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Original article

Vitamin C alleviates ototoxic effect caused by coadministration of amikacin and furosemide

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ABSTRACT

Background: Drug-induced ototoxicity is still a main clinical problem in otolaryngology. It is widely known that aminoglycoside antibiotics combined with loop diuretics significantly contribute to permanent ototoxicity. The aim of this study was to find out whether ascorbic acid (vitamin C) is able to reverse or alleviate ototoxicity evoked by systemic (*ip*) administration of combination of amikacin and furosemide in experimental male albino Swiss mice.

Methods: Ototoxic combination of amikacin and furosemide was isobolographically evaluated based on the hearing threshold decreasing doses by 20% and 50% (TDD_{20} and TDD_{50}), respectively. Linear regression analysis was used to determine the TDD_{20} and TDD_{50} values for amikacin, furosemide, vitamin C administered alone and in combination (at the fixed-ratio of 1:1).

Results: Vitamin C (in a dose of 500 mg/kg, *ip*) alleviated the impairment in hearing threshold evoked by combined *ip* administration of amikacin and furosemide (at the fixed-ratio of 1:1) in mice by reducing TDD_{50} values from 49.82 to 21.56 ($p < 0.01$). In contrast, vitamin C (500 mg/kg, *ip*) had no significant effect on TDD_{20} values for the combination of amikacin and furosemide at the fixed-ratio of 1:1.

Conclusions: Vitamin C administered together with ototoxic drug combination of amikacin and furosemide reduced ototoxicity evoked by this two-drug combination in the experimental mice.

Introduction

Ototoxic effects after exposure to aminoglycoside antibiotics and loop diuretics are extensively examined by researchers. Generally, aminoglycoside antibiotics produce vestibular dysfunction due to hair cell loss (i.e., gentamicin) or evoke cochleotoxic effects (i.e., kanamycin) contributing to the permanent loss of hearing [1–4]. Aminoglycoside ototoxicity requires the interaction of the drug with metal ions [5], resulting in the formation of free radicals and highly reactive oxygen species [6], which has been previously investigated and confirmed by many authors [7–11].

To reduce and/or minimize ototoxic effects evoked by aminoglycoside antibiotics in the inner ear of experimental animals, some pharmacological treatments with antioxidants and other compounds that counteract the damage produced by reactive oxygen species, are recommended [6,12–17]. For instance, some antioxidants, including resveratrol, N-acetylcysteine and ascorbic acid (vitamin C) reduced the ototoxic effects produced by some aminoglycoside antibiotics (kanamycin, neomycin, gentamycin) in various preclinical studies [18–20]. Moreover, it was observed that amikacin (another aminoglycoside antibiotic) considerably lowered the activity of various cochlear antioxidant enzymes including, superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase in guinea-pigs [21].

On the other hand, loop diuretics (including, etacrynic acid and furosemide) evoke edema of the stria vascularis and decrease of the endocochlear potential, contributing to hearing deficits [22]. Experimental evidence indicates that a single intravenous injection of furosemide produced significant changes in auditory brainstem responses (ABRs) in rats [23]. The ototoxic effect of loop diuretics is related to inhibition of Na+, K+-ATP-ase, adenylate cyclase and succinate dehydrogenase in the stria vascularis [24].

Experimentally, it has been proved that kanamycin administered together with furosemide produced irreversible changes in cochlear function [25]. Repeated treatments of kanamycin with furosemide exerted near total loss of outer hair cells and negatively affected the stria vascularis and spiral limbus, resulting finally in

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hearing loss in animals [26]. Moreover, kanamycin combined with furosemide exerted permanent cochlear damage in guinea pigs [27]. These data confirm that ototoxic effects of aminoglycoside antibiotics may be potentiated by loop diuretics.

The explanation of this severe ototoxic interaction between the loop diuretic (furosemide) and aminoglycoside antibiotics (kanamycin, streptomycin and gentamicin) has been presented by some authors, who reported damage not only in the inner ear, but also in the kidney of experimental animals. For instance, it has been found that kanamycin concentrations after co-administration of furosemide significantly increased not only in the perilymph, but also in the cerebrospinal fluid and serum of experimental rabbits [28].

