

Reducing Aminoglycoside Ototoxicity

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ABSTRACT:

Objective: The objective of this project was to determine whether sodium thiosulphate (STS) could be used clinically to reduce hearing loss caused by gentamicin, an aminoglycoside. First, we established the appropriate dose of gentamicin to induce a mild to moderate sensorineural hearing loss and the most appropriate time to test the hearing loss. Second, we used this model to assess the protective effects of STS and α -tocopherol on gentamicin induced ototoxicity.

Methods: C57 male mice (4 – 5 weeks old, 150 – 200g).

Phase 1: Thirty mice received gentamicin in increasing doses from 160-220 mg/kg and underwent auditory brainstem response (ABR) testing to assess hearing changes over 3 months. This dose-response relationship was used to optimize Phase2.

Phase 2: Six groups of 64 mice were studied. Group1: gentamicin 220 mg/kg i.p. with isotonic (25%) STS 1600 mg/kg. Group2: gentamicin 220 mg/kg i.p. with α -tocopherol 400 mg/kg. Group3: STS 1600 mg/kg i.p. Group4: α -tocopherol 400 mg/kg i.p. Group5: saline i.p. Group 6: gentamicin 220 mg/kg i.p. ABRs were recorded at baseline and at 30 days.

Results: Phase 1: The optimal mouse model for this type of research should employ a dose of 220 mg/kg and study animals at one month.

Phase 2: Threshold changes for the treatment group that received STS with gentamicin were not statistically significantly different from gentamicin without STS. Gentamicin induced threshold shifts of about 15 dB at one month and great variability was found in the response.

Conclusion: STS does not offer protection against gentamicin induced hearing loss.

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INTRODUCTION:

Aminoglycosides are a class of antibiotics that are highly effective at treating gram negative infections and tuberculosis (1,2). Despite their benefits, aminoglycosides have long been recognized to cause nephrotoxicity, vestibulotoxicity, and ototoxicity (3). Ototoxicity is a major side effect of aminoglycosides – it affects up to 33% of patients receiving aminoglycoside (4-8). Ototoxicity, in this paper, is defined as cochlear injury which can manifest as hearing loss and or vestibular dysfunction.

Since hearing loss is not visibly apparent, the extent to which such changes affect patients' lives and society may be overlooked. Patients with acquired hearing loss often withdraw from social situations in an attempt to avoid the difficulties of trying to converse (9). This narrowing of interpersonal and social life leads to increased feelings of frustration, sadness and isolation (9-12). Studies show that hearing loss is consistently associated with increased levels of depression and reduced quality of life (9,13-15). Research showed that the incidence of mental illnesses among deaf people is four times greater than the incidence in the general population (9). Additionally, in children, hearing loss leads to difficulties with speech and language development, learning, and communication (16). The impacts of hearing loss extend even beyond the individual and the loss of productivity in patients with severe to profound hearing loss costs society an estimate of \$297 000 throughout a patient's lifetime (17).

In the past, despite their toxicities, aminoglycosides have been widely used because they are extremely efficacious antibiotics (1-3). Currently, as new expensive antibiotics with fewer side effects enter the market, patients in developed countries can afford to make a change, while patients in developing countries continue to depend on the only economically affordable antibiotic – aminoglycosides (4,18,19). Unfortunately, in developing countries, these drugs are loosely regulated, and thus, have resulted in a significantly higher incidence of drug toxicity, with estimates suggesting that these drugs are responsible for up to 66% of all deaf-mutisms in those countries (20). If a simple and effective method of reducing ototoxicity can be implemented, many of the negative social, economic, psychological, and emotional impacts of hearing loss could be alleviated, if not eliminated.

Unfortunately, the mechanism of ototoxicity has not been thoroughly elucidated. Much of the current research suggests that aminoglycosides generate free radicals which cause the sensory cells in the cochlea to undergo apoptosis, eventually leading to hearing loss (21-24). This theory is supported by the discovery of gentamicin's ability to chelate iron and form a compound with oxidative properties (23). By binding iron, the aminoglycoside becomes redox-active and can form free radicals which subsequently damage various components of cells such as lipids, proteins, and DNA, leading to permanent hearing loss (25,26). This theory is further supported by histological findings of hair cells that appear to have undergone apoptosis rather than necrosis and the presence of oxygen and nitrogen free radical species in the cochlear hair cells (26).

