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# Dose-dependent protection on cisplatin-induced ototoxicity – an electrophysiological study on the effect of three antioxidants in the Sprague-Dawley rat animal model

## Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
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## Summary

### Background:

Sprague-Dawley rats were used as an acute cisplatin ototoxicity model to compare the chemo-protective efficacy of 2 sulphur-containing antioxidants (D-methionine, N-L-acetylcysteine) and 1 selenium-organic compound (ebselen). Each putative chemo-protective agent was tested at 3 different dosages in order to assess the influence of dose on auditory preservation.

### Material/Methods:

A total of 40 Sprague-Dawley albino male rats were used in the study. Animals were divided into 10 groups, 3 groups of different doses for each protective agent and a cisplatin-treated control group. The animals were weight-matched before drug exposure to ensure similar weights in all groups. Auditory function was assessed with auditory brainstem responses and distortion product otoacoustic emissions at time zero and at 96 hours post-treatment.

### Results:

At the post-treatment follow-up no significant threshold change at 8 kHz was found in the D-Met- and NAC-treated groups. All ebselen-treated animals presented significant threshold elevations. At 12 and 16 kHz, only the groups treated with 300, 450 mg/kg of D-Met and 475 mg/kg of NAC presented thresholds comparable to the pre-treatment ABR data. The ebselen-treated animals presented significant threshold shifts and showed the highest threshold elevations. The DPOAE data analysis showed that only the animals from the 350 mg/kg D-met group presented lack of statistical differences between the pre and post recordings.

### Conclusions:

Considering the outcome from the ABR and DPOAE analyses together, only the 350 mg/kg D-met group presented a complete auditory preservation against the 14 mg/kg cisplatin administered i.v. Data from ebselen pre-treated Sprague-Dawley albino male rats demonstrate that ebselen dosages up to 12 mg/kg given by i.p. administration lack auditory preservation in this species.

### key words:

**cisplatin ototoxicity • auditory brainstem responses • distortion product otoacoustic emissions • D-methionine • N-L-acetylcysteine • ebselen**

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## BACKGROUND

Cisplatin is an antineoplastic drug commonly used in chemotherapy worldwide. However, this chemotherapeutic agent is well-known to generate adverse effects such as nephrotoxicity [1–5] and ototoxicity [6–13]. High-dose cisplatin treatment typically induces a high-frequency hearing loss, which can gradually or suddenly extend to lower frequencies during subsequent courses [14–16]. In animal studies, cisplatin produces multiple toxic effects on the guinea pig and rat cochlea, which are characterized by lesions to the outer hair cells [13,17–19] the stria vascularis [8,9,12,20–23] and the auditory neurons [24]. Protection from these adverse effects has been widely studied, because hearing preservation is crucial for the patient's quality of life. However, no effective otoprotective treatment for cisplatin ototoxicity is currently in clinical use.

Among the numerous agents that have been proposed as effective oto-protectors are D-methionine, N-L-acetylcysteine and ebselen. These drugs can be administered systemically (i.p., i.v., or orally) or topically.

D-methionine (D-met), whether administered systemically or through the round window membrane, has been shown to protect from cisplatin-induced ototoxicity [13,23–30]. D-met is a sulphur-containing nucleophile and antioxidant with multiple protective mechanisms. D-met may protect against cisplatin ototoxicity by reversing cellular platinum-thiol complexes [31], protecting the essential amino acid L-methionine, and also functioning as an antioxidant [25,32]. Campbell [13] reported D-met pre-treatment provided complete protection *in vivo* against cisplatin-induced hearing loss and outer hair cell loss in the rat at 72 hours post-administration. Gabaizadeh [24] demonstrated that in combination with brain-derived neurotrophic factor, D-met also protects against cisplatin-induced loss of auditory neurons. Further, D-met does not interfere with cisplatin's anti-tumor action [33,34]. Ekbom [35] found that pre-treatment with D-met in guinea pigs affects the concentration of free cisplatin in the systemic circulation. Ekbom assumed that a cisplatin-methionine complex would not be cytotoxic for cancer cells, but Deegan [36] documented that a cisplatin-methionine complex is significantly cytotoxic for cancer cells *in vitro* but lacks the associated renal toxicity.