However, up-to-date there is no data showing protection from both, amikacin and furosemide evoked ototoxicity. This was the main reason to investigate protection from this drug combination. Therefore, we intend in this study to examine ascorbic acid (vitamin C – a widely known antioxidant) as a protective drug against ototoxicity evoked by the combination of amikacin (an aminoglycoside antibiotic) and furosemide (a loop diuretic) in naïve male albino Swiss mice.

Materials and methods

Animals and experimental conditions

Male Albino Swiss outbred mice, at the age of 14–21 days were used in this study, as recommended earlier [29,30]. The animals were kept under laboratory conditions (natural light-dark cycle, temperature of $23 \pm 1\,^{\circ}C$, relative humidity of $55 \pm 5\%$). All experiments were conducted on animals between 8 a.m. and 3 p.m. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures described herein were approved by the First Local Ethics Committee.

Drugs

Amikacin (Biodacyna, Polpharma, Poland), furosemide (Furosemidum, Polpharma, Poland), and vitamin C (Acidum ascorbicum, Pliva, Poland) were given intraperitoneally (*ip*) as follows: vitamin C at 30 min. before amikacin and furosemide administration, while amikacin and furosemide were administered *ip* either 15, or 30 min. before hearing measurement. During the evaluation of hearing threshold all the mice were anesthetized with combination of xylazine (Xylazinum, Biowet Pulawy, Poland) in a dose of 20 mg/kg (*ip*) and ketamine (Ketamini hydrochloridi, Vetoquinol Biowet, Poland) in a dose of 100 mg/kg (*ip*). More specifically, the animals were anaesthetized and a pre-treatment hearing threshold was measured in each mouse. This baseline hearing threshold in each animal was considered as a control value. Next, the animals were randomly divided into two groups, the first group of animals received vitamin C (in a constant dose of 500 mg/kg) and the second group was injected with vehicle. After next 15 or 30 min. furosemide, amikacin, or mixture of both drugs were administered *ip* to the respective groups of animals, and finally, re-evaluation of the hearing threshold (post-treatment) was performed. To evaluate dose-response relationships for amikacin, furosemide and their mixture at the fixed-ratio of 1:1 (with respect to impairment in hearing threshold), the drugs were administered *ip* at increasing doses and their corresponding hearing thresholds were determined for each dose and each drug separately. However, at least 3 groups of animals were required to determine the shift in hearing threshold for increasing doses of each drug. Therefore, in this study, 6 groups of animals received furosemide alone, 6 – amikacin alone and 6 – the combination of amikacin and furosemide at the fixed-ratio of 1:1. Thus, 18 groups of animals received vehicle and 18 – vitamin C. Total number of animals used in this study amounted to 288 mice (36 groups with 6–10 animals per experimental group).

Auditory brainstem responses (ABR)

Every anesthetized mouse has had hearing threshold evaluated before application of drugs or their combination (the pre-treatment hearing threshold). ABR data collection was obtained with computerized Interacoustics Eclipse EP15 evoked potential unit (Middelfart, Denmark). Alternating click stimuli were presented to the left ear of the animal. The click has substantial energy in the 2–8 Hz range, as suggested elsewhere [30]. ABR responses were recorded *via* subcutaneous electrodes placed near the ipsilateral pinna, vertex and contralateral pinna with the ground electrode placed along the trunk. The responses were amplified, filtered, averaged by a computer and displayed on the computer screen. The sound level of the stimuli decreased from 90 dB sound pressure level (SPL) to 20 dB SPL in 10 dB steps and finally in 5 dB steps to identify the lowest intensity at which an ABR wave V was detectable (see: Supplementary Figs. 1–3). At each sound level up to 1000 responses were averaged and analyzed. If the repeatability of the recorded wave exceeds 95%, the system automatically starts the stimulation with the next programmed volume level. Stimuli were presented at the rate of 39 Hz and recorded for 10 ms duration. Hearing threshold was determined by a single observer, who noted the lowest sound level at which a recognizable waveform was seen on a screen from the highest to the lowest sound levels. Waveforms were confirmed as auditory evoked responses by their decreasing amplitude and increasing latency with decreasing sound intensity of the stimulus and repeatability at least 95%. Rectal temperature in animals was monitored throughout recordings with animal temperature maintained by a warming pad. All animals were fully anesthetized throughout all ABR procedures.