With this mechanism in mind, researchers sought out ways of protecting against ototoxicity. Extensive research has led to the discovery of at least 30 compounds that have some protective effects against ototoxic compounds (27). One compound that may be promising is sodium thiosulphate (STS). We theorize that the antioxidant properties of STS will neutralize the free radicals thought to be central to the development of ototoxicity. In addition, previous studies have demonstrated that STS protects against cisplatin induced ototoxicity (28-30). Because the pathophysiological mechanism of both cisplatin and gentamicin involves the generation of free

radicals, we hypothesize that STS will also protect against gentamicin induced ototoxicity (28-30). Another antioxidant that also may be promising is α -tocopherol – vitamin E. There are studies that suggest that α -tocopherol also protects against both gentamicin and cisplatin induced ototoxicity (31,32). The purpose of this study is to evaluate the protective effects of STS and α -tocopherol against gentamicin induced ototoxicity in a mammalian model.

MATERIALS AND METHOD

In this study, male mice (5 weeks old, 150-200g) were used. The University of Manitoba Animal Research Ethics Board approved the procedures and use of animals in this study according to their guidelines. All animals were handled with care and in a humane way.

Functional evaluation of hearing

Hearing loss can be objectively measured with auditory brainstem response (ABR) testing, which is the measurement of electrical response of the cochlea and its brainstem pathway (33). Although other techniques do exist, ABR testing is more feasible in this situation due to its lower variability and ease of performance (34).

ABR testing was done on each mouse for the functional evaluation of hearing at baseline (day 0) and again at day 30. Each mouse was put under a general anaesthetic, using i.p. ketamine and Rompun in a 10:1 ratio to prevent movement. The animal was placed on a warming blanket to maintain body temperature at 37°C. To minimize electrical and acoustic interference, the ABRs were recorded inside an Eckel AB2000 sound booth with added copper electrical shielding, grounding and echo-reducing carpet lining. ABRs were recorded using 3 subcutaneous electrodes. The recording electrode (positive) was inserted immediately medial to the pinnae and was secured in a position that allowed the tip to be in direct contact with the skull. The reference electrode (negative) was placed subcutaneously posterior to the contralateral ear. The ground electrode (neutral) was placed subcutaneously in the contralateral flank.

Using high frequency transducers from Intelligent Hearing Systems, 256 pure tone pip stimuli were delivered at 31.3 sec⁻¹ while averaging the 12 ms. evoked EEG waveform. Auditory thresholds were obtained at 6, 8, 12 and 24 kHz using intensities decreasing from 90 dB SPL to 10 dB SPL in 10 dB SPL steps. The responses were band-pass filtered from 300-3000 Hz using the system II PC computer-based auditory research system from Tucker-Davis Co. (Gainesville, FL) and stored on diskette for analysis later. Using differential recording, the preamplifier amplified the two channels by a hundred fold before attenuation to optimize digitization and the analog to digital conversion. The auditory threshold was determined to be the highest sound threshold where the ABR waveform is not appreciable.

Phase 1: Establishing an animal model

First, we established the optimal dose of gentamicin that would yield a statistically significant change in hearing, and determined the optimal time to assess this change. The data collected, were used to determine two criteria: the dose of gentamicin that would yield a moderate degree of hearing loss without fatal toxicities, and the time it took for the hearing threshold to peak. In clinical practice most patients have minimal hearing loss, but a few patients have large hearing losses. For this reason we wanted to create small but measurable changes in hearing, anticipating that many animals would not have significant hearing loss. We hoped to establish an animal model that would mimic several observations from clinical practice.

An initial dose of gentamicin was determined by a literature search of auditory changes in mice given intraperitoneal (i.p.) gentamicin injections. Reported LD50 values of i.p. gentamicin injections in mice were found to range from 245mg/kg to 305 mg/kg (35-37). In addition, a study showed that C57 mice, with either single or 19 daily doses of 120mg/kg, incurred a slight but statistically similar degree of hearing loss (38). Based on these values, an initial injection of 140 mg/kg was selected and given to the first group of five mice. This procedure was repeated with subsequent groups of five mice receiving a systematically higher dose than the previous group until the mice achieved either a significant degree of hearing loss or showed signs of organ specific toxicities. We used a total of 6 different groups of 5 mice, with each group given a corresponding injection of 140, 160, 180, 200, 210 and 220 mg/kg of gentamicin. At each increment we monitored the mice for 30 minutes post injection for signs of acute gentamicin reaction in the form of a neuromuscular blockade manifesting as acute respiratory failure (39).