N-L-acetylcysteine (NAC), a precursor of glutathione, is an antioxidant that limits the extent of the oxidative stress damage to the cell and is able to improve the oxidant/antioxidant cellular balance [37]. The antioxidative effect has been widely documented in a number of experimental studies using various stressors. Pre-treatment with NAC was shown by Dickey [38] to protect against cisplatin ototoxicity in the Long-Evans rat model. The major mechanism of NAC cyto-protection seems to be mediated via inhibition of the effects induced by reactive oxygen species.

Of the 3 otoprotectors used, ebselen has the least documented otoprotective effect. Ebselen, an anti-inflammatory agent, has been shown to reduce cisplatin ototoxicity [39] in Wistar rats after high doses (16 mg/kg). It has also been demonstrated in Fisher 344 rats that ebselen alone (16 mg/kg) or in combination with allopurinol (each compound was given at 8 mg/kg) can protect against cisplatin-induced ototoxicity

[40]. However, a narrow range for otoprotection of ebselen has been documented in guinea pigs exposed to acoustic trauma [41].

Based on the experience in this laboratory that many pharmacological agents show a species dependence [42–44], the Sprague-Dawley rat was used as an acute cisplatin ototoxicity model [45–47] to compare the otoprotective efficacy of 2 sulphur-containing antioxidants, D-methionine, N-L-acetylcysteine, and 1 seleno-organic compound, ebselen. Each putative protective agent was tested at 3 different dosages in order to assess the influence of dose on auditory preservation. The dosages were derived from the literature or from data collected in this laboratory (D-met, NAC).

## MATERIAL AND METHODS

### Animals

Forty male albino Sprague-Dawley rats (Charles River, Italy) were divided into 10 groups; 3 groups for each protective agent and a cisplatin-treated control group.

### Experimental protocol

The experimental protocol (applied to all animals) included these steps:

1. Anesthesia with a ketamine-xylazine cocktail.
2. Assessment of the auditory function, including auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) recordings.
3. i.p. injection of the tested protector (D-met, NAC, ebselen, and saline)
4. After 1 hour delay, cisplatin (14 mg/kg) was administered to each animal by a slow i.v. infusion (for details see the next section on cisplatin).
5. ABR and DPOAE data (PS96 recordings) were acquired after 96 hours from the time cisplatin was administered.

### Cisplatin

In previous papers the authors [13,23,25,45,47] have shown that a cisplatin dosage of 16 mg/kg damages the rat cochlea. This dosage serves for an acute ototoxicity model, but it is not well related to the dosages administered in humans. Furthermore, the higher the dosage of cisplatin the higher the required dosage of the oto-protector. In order to better evaluate the behavior of the tested otoprotectors in the Sprague-Dawley rat, a lower cisplatin dosage (14 mg/kg) was administered to the tested animals. Cisplatin (Cisplatino, Ebewe, Italy) was delivered by slow infusion (0.1 ml/min.) in the caudal vein (i.v.) at a concentration of 1 mg/ml. A 1-hour interval (between the i.p. injection of each protective agent and the i.v. administration of cisplatin) was chosen on the basis of earlier results [48].

### Otoprotectors

The otoprotectors were administered as an intraperitoneal injection (i.p.) in all 10 experimental groups, 1 hour before cisplatin administration.

D-methionine (D-met; Sigma Chemical Co.) was dissolved in saline (50 mg/ml) and administered as a bolus i.p. injection.

D-met treated animals were divided into 3 groups (4 animals per group) according to dosage: groups 1, 2 and 3 received 300, 350 and 400 mg/kg D-met, respectively (dosages derived from previous pilot studies in this laboratory).

N-L-acetylcysteine (NAC; Sigma Chemical Co.) was dissolved in saline (100 mg/ml) adjusted to pH 7.0 and administered as a bolus i.p. injection. NAC-treated animals were divided into 3 groups (4 animals per group) according to dosage: groups 4, 5 and 6 received 275, 375 and 475 mg/kg NAC, respectively (dosages derived from previous pilot studies in this laboratory).

