatonin alone affected IPLs, the most notable finding was the significant reduction in I-V and I-III IPLs between the cisplatin and cisplatin + melatonin groups ($p<0.05$), indicating a protective effect of melatonin. Additionally, the cisplatin + melatonin group showed significantly different I-V and I-III IPLs compared to the cisplatin + ALA group ($p<0.05$).

Hearing Thresholds

Table 3 shows the hearing thresholds in all experimental and control groups at different time points (days 0, 3, 7, and 15). Cisplatin significantly elevated hearing thresholds at days 3, 7, and 15 ($p<0.05$). ALA and melatonin alone also induced changes in hearing thresholds. However, the cisplatin + melatonin group demonstrated significantly lower thresholds compared to the cisplatin-only group ($p<0.05$, Figure 1), confirming melatonin's protective effect. No significant difference was observed between the cisplatin and cisplatin + ALA groups. Consistent with the wave V latency findings, the cisplatin + melatonin group exhibited significantly lower hearing thresholds compared to the cisplatin + ALA group ($p<0.05$).

Waveform Morphology

Waveform morphology analysis revealed transient distortions in all treatment groups. The highest incidence was observed in the cisplatin group, while the combination treatment groups, particularly cisplatin + melatonin, showed a tendency toward recovery by day 15.

DISCUSSION

Cisplatin is a highly effective chemotherapeutic agent

Table 2. Interpeak Latencies (IPLs) for I-III, I-V, and III-V in all experimental and control groups at days 0, 3, 7, and 15.

	Day 0. (Mean±sd)	Day 3. (Mean±sd)	Day 7. (Mean±sd)	Day 15. (Mean±sd)	p
I-V IpL					
Control	4.01±0.27		4.00±0.16	3.92±0.21	
CP	3.95±0.25	4.02±0.15	3.97±0.31	4.00±0.14	
ALA	3.99±0.21	4.05±0.12	4.10±0.12	4.10±0.30*	0.002
Mel	3.94±0.15	3.98±0.21	3.91±0.23	4.03±0.16*	0.017
CP+ALA	3.98±0.22	3.95±0.15	4.02±0.10	4.04±0.14*	<0.001
CP+Mel	4.14±0.20	4.02±0.22*	4.11±0.16	4.03±0.15*	<0.001/<0.001
I-III IpL					
Control	2.03±0.29		1.89±0.19	1.86±0.21	
CP	2.05±0.33	1.94±0.20	2.05±0.23	2.00±0.15	
ALA	2.10±0.43	2.11±0.18	2.13±0.12	2.08±0.14	
Mel	1.88±0.23	2.00±0.12*	2.00±0.11*	1.91±0.18	0.007 / 0.050
CP+ALA	2.16±0.30	1.95±0.17*	1.96±0.12*	1.97±0.13*	<0.001/<0.001/<0.001
CP+Mel	2.19±0.24	2.07±0.21*	2.00±0.17*	2.03±0.16*	<0.001/<0.001/<0.001
III-V IpL					
Control	1.97±0.22		2.12±0.18	2.05±0.17	
CP	1.90±0.27	2.07±0.15*	1.98±0.24*	2.00±0.12	<0.001 / 0.049
ALA	1.90±0.35	1.93±0.13	1.95±0.12	2.03±0.28	
Mel	2.04±0.14	1.97±0.16*	1.91±0.28*	2.11±0.09*	0.001/0.002/0.001
CP+ALA	1.82±0.35	2.00±0.18*	2.07±0.14*	2.08±0.17*	<0.001/ <0.001/ <0.001
CP+Mel	1.91±0.22	2.01±0.16*	2.10±0.13*	1.99±0.13*	<0.001/ <0.001/ 0.001

CP: Cisplatin ALA: Alpha-Lipoic Acid Mel: Melatonin *: Indicates a statistically significant difference compared to Day 0 (P < .05).

Table 3. Hearing thresholds in all experimental and control groups at days 0, 3, 7, and 15.