To assess hearing and to determine the best time to appreciate the hearing changes, ABRs were recorded immediately prior to the injection (baseline) and again at 14, 30, and 60, days.

Analysis of variance (ANOVA) for repeated measures was used to determine whether the changes in threshold were significant across frequency and gentamicin dose (SPSS, Inc., Chicago, IL). The data were analyzed with the Greenhouse-Geisser test for sphericity and adjusted for the degrees of freedom accordingly. A significance of $p < 0.05$ was set. Age and frequency were set as within-subject factors and treatment group was set as a between-subject factor. The 30 mice that established the animal model were analyzed using a full-factorial univariate ANOVA for repeated measures.

The dose-response relationship was assessed using GraphPad Prism software package. Hearing loss at 30 days as a function of dose of gentamicin (mg/kg) versus hearing threshold across all frequencies. We fit the data using the sigmoid shape of the log of the concentration because pharmacologic data is typically fit to this shape. The curve-fitting algorithm is performed by fitting the data to equation 1.

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{-(\text{LogEC50} - X) * \text{HillSlope}}) \quad \text{eq. 1}$$

Where **EC50** is the concentration of gentamicin that gives a response half way between Bottom and Top. Prism reports both the EC50 and its log. **HillSlope** is the steepness of the curve. A Hill slope greater or less than 1.0 is steeper or shallower respectively. **Top** and **Bottom** are plateaus in the units of the Y axis.

Phase 2: Determining protective effects against ototoxicity

Six treatment groups of C57 mice were used in this study. They were randomly divided (Table 1). Equal volumes of 0.8 cc were delivered to all groups to allow for hydration as well as for medication delivery:

1. Gentamicin 220 mg/kg i.p. preceded by isotonic (25%) sodium thiosulfate 1600 mg/kg.
2. Gentamicin 220 mg/kg i.p. preceded by α -tocopherol 400 mg/kg.
3. Sodium thiosulfate 1600 mg/kg i.p. only
4. α -tocopherol 400 mg/kg only
5. Negative control: saline i.p. only
6. Positive control: gentamicin 220 mg/kg i.p. only

According to the formula for comparing the means of two normally distributed samples of equal size using two-sided test rounds, and based on sample standard deviation of ABR data from

previous work with the same protocol. A sample size of 8 was needed to achieve a significance of $p < 0.05$, and a power of 80% that can detect a 20dB difference between 2 groups (40). Since there is an expected 25% rate of data loss due to other diseases, toxicity-related death, pre-existing hearing loss and/or serous otitis media, a sample size of up to 12 mice per group was chosen. Fortunately, with fewer deaths than expected the sample sizes per group were set to 11 mice (Table 1).

Our hope was to create a model that reflects the clinical situation for gentamicin in at least two respects. Firstly, in clinical practice hearing changes are usually small and, secondly, many patients do not experience hearing changes. Although gentamicin is ototoxic, only a small number of patients experience profound hearing loss but these are the patients that we are aware of. Phase I of this project seemed to provide a model that met these two criteria. The anticipated small changes in hearing mandated a fairly large number of tests.

For this project, the term “condition” refers to a specific test parameter or factor to be studied such as a particular animal, auditory frequency tested (6, 8, 12 or 24 kHz), ear (right or left), treatment group, and day of testing (baseline or 30 days after treatment). Our dataset contained almost 1000 ABRs considering all mice, groups, days, and ears.

Data reduction and simplification:

Although we have valid data to address several questions, thoroughly dealing with each of them in one report would result in a prohibitively large, unfocussed report. The objective of this project is to assess the possible effect of STS as a protective agent so the analysis will focus on that topic, briefly mentioning other possible question such as the possible effect of α -tocopherol on auditory threshold, influence of gentamicin across different frequencies, and right/left ear differences and alternative analytic techniques. Results are therefore presented first for overall thresholds 30 days after treatment for all treatment groups, then after data reduction, for STS and gentamicin only.