Ebselen (Sigma Chemical Co.) was dissolved in pure dimethylsulfoxide (DMSO) at 20 mg/ml and stored at 20°C. Ebselen-treated animals were divided in 3 groups (4 animals per group) according to dosage: groups 7, 8 and 9 received 4, 8 and 12 mg/kg ebselen, respectively, by i.p. bolus. The dosages for the ebselen groups were derived from data in the literature, taken with some conservation. For example, Lynch [40] reported that 16 mg/kg of ebselen can provide protection against 16 mg/kg of cisplatin in the Fischer-344 rat. The same authors also suggested that 8 mg/kg of ebselen and 8 mg/kg of allopurinol can provide similar effects. Pourbakht and Yamasoba [41] have shown that a lower concentration of ebselen (10 mg/kg) performs better than a higher concentration (30 mg/kg) of ebselen in protecting Guinea pigs exposed to noise. Considering this information, it was decided that it was safer for the objectives of the study (ie, using cisplatin at a lower concentration) to set 12 mg/kg as the maximum ebselen dose.

The last group (n=4) received an equivalent volume saline solution and served as the control group.

#### Acoustical and electrophysiological measurements

To partially compensate for the small number of animals in each tested group, the ABR threshold recordings at low stimulus levels and all DPOAE responses were recorded twice and the responses were averaged.

ABR responses were recorded by 3 platinum-iridium needle electrodes, placed subdermally over the vertex (positive), the mastoid (negative) and the dorsum area (reference/ground) of the animal. The sound transducer of a Motorola tweeter (flat response  $\pm 1.5$  dB from 4.0 to 35 kHz), was placed at a distance of 4 cm from the rat's ear. The ABRs were amplified 20 000 times and filtered from 20 to 5000 Hz. Each recording was the average of 1000 individual responses. The ABRs were elicited by 8, 12 and 16 kHz tone pips (1 ms rise-fall time, 10 ms plateau), over the intensity range of 30–110 dB SPL. The stimulus sound intensity was varied in 5 dB intervals. Threshold was based on the visibility and reproducibility of Wave III. The electrophysiological hearing threshold was defined as the lowest intensity at which a replicable ABR wave was seen in 2 averaged runs. As in previous studies [45,47], the threshold level of the Sprague-Dawley rat at frequencies up to 16 kHz was found to be approximately at 35–40 dB SPL. Ear plugs were used to occlude the contralateral ear in order to avoid binaural stimulation.

The recordings of the distortion product otoacoustic emissions (DPOAE) were conducted by a Starkey 2000 (Starkey

Labs, USA) device. The DPOAE amplitudes were analyzed in the frequencies from 6.0 to 17.0 kHz (referred to as f2) and 5 frequency points were sampled. The frequency ratio between primaries was fixed to 1.21. Each recording was made on the average from 4 seconds of data sampling, and the noise tolerance was fixed at  $-15$  dB SPL. The recordings were elicited by asymmetrical DPOAE protocol where  $L1 > L2$  ( $L1=50$  and  $L2=40$  dB SPL). Asymmetric protocols are generally considered a better choice to identify cochlear dysfunction [49,50].

During the electrophysiological recordings (ABR and DPOAE) the body temperature of the animal was maintained at  $37 \pm 0.5^\circ\text{C}$  by the use of a temperature control device (Harvard Apparatus, USA). A rectal probe was placed in order to assess the rat's body temperature changes, and a homoeothermic blanket under the rat's body regulated the heating to keep the body temperature constant for the time needed for the acquisition of recordings. All measurements were conducted at the right ear of each tested animal in a soundproof chamber.

The levels of all the stimuli used in the present study were checked by the use of a Bruel and Kjaer impulse precision sound level meter type 2209, coupled with an 1-inch condenser microphone Bruel and Kjaer type 4145 for free field use, which had a normal incidence-free field response linear from 1 to 2 Hz ( $-3$  dB) to 18 kHz ( $\pm 1.5$  dB) and meets the requirement of the ANSI (American National Standards Institute) for laboratory standard type L microphone. In addition, a Bruel and Kjaer 1/3 octave filter set (type 1616 for 1/3 octave analysis in the range 18 Hz–44 kHz covered by 34-pass band filters) was used in conjunction with the precision sound level meter type 2209.