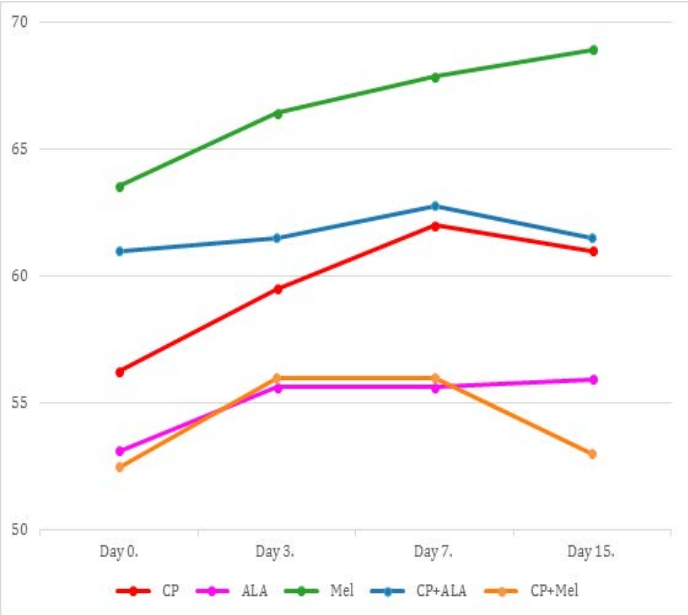
	Day 0. (Mean±sd)	Day 3. (Mean±sd)	Day 7. (Mean±sd)	Day 15. (Mean±sd)	p
Control	56.25±6.46	56.75±4.94	57.00±5.48		
CP	56.75±11.80	59.50±11.54*	62.00±12.24*	61.00±10.69*	0.001/<0.001/<0.001
ALA	53.12±5.31	55.62±5.00*	55.62±4.67*	55.94±4.44*	<0.001/<0.001/<0.001
Mel	63.57±8.22	66.43±9.83*	67.86±9.31*	68.93±10.51*	<0.001/<0.001/<0.001
CP+ALA	61.00±10.95	61.50±11.12	62.75±12.94*	61.50±10.18	<0.001
CP+Mel	52.50±7.36	56.00±7.21*	56.00±8.79*	53.00±6.24	<0.001/<0.001

CP: Cisplatin ALA: Alpha-Lipoic Acid Mel: Melatonin *: Indicates a statistically significant difference compared to Day 0 (P < .05).

used to treat a wide variety of malignancies (12). However, its clinical efficacy is often limited by dose-dependent and often irreversible ototoxicity, primarily manifesting as sensorineural hearing loss (13). This study investigated the potential protective effects of melatonin and alpha-lipoic acid, two potent antioxidants, against CP-induced ototoxicity in a rat model using BAEPs.

Our findings demonstrate that a single i.p. injection of 10 mg/kg CP resulted in a significant increase in BAEP wave V latency at day 3, a commonly used indicator of cochlear and auditory nerve dysfunction (14). This observation aligns with previous studies that have established the ototoxic nature of CP, particularly targeting the outer hair cells of the cochlea's basal turn, leading to high-frequency hearing loss that can progress to lower frequencies over time (15,16). The prolonged latency observed in our study indicates a delay in the transmission of auditory signals through the auditory pathway, likely due to CP-induced damage to cochlear structures and potentially the auditory nerve.

Interestingly, the groups receiving melatonin or ALA alone exhibited significant changes in BAEP wave latencies compared to the control group. ALA administration resulted in a statistically significant shortening of wave I latency on days 3 and 15, while melatonin administration led to a significant prolongation of wave III latency on days 3 and 7. These findings suggest that both melatonin and ALA, when administered independently, may have an effect on auditory signal processing within the brainstem. However, the exact mechanisms underlying these observed changes warrant further investigation. For instance, ALA's ability to shorten wave I latency could indicate an enhanced excitability of the auditory nerve, potentially through its known neuroprotective



CP: Cisplatin ALA: Alpha-Lipoic Acid Mel: Melatonin

Figure 1. Comparison of hearing thresholds between all experimental groups at days 0, 3, 7, and 15

effects (2). Conversely, the prolongation of wave III latency with melatonin could be attributed to its modulation of neurotransmitter systems in the brainstem, a well-documented effect of melatonin (17,18).