Excluding the α -tocopherol data, 43 mice in the first 3 groups of Table 1 remained. The change or shift in hearing over 30 days was assessed by subtracting final threshold from initial or baseline threshold. This simplified analysis and reduced the number of measurements by half while preserving information. Right and left ears were included as separate measurements. For 43 mice X 2 ears X 4 frequencies 344 conditions were studied. Data storage failure resulted in preclusion of 2 ABR recordings, thus, yielding 342 data runs for analysis

The nonparametric Kruskal-Wallis and Median tests were used to assess STS protection against gentamicin-induced hearing loss. The two tests are similar in some ways but yield different information. The Kruskal-Wallis test ranks all the data (in this case the threshold shift) and determines whether or not there is a significant difference in rank across treatment groups. The median test assesses the median, rather than the mean as in parametric tests, of each treatment group and determines whether the median differs significantly across the groups.

RESULTS

Part 1: An animal model

When we compared different concentrations of i.p. gentamicin injections and the hearing thresholds at 14 days, 30 days, and 60 days, two trends are seen (Figures 1, 2). First, hearing loss is maximal at 1 month. Second, a dose at 220 mg/kg yielded the highest degree of hearing loss, around 20 db. At 240 mg/kg, 4 mice exhibited signs of acute gentamicin toxicity and were

humanely terminated. Significant values were found for frequency ($p < 0.001$) and the interaction between frequency by month ($p = 0.049$). At 5 months, 10 mice were assessed again and exhibited no physical signs of long term gentamicin toxicity. There were no statistically significant changes in the hearing thresholds at 5 months compared to 1 month ($p = 0.106$).

The dose-response relationships shown in Figure 1 are between hearing threshold at 30 days and dose of gentamicin (mg/kg) across all frequencies. Pharmacologic data is typically fit using the sigmoid shape of the log of the concentration for classical dose-response curve as illustrated in Figure 1b. The EC 50 was 174-224 mg, suggesting that our dose of 220 mg/kg is appropriate. Figure 1a may be easier to interpret than Figure 1b, so both are shown.

These results suggest that the optimal animal model to assess the auditory effects of gentamicin should employ a dose of 220 mg/kg of gentamicin and assess the response at 30 days.

Part 2: Protective effects against ototoxicity

As predicted from our model, the mice had small hearing losses and for most conditions there were no changes. Three animals died during the anaesthetic. Of 342 ABRs there was no threshold shift in 241 (70%). Figure 3 shows the overall final thresholds 30 days after treatment and tables 2 and 3 provide the mean and 95% CI thresholds by group (table 2) and frequency (table 3). Figure 1a and Tables 2 and 3 are meant for illustration only, not for statistical interpretation. It should be pointed out that although this is the usual format for presentation of scientific data, our data are not normally distributed so the use of 95% C.I. may be misleading.

Are the data normally distributed?

A requirement for parametric statistical analysis is the assumption that the data conform fairly well to a normal distribution. If the data are not normally distributed a non-parametric method of analysis should be used. Although we should presume that our data are not normally distributed simply because 70% had no shift in threshold, a more quantitative assessment seems proper. SPSS offers two quantitative methods of assessing the departure from normality. The first is to divide the skewness statistic by the standard error of the statistic and if the result is greater than 2.5, normality cannot be assumed. The second method is to simply use the skewness statistic alone. If the absolute value of the skewness statistic is greater than 1.0, normality cannot be assumed (41). Across the six treatment groups, four frequencies and right versus left ears, calculations for each (96 calculations, not shown) of the assumptions of normality appeared to be violated in 89 so clearly, a non-parametric analysis for the results of STS and gentamicin-induced hearing loss is indicated.

Is STS otoprotective?

Figure 4 displays the threshold shift (not absolute threshold as in Figure 3) by frequency for the four treatment groups including all data. Inspection of the graph does not suggest striking differences but the two treatment groups without gentamicin (STS and saline) had less hearing loss than the two groups with gentamicin (gentamicin and gentamicin + STS). If STS is protective we would expect that the STS + gentamicin group would have less hearing loss than the gentamicin alone group, but this is not the case.

Table 4 shows the results of the Kruskal-Wallis test to assess the effect of STS on gentamicin-induced hearing loss. The results suggest that there are no significant differences across treatment groups ($p = 0.075$) but the median test (Table 5) suggests that there are highly significant differences ($p < 0.001$). In this project our main interest lies in whether there is a significant difference between the STS + gentamicin treatment and the gentamicin alone

treatment. If there is a difference then it would suggest that STS is protective, which the main question of this project. In order to address this discrepancy between the Kruskal-Wallis test and median test, pair-wise tests were performed for each of the pairs of treatment groups. These are shown in Table 6. These data suggest that the four treatment groups can be considered as two sub-groups of two treatments. The two treatments that included gentamicin (gentamicin and gentamicin + STS) had significantly greater hearing loss than the two treatment groups without gentamicin (saline and STS only). Our data shows that there are no statistical differences in hearing loss by ear or across frequencies for all doses. These data suggest that STS is not protective against gentamicin-induced hearing loss.