#### Measurement of weight

Each animal was weighed on the day before the pre recordings and 4 days after cisplatin administration before the post recordings.

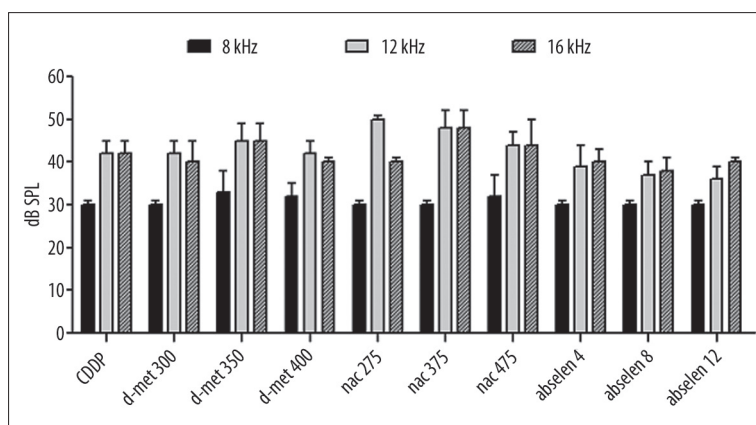
#### Statistical analysis

The ABR and DPOAE variables were evaluated for statistical significance and post-pre differences were evaluated. A 1-way ANOVA, with treatment as the factor, was fit for each protocol and response variable. Levene's test was used to check equality of variances. Estimates and confidence intervals were obtained for mean differences per treatment and for pairwise differences between mean differences for different treatments. Tukey intervals were used to maintain an overall confidence level for each variable, and a Bonferroni adjustment was made to ensure an overall 0.05 level for all intervals per interval type (mean difference or pairwise difference) and protocol.

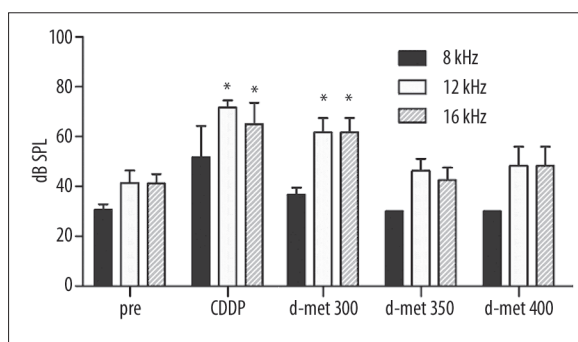
For the analysis of body weight alterations, a paired t-test was performed between the body weight at pre-and post-cisplatin administration of each experimental group.

#### RESULTS

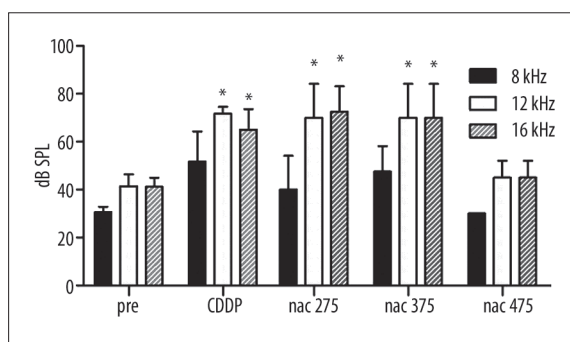
Figure 1 shows the pre-treatment hearing levels of all animals, divided per group at 8, 12 and 16 kHz. The hearing



**Figure 1.** ABR Threshold data at 8.0, 12.0 and 16 kHz for all the pre-recordings of the ten tested groups. The Y-axis depicts threshold levels in dB SPL. No statistical differences are present. Histograms are shown with standard error bars.