Importantly, our study provides evidence for the protective effect of melatonin against CP-induced ototoxicity. Rats receiving melatonin concomitantly with CP exhibited significantly shorter wave V latencies compared to the CP-only group, particularly at days 7 and 15. This suggests that melatonin pre-treatment may mitigate the delayed signal transmission caused by CP, thus preserving auditory function. These results are consistent with previous research highlighting melatonin's potent antioxidant properties and its ability to protect against oxidative stress-mediated damage in various tissues, including the cochlea (19). Melatonin's multiple mechanisms of action, including direct scavenging of reactive oxygen species, upregulation of antioxidant enzymes, and inhibition of pro-oxidant enzymes, likely contribute to its protective effects against CP-induced ototoxicity (20).

Surprisingly, ALA, despite its well-established antioxidant properties (12), did not demonstrate significant protection against CP-induced changes in wave V latency in our study. This unexpected finding suggests that the complex interplay between CP's ototoxic mechanisms and ALA's antioxidant and metabolic effects might contribute to this result. It is plausible that ALA's pro-oxidant potential under certain conditions, particularly in the presence of free iron (21), might have played a role in negating its protective effects against CP-induced ototoxicity. Further investigations are needed to clarify the specific interactions between CP and ALA in the context of cochlear damage.

Moreover, the study revealed significant differences in BAEP hearing thresholds between the groups. The CP-only group demonstrated a progressive increase in hearing thresholds over the 15-day observation period, confirming the development of SNHL. Notably, the group receiving CP and melatonin had significantly lower hearing thresholds compared to the CP-only group, further supporting the protective effect of melatonin against CP-induced ototoxicity. However, the CP+ALA group did not exhibit a significant difference in hearing thresholds compared to the CP-only group, further reinforcing the lack of protective effect of ALA in our study model.

Analysis of BAEP waveform morphology revealed transient distortions in a proportion of ears across different groups, with a higher incidence observed in the CP-only group. These distortions, often characterized by reduced wave amplitudes and altered peak latencies, provide additional evidence of CP-induced alterations in auditory signal processing within the brainstem (22). The lower incidence of waveform distortions in the melatonin and ALA pre-treatment groups, especially the normalization observed in some ears over time, further suggests a potential for these antioxidants to attenuate the severity of CP-induced ototoxicity.

This study has several limitations that should be considered. First, the study used a single dose of CP, limiting the ability to draw conclusions about the effects of cumulative CP doses, which are commonly used in clinical settings. Future studies using multiple CP doses are needed to further assess the long-term protective effects of melatonin and ALA. Second, the study only evaluated BAEPs and hearing thresholds as measures of ototoxicity. Future research incorporating histological analysis of cochlear structures would provide more detailed insights into the specific cellular and molecular mechanisms underlying the protective effects of melatonin and ALA. Finally, the relatively small sample size of each group, particularly after the loss of some rats due to peritonitis, may have limited the statistical power to detect subtle differences between the treatment groups.

Despite these limitations, this study provides valuable insights into the potential of melatonin and ALA as protective agents against CP-induced ototoxicity.

CONCLUSION

Our findings suggest that melatonin pre-treatment may effectively reduce CP-induced hearing loss in rats, potentially through its potent antioxidant properties. This finding has important implications for clinical practice, as it highlights a possible strategy to mitigate the debilitating side effects of CP treatment, improving the quality of life for cancer patients. The unexpected lack of protective effect of ALA in our study model necessitates further investigations to elucidate the complex interactions between CP and ALA in the context of cochlear damage. Future research should focus on optimizing dosing regimens, exploring combination therapies, and investigating the underlying molecular mechanisms of action to translate

these pre-clinical findings into effective clinical interventions for the prevention and management of CP-induced ototoxicity.

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