A possible criticism of our data could be that some of the mice had poor baseline hearing and this could have affected our results. In fact, we had planned to exclude mice with hearing worse than 30 dB. For this reason the data was analyzed again using data only for mice with baseline hearing <30 dB, but the Kruskal-Wallis and median tests results did not change. The threshold shift across frequencies was not significant by either the Kruskal-Wallis test ($p = 0.613$) or the median test ($p = 0.561$).

DISCUSSION

Globally, every year, millions die from gram-negative sepsis and about a third of the world's population is affected by tuberculosis (42). Aminoglycosides are an inexpensive antibiotic capable of effectively treating these infections, but its use is limited by ototoxicity (1-8).

Recently, much research has been done to try to elicit the exact mechanism of aminoglycoside induced ototoxicity and ways to attenuate it. The current theory of the mechanism suggests that aminoglycosides chelate iron to generate reactive oxygen species within the inner ear leading to the activation of apoptosis and permanent damage of hair cells and neurons (21-24). Based on these mechanisms, several studies have evaluated the effectiveness of various iron chelators and antioxidants at protecting against aminoglycoside induced ototoxicity (4,20,29,43-47). Despite there being a few studies on protectants, there is still a lack of high quality data that could justify the use of a protectant in widespread clinical trials.

Before assessing the protective effects of various substances on gentamicin induced ototoxicity, we established a dose response curve that would guide us in determining the appropriate dose of gentamicin and testing day. The results from this could significantly contribute to the literature for three main reasons. Firstly, this curve would allow researchers to predict the expected degree of hearing loss with corresponding injections of gentamicin in mice. Secondly, any past or current inconsistencies in the literature will become apparent. This is something that must be addressed due to the wide variability of gentamicin dosages used in studies in the literature. Thirdly, this animal model would provide the backbone for future investigation of the effectiveness of various other protectants. Because the protocol has already been laid out, by interchanging promising protectants, we could facilitate the process of discovering the most clinically and economically effective substance.

We studied the protective effects of two antioxidants, STS and α -tocopherol, on gentamicin induced ototoxicity. Because it is proposed that ototoxicity occurs due to the generation of reactive oxygen species, we considered the possibility that the two antioxidants could attenuate the ototoxicity (21-24). However, the scope of this paper focuses on the protective effects of STS (and not α -tocopherol) on gentamicin induced ototoxicity.

Our data suggest that across all frequencies with treatments of STS there are no statistical differences in hearing loss. Because there are no differences across frequencies, it would be valid to analyze the data by combining the data across the frequencies, thus, simplifying the analysis.

Phase 2 of our results showed that STS does not protect against gentamicin induced ototoxicity. The results from this project suggest that it would not be wise to pursue testing in a clinical setting. The protocol from this project could be easily adopted and be used to assess protective effects of other protectants. Because the dose-response curve is already established in phase 1, only phase 2 needs to be repeated with other possible protectants.

Undoubtedly, the results of studies on hearing loss from gentamicin would help prevent countless patients in the future from developing aminoglycoside induced ototoxicity. If it becomes possible to clinically prevent ototoxicity, it would be beneficial to use higher dosages of aminoglycosides because their bacteriocidal effect is concentration-dependent rather than time-dependent (48). Patients would benefit because higher dosages would lead to faster recoveries from infections, fewer complications and lower chances of creating resistant microbes. Higher dosages could be especially beneficial in the treatment of tuberculosis, where drug resistance is a major problem (25). Because, both STS and α -tocopherol are already approved for human use, the results from this project could be readily adopted world-wide.

In addition, if ototoxicity could be attenuated or eliminated, patients who would otherwise develop hearing loss would evade the negative impacts of acquired hearing loss (9-16). Overall, this could help prevent patients from developing depression, and other mental illnesses associated with acquired hearing loss (9-15). Children, not only would benefit from the effective treatment of infections, but also would save themselves from the loss of sense at a time where auditory learning and exploration is crucial to normal development (16). Of course, the development of protectants against gentamicin induced hearing loss would obviously save countless people from hearing loss, but that is only secondary. Ultimately, due to the cost and therapeutic effectiveness of gentamicin, and a protective agent, the results from this project could be used to develop an inexpensive combination of drugs that would globally help to improve people's health and productivity.