**Figure 2.** ABR Threshold data at 8.0, 12.0 and 16 kHz for pre-treatment, cisplatin treated animals and the D-met groups. The Y-axis depicts threshold levels in dB SPL. Statistical differences from the pre-recordings are indicated by asterisks. Histograms are shown with standard error bars.



**Figure 3.** ABR Threshold data at 8.0, 12.0 and 16 kHz for pre-treatment, cisplatin treated animals and the NAC groups. The Y-axis depicts threshold levels in dB SPL. Statistical differences from the pre-recordings are indicated by asterisks. Histograms are shown with standard error bars.

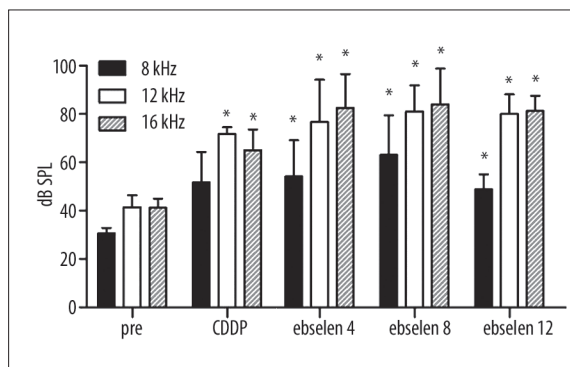
levels at 8 kHz were consistently lower than the other 2 tested frequencies, across all groups. The hearing levels at 12 and 16 kHz were very similar across all groups with the exception of the 275 mg/kg NAC group.

At 8 kHz no significant threshold change was found in the D-Met- and NAC-treated animals. In contrast, in the 3 groups pre-treated with ebselen significant hearing threshold elevations were observed. The animals treated with 12 mg/kg of ebselen presented the lowest threshold shifts among the ebselen groups, which were statistically significant in comparison to the pre-treatment data.

At 12 and 16 kHz, only 3 groups presented comparable thresholds to the pre-treatment data – the animals that received 350 and 400 mg/kg of D-met and 475 mg/kg of NAC. The ebselen-treated animals presented significant threshold shifts and showed the highest threshold elevations in the treated groups. At these frequencies the hearing thresholds across the 3 ebselen groups were similar, although the protector doses were increased 2- and 3-fold (from 4 mg/kg to 12 mg/kg).

Figures 2–4 and Table 1 summarize the ABR data at the 3 tested frequencies across the 10 treatment groups.

The DPOAE analysis showed that cisplatin causes an average negative shift in the DPOAE amplitude in the order of 20 dB in the frequency span from 6.5 to 17 kHz. Figures 5

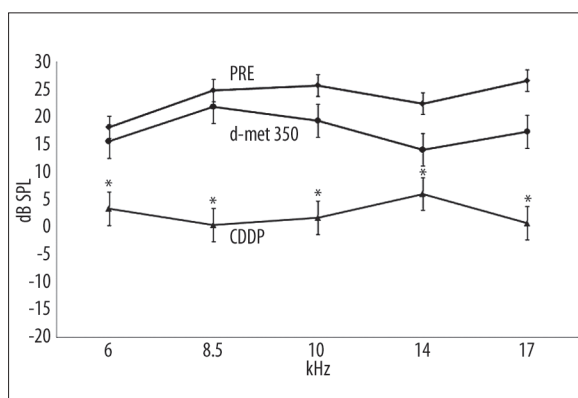


**Figure 4.** ABR Threshold data at 8.0, 12.0 and 16 kHz for pre-treatment, cisplatin treated animals and the ebselen groups. The Y-axis depicts threshold levels in dB SPL. Statistical differences from the pre-recordings are indicated by asterisks. Histograms are shown with standard error bars.

and 6 summarize the DP-Gram information from untreated and treated animals with cisplatin and otoprotectors. Only the animals from the group treated with 350 mg/kg of D-met presented lack of statistical differences between the pre and post DPOAE recordings. All other groups showed significant alterations in DPOAE amplitude, including groups 3 and 6 (400 mg/kg D-met and 475 mg/kg NAC). The 3 ebselen-treated groups showed statistical differences

**Table 1.** Post-pre ABR threshold differences of each treatment and at each tested frequency. The star symbol indicates a significance difference. The dash symbol “–” indicates no significant difference.