CONCLUSIONS:

1. STS is not protective against gentamicin-induced hearing loss even when the losses are small.
2. Other compounds should be studied to assess the possibilities for protection against gentamicin ototoxicity.
3. Gentamicin may cause hearing losses in some mice but the magnitude and incidence are only about 10 dB and 30% respectively in mice using this model. These findings are analogous to the clinical situation and we feel are meaningful.
4. An i.p. dose of 220mg/kg of gentamicin is ideal for inducing a moderate level of hearing loss in C57 mice while minimizing fatal toxicities. At this dose, hearing loss peaks at approximately 30 days post injection. STS and α -tocopherol may be useful as a preventive agent against gentamicin induced ototoxicity.

TABLES AND FIGURES:

	n	Deaths (excluded from “n”)
Saline	5	
Gentamicin	17	1
Sodium thiosulphate (STS)	10	1
Gentamicin + STS	11	
α -tocopherol	10	
α -tocopherol and Gentamicin	11	1
TOTAL	64	3

Table 1. Assignment of mice to overall Treatment groups. In analysis of the protective effect of STS against gentamicin-induced hearing loss the vitamin E and vitamin E + gentamicin groups were excluded, leaving 43 mice.

Treatment Group	Mean threshold (dB SPL)	95% C.I.
Saline	4.5	0.14-8.86
Gentamicin	12.55	0.52-16.58
STS	4.50	1.63-7.37
STS + gentamicin	14.46	9.93-18.99
Vitamin E	11.44	7.01-15.86
Vitamin E + gentamicin	8.01	4.66-11.37

Table 2: Thresholds at 30 days by treatment group. On this SPL scale we consider 0 dB as normal. Thresholds at 30 days were not greatly elevated.

Test Frequency (Hz)	Mean Threshold (dB SPL)	95% C.I.
6000	5.36	3.08-7.64
8000	4.49	2.58-6.40
12000	14.77	10.70-18.84
24000	14.68	10.67-18.69

Table 3 – Thresholds at 30 days by test frequency including all treatment groups. Although thresholds are slightly greater for higher frequencies, these differences were not statistically significant (see Kruskal-Wallis and median test results below)

Table 4a: Rank

	Treatment Group	N	Mean Rank
Diff	Saline	40	172.94
	Gentamicin	134	179.77
	STS	80	148.82
	STS and Gentamicin	88	178.87
	Total	342	

	Diff
Chi-square	6.908
Df	3
Asymp. Sig.	.075

Table 4 – Kruskal-Wallis test results for all treatment groups. Table 4a shows the rank levels for the various groups. The gentamicin group had the highest scores, followed closely by the STS plus gentamicin group, suggesting that STS is not protective. Table 4b indicates that the differences among the groups are not significant ($p=0.075$).

		Treatment			
		Saline	Gentamicin	STS	STS and Gentamicin
Diff	> Median	6	44	8	30
	<= Median	34	90	72	58

	Diff
N	342
Median	.00
Chi-square	19.528
df	3
Asymp. Sig.	.000

Table 5 – Median test results for all treatment groups. Table 5a shows the distribution of the medians for the various groups. The gentamicin group had the highest percent of measures above the median, followed closely by the STS plus gentamicin group, suggesting that STS is not protective. Table 5b indicates that the differences among the groups are highly significant ($p<0.001$). This result is in conflict with the data on Table 4. In order to resolve this conflict, median tests were conducted pair-wise for each pair of the four treatment groups.

	Gentamicin	STS	Gentamicin + STS	saline
Gentamicin	-	0.021	0.846	0.047
STS		-	0.000	0.421
Gentamicin + STS			-	0.026
saline				-

Table 6 – Pair-wise comparisons across groups for the median test. This table reveals that the significant differences from table 5 arise between either of the treatment groups with gentamicin and the STS or saline treatments. The gentamicin treatment does not differ from the gentamicin and STS group indicating that STS does not have a protective effect against gentamicin-induced hearing loss.