Treatment	Freq. (kHz)		
	8	12	16
D-met 300	–	*	*
D-met 350	–	–	–
D-met 400	–	–	–
NAC 275	–	*	*
NAC 375	–	–	*
NAC 475	–	–	–
CDDP 14	*	*	*
Ebselen 4	*	*	*
Ebselen 8	*	*	*
Ebselen 12	*	*	*

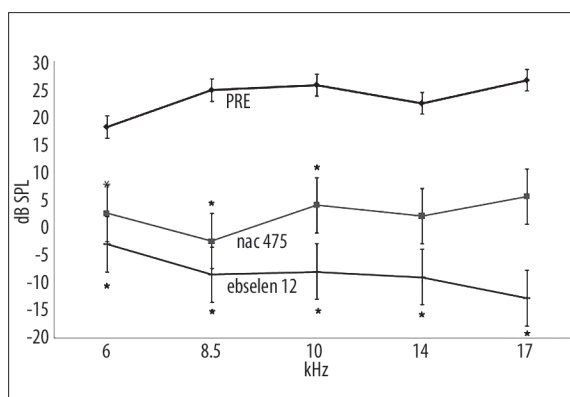


**Figure 5.** DP-Grams from untreated animals, animals treated with cisplatin (CDDP) and cisplatin plus D-met (d-met 350). The top curve (pre) represents the DPOAE responses from all tested animals. The vertical bars indicate standard error measurements. Significant differences are marked by an asterisk. The significant differences per frequency and per protector are reported in Table 2.

at all tested frequencies, and in a number of animals it was not possible to record a post-treatment DPOAE response. The DPOAE data are also summarized in Table 2. Table 3 shows the average weight loss of the experimental animals per treatment group. The data indicate that only the 375 mg NAC treatment provides partial protection against cisplatin-induced weight loss. Animals treated by 275 mg of NAC had a borderline ( $P=0.058$ ) weight loss, whereas animals included in all the remaining treatment groups showed a significant CDDP-dependant weight loss.

## DISCUSSION

We used the Sprague Dawley rat model to compare the efficacy of different dosages of 3 pharmacological agents that



**Figure 6.** DP-Grams from untreated animals and animals treated with cisplatin/NAC (nac 475) and cisplatin/ebselen (ebselen 12). The top curve (pre) represents the DPOAE responses from all tested animals. Significant differences are marked by an asterisk. The significant differences per frequency and per protector are reported in Table 2.

**Table 2.** Post-pre DPOAE amplitude differences of each treatment and at each tested frequency. The star symbol indicates a significance difference. The dash symbol “–” indicates no significant difference.

Treatment	Freq. (kHz)				
	6	8.5	10	14	17
D-met 300	*	–	–	*	*
D-met 350	–	–	–	–	–
D-met 400	–	–	*	*	–
NAC 275	–	–	–	*	–
NAC 375	–	*	*	–	–
NAC 475	*	*	*	*	*
CDDP 14	*	*	*	*	*
Ebselen 4	*	*	*	*	*
Ebselen 8	*	*	*	*	*
Ebselen 12	*	*	*	*	*

have been reported to exert a protective effect against cisplatin ototoxicity in other strains of rats. Experimental studies have shown that cisplatin administration causes the formation of reactive oxygen species in the inner ear, leading to lipid peroxidation, triggering of apoptosis and a significant alteration of the auditory function [11,51]. The ototoxic effect in experimental animals is dose-dependent and is manifested as an increase of the electrophysiological hearing threshold.

There are numerous reports in the literature [13,23–30,47,52] describing the protective effects of various oto-protectors against cisplatin, usually evaluated in a short time-period (e.g., 72 hours). In the majority of studies the ABR has been utilized to assess the hearing threshold of the tested animals. In this study the efficacy of the tested pharmacological



**Table 3.** Animals were weighed on the day of the electrophysiological recordings and 4 days after cisplatin treatment (96 h). In the first column is reported the average weight loss per group in gr. A paired-t-test on the body weight data was performed on each group. The star symbol indicates a significance difference. The dash symbol “-” indicates no significant difference. BL means border line difference.