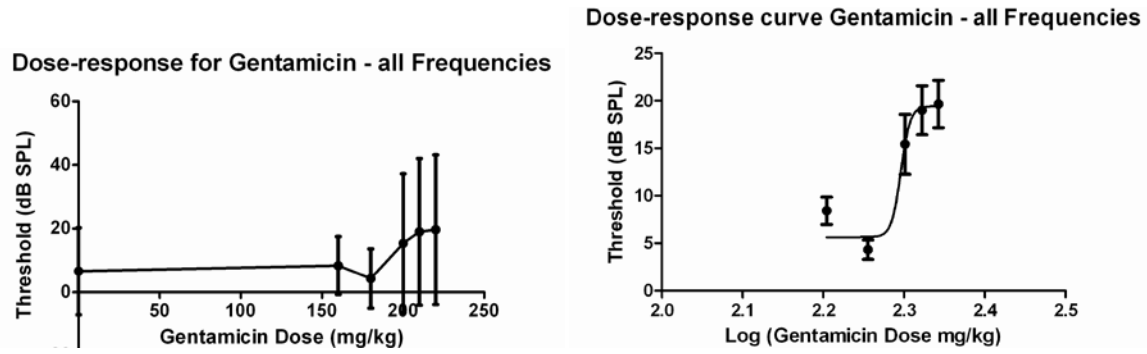


Figure 1 – Dose-response relationship between gentamicin dose and ABR threshold for all frequencies together. Figure 1a: Dose response curve for gentamicin. Figure 1b: Classic dose-response curve for Log(gentamicin) and auditory threshold. Note that the dose must be greater than 180 mg/kg and that maximal hearing loss occurred at 220 mg/kg – the highest dose. We cannot deliver higher doses than this as the LD50 for gentamicin (depending on the source, is from 224-245 mg/kg for mice. Even at the maximum dose the changes in hearing are only about 20 dB. Large doses cause death by inducing neuromuscular paralysis. There appears to be a typical sigmoid-shaped curve with the EC50 at 174-224 mg/kg.

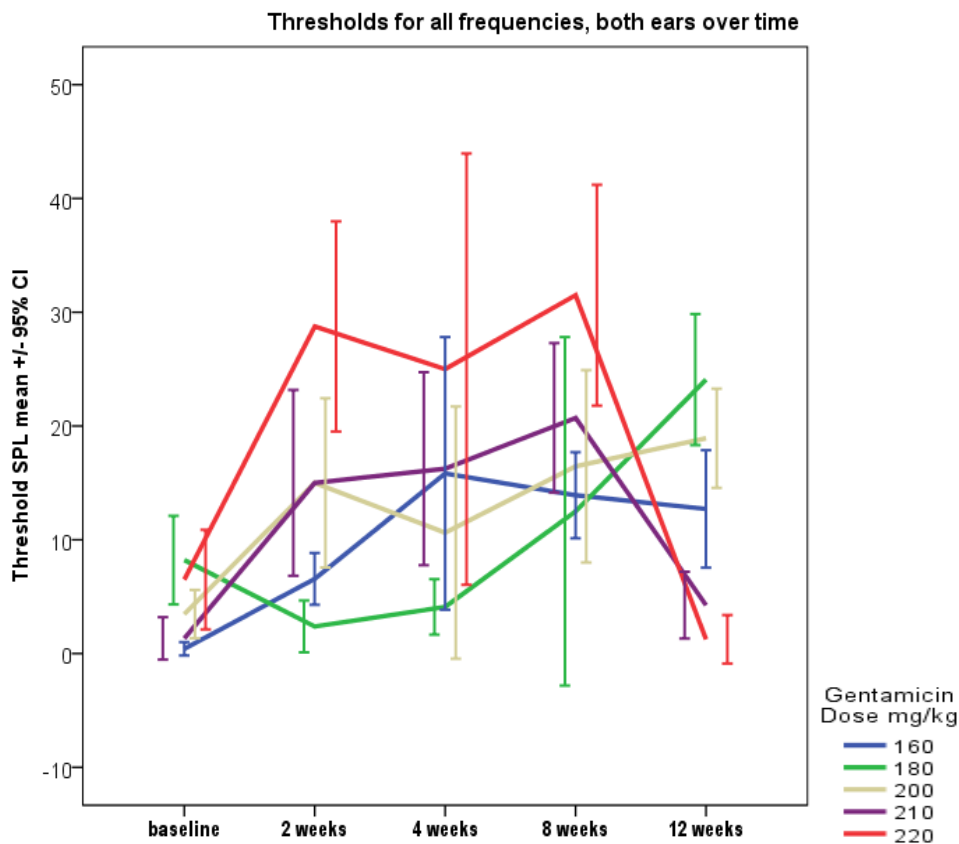


Figure 2: Auditory threshold for mice at all frequency (6 kHz, 8 kHz, 12 kHz, and 24 kHz) with gentamicin doses of 160 mg/kg, 180 mg/kg, 200 mg/kg, 210 mg/kg and 220 mg/kg.