	Weight loss (gr)	STD	P	
D-met 300	60.00	11.14	0.011	*
D-met 350	31.25	11.35	0.012	*
D-met 400	45.00	8.66	0.012	*
NAC 275	38.00	2.83	0.058	BL
NAC 375	17.00	7.07	0.182	-
NAC 475	52.67	12.06	0.017	*
CDDP	73.33	15.28	0.014	*
Ebselen 4	44.43	17.36	0.000	*
Ebselen 8	58.00	14.42	0.000	*
Ebselen 12	41.25	4.79	0.000	*

agents was additionally evaluated with distortion product otoacoustic emissions. The combined information from both measurements provides an enhanced understanding of the events related to inner ear and neural fiber otoprotection.

The ABR data from the D-met group confirm the previous findings of Campbell [13,23] in the Wistar rat, suggesting that dosages of 300–400 mg/kg have the potential to protect the inner ear. In terms of performance, the higher dosages (350 and 400 mg/kg) showed better auditory preservation across all the tested ABR frequencies. The DPOAE responses (shown in Table 2) revealed a different scenario. Of the 3 tested protocols, only the moderate dose (350 mg/kg) group generated responses with no significant pre-post differences, and in light of this only the moderate dose can be considered as a candidate for auditory preservation. The data from the D-Met DPOAE responses suggest that increasing the amount of otoprotector does not necessarily increase the index of auditory preservation.

The ABR data from the NAC group showed a partial auditory preservation in the 275 and 375 mg/kg groups and a complete preservation in the 475 mg/kg group. These findings are similar to those of Dickey [38] in which a pre-treatment injection of 400 mg/kg NAC was able to prevent ototoxicity at a considerably lower dose of cisplatin (6 mg/kg) in the Long-Evans rat. In their study, NAC administration was given 30 minutes and 4 hours before cisplatin injection to reduce ototoxicity. The DPOAE responses from the NAC treated animals showed significant amplitude changes across many frequencies. In terms of performance, the best results were observed in the 375 mg/kg group. By integrating the information from the ABR and DPOAE data, we conclude that the NAC protocols do not seem to offer complete auditory preservation in dosages of up to 400 mg/kg.

An explanation for the observed discrepancy between the ABR and DPOAE findings in the D-met and NAC groups cannot be given only from the electrophysiological findings; however, it is reasonable to speculate that the effect on the DPOAE recordings could be a result of minor loss of outer hair cells in the treated animals. Considering the time window of observation (96 hours) the DPOAE data might indicate cell death of the outer hair cell population. To elucidate this argument further additional studies utilizing longer observation windows (168 hours or longer) are required in order to verify the assumed apoptosis scenario.

Results of the present study clearly show that ebselen pre-treatment did not protect the cochlea in the Sprague Dawley albino male rat. This finding is contradictory to earlier reports, showing amelioration of cisplatin ototoxicity in the Fischer 344 rat given a 16 mg/kg dose of cisplatin [40]. Moreover, Rybak [39] showed that the hearing of the Wistar rat was protected by ebselen (16 mg/kg) in connection to cisplatin treatment. Lynch et al. [40] have shown that in the Fischer 344 rat, 8 mg/kg of Ebselen administered orally with 8 mg/kg of allopurinol offers protection similar to a single 16 mg/kg ebselen dose. The ABR and DPOAE data from this study show that in the Sprague Dawley rat there is no significant otoprotection from an i.p. administration of ebselen in dosages of up to 12 mg/kg. The maximum ebselen dosage used in this study is lower than the dosages reported in the literature (16 mg/kg) to compensate for the lower cisplatin dosage employed (14 vs. 16 mg/kg). In addition, the pattern of the ABR/DPOAE data from the ebselen-treated animals was different than the data from the other 2 protectors, even at dosages that did not offer protection at all frequencies. Non-optimized dosages of D-met or NAC presented some protection in 1 of the tested ABR/DPOAE frequencies, but this was not the case for ebselen.