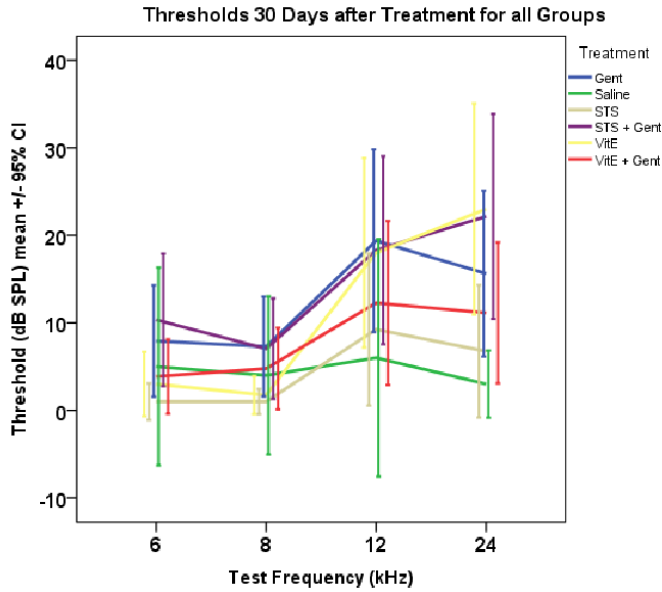


Figure 3 – ABR Thresholds 30 days after Treatment across the frequencies tested. Groups were treated were divided according to table 1. Mean hearing loss was small and many animals had minimal hearing loss, which reflects the clinical situation. Relatively large thresholds occurred for any group that included gentamicin. It appears from the graph that neither STS nor vitamin E was protective against gentamicin-induced hearing loss and this is supported by the non-parametric analysis.

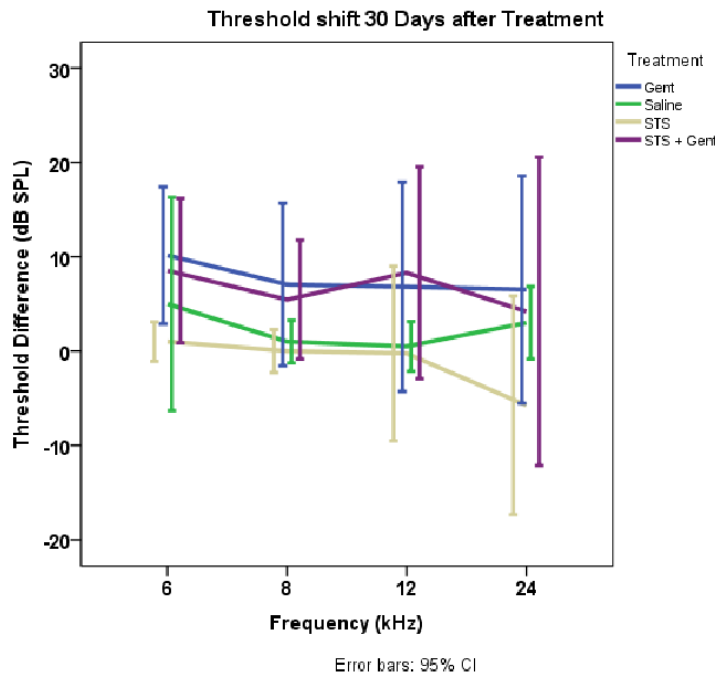


Figure 4. Threshold Shift 30 days after treatment. This figure shows data for all animals excluding α -tocopherol data. The difference across frequencies is not statistically significant. Among the four treatment groups the gentamicin and STS+gentamicin groups differ from the saline and STS groups due to the effect of gentamicin on hearing, but the important finding here is that STS did not protect against gentamicin-induced hearing loss when administered together.

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