One may speculate whether less favorable pharmacokinetics or pharmacodynamic parameters influenced the putative protective effect of ebselen in the cochlea after the i.p. injection. The earlier studies reporting positive inner-ear protection effects have all used the same administration route for both ebselen and cisplatin. The findings of amelioration of ototoxicity in these studies might be explained by a direct drug interaction in the blood compartment not obtained by the present treatment protocol. Possible interactions between ebselen and cisplatin in the systemic circulation could lower the level of free cisplatin and thereby result in less cochlear injury. The key element of this hypothesis is the absorption time of ebselen from the intraperitoneal cavity of the employed animal model. Longer absorption times would promote stronger interaction effects between the 2 pharmacological agents, ebselen and cisplatin, whereas rapid uptake of ebselen and a fast elimination would promote higher concentrations of free cisplatin to reach the inner ear. Another explanation is that the levels of ebselen could be affected by first-pass hepatic losses. The pharmacokinetics of ebselen after i.p. administration has not been studied in the rat. Strain-specific differences for otoprotection are less plausible in explanation of lack of otoprotection, although species specific differences in otoprotection are not uncommon. For example, Duan [53] has reported species otoprotection variability against impulse noise using NAC. The data from the present study strongly suggest that additional studies are needed on strain-specific

otoprotection to better define the benefits with systemic ebselen otoprotection.

One of the methodological objectives of the study was the minimization of the drug interaction between cisplatin and the otoprotectors. The reasons behind this objective are rooted in the clinical environment, where it is essential to obtain maximum chemotherapeutic effect while preserving the hearing of patients treated with cisplatin-based chemotherapy. There are grounds for speculation that the time interval between administration of an otoprotector and administration of cisplatin, as well as the mode of drug administration, affect the outcome. The risk of systemic drug interaction suggests that an otoprotector and cisplatin should be given separately, not only in time, but also, ideally, by separating the routes of administration. This is why cisplatin was given i.v. and the otoprotective substances were given i.p. in the Sprague-Dawley albino male rats. In humans, 2 modes of systemic administration can be achieved by using i.v. and intra-arterial administration when possible. Intra-arterial infusions of cisplatin have been given to patients with advanced hypopharyngeal cancer concomitant with i.v. sodium thiosulfate to reduce adverse effects [54]. Pre-administration of the thiol-containing antioxidant was used allow the protective agent to accumulate in the inner ear before cisplatin reached the target cells. No pharmacokinetics analysis was undertaken and therefore no such data can be given to explain our findings. As an i.p. administration of the otoprotector was used, one can speculate that the peak concentration in the blood of otoprotector and cisplatin was reached within 1 hour. Another to consider in otoprotection is the transport of the drugs over the blood-labyrinth barrier. Cisplatin is reported to have a peak concentration in the scala tympani perilymph 20 minutes after an i.v. injection [55]. No information can be found in the literature on the cochlear kinetics of the used otoprotectors after the i.v. administration. Thio-sulfate, another thiol-containing antioxidant, is readily transported to the inner ear and reaches its peak concentration in scala tympani perilymph within 10 minutes after an i.v. injection [56]. By the use of round window membrane administration, the terminal half-life of D-met in scala tympani perilymph has been estimated to 0.6 hour [57]. To obtain maximal otoprotection and minimal drug interaction between a thiol-containing antioxidant and cisplatin, it is necessary to acquire data from additional studies on blood and inner ear pharmacokinetics.

## CONCLUSIONS

- The ABR data show that animals given 350 and 400 mg/kg of D-met and 475 mg/kg of NAC presented significantly better auditory preservation than the other groups.
- The DPOAE data show that only animals receiving 350 mg/kg of D-met presented no significant differences between the pre and the post recordings. Combining this information with the ABR data, it can be concluded that only D-met shows a complete auditory preservation 96 hours after cisplatin administration.
- Findings from ebselen pre-treated Sprague-Dawley albino male rats demonstrate that ebselen dosages up to 12 mg/kg given by i.p. administration lack auditory preservation effects in this species.

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