Calculations and isobolographic analysis

The impairments in the hearing threshold (in %) were calculated by comparing the pretreatment hearing threshold with that determined after the drug administration. The percentage of hearing threshold impairment, corresponding to the respective dose of a drug (amikacin or furosemide) was plotted into the Cartesian system of coordinates and assessed with linear regression analysis, from which the threshold decreasing doses by 20% and 50% (TDD_{20} and TDD_{50}) were calculated for amikacin and furosemide, separately. Of note, the pretreatment hearing threshold was accepted as 100% and any changes in animals (receiving amikacin or furosemide) were expressed as % of impairment in the hearing threshold. In other words, the same animals were both, the control and experimental groups by themselves. Subsequently, the two-drug mixture of amikacin and furosemide at the fixed-ratio combination of 1:1 was determined isobolographically and the mixture was administered to the animals. Any changes in the hearing threshold were calculated using linear regression analysis. Experiments allowing for the determination of TDD_{20} and TDD_{50}, after *ip* administration of amikacin, furosemide and their combination at the fixed-ratio of 1:1, were performed separately. The TDD_{20} and TDD_{50} values were determined independently from two separate sets of experiments on animals. The isobolographic analysis of interaction is a golden standard for an exact classification of interaction between drugs. Generally, type I isobolographic analysis is applied if all the tested drugs produced the same definite effect, which in this study was defined as the shift in hearing threshold in experimental animals. Doses of the studied drugs that impaired the hearing threshold by 20% and 50%,

as compared to the pre-treatment hearing threshold, were linearly related and analyzed, as described earlier [31–33]. The TDD_{20} values were calculated from the animals' groups that received drugs at 15 min. prior to the evaluation of the hearing threshold, whereas the TDD_{50} values were determined from the animals injected *ip* with the studied drugs at 30 min. before the measurement of the hearing threshold. Subsequently, after computing the TDD_{50} and TDD_{20} values for amikacin and furosemide alone, the doses of each drug used in the mixture (at the fixed-ratio of 1:1) were calculated. Next, the doses of both drugs were administered to animals and the TDD_{20} and TDD_{50} values for the mixture were determined. To evaluate the influence of vitamin C on the hearing threshold in experimental animals, a constant dose of vitamin C (500 mg/kg, *ip*) was added to the mixture of amikacin and furosemide that experimentally impairs the hearing threshold by 20% and 50% (TDD_{20} and TDD_{50}), respectively, as recommended elsewhere [29]. In animals receiving the mixture of amikacin, furosemide and vitamin C the threshold hearing was determined and statistically compared to that denoted for the mixture of amikacin and furosemide alone at the fixed-ratio of 1:1. Statistical analysis of data was performed with one-way ANOVA followed by the *post-hoc* Sidak's multiple comparisons test.

Results

Evaluation of hearing threshold in mice received amikacin and furosemide either alone or in combination with vitamin C

Amikacin and furosemide, when administered (*ip*) alone at 15 min. before the hearing threshold evaluation, produced in a dose-dependent manner the impairment in the hearing threshold in experimental animals. Doses of amikacin and furosemide were linearly related to their impairment in hearing thresholds in mice. The experimentally denoted doses of drugs that impaired the hearing threshold by 20% (TDD_{20}) in mice were 0.797 mg/kg for amikacin and 56.25 mg/kg for furosemide (Fig. 1A–B). Similarly, amikacin combined with vitamin C (500 mg/kg) and furosemide combined with vitamin C (500 mg/kg), impaired dose-dependently the hearing threshold in experimental animals. Doses of amikacin and furosemide were also linearly related to their impairments in hearing thresholds in mice. The experimentally denoted TDD_{20} values for the combination of amikacin with vitamin C and furosemide with vitamin C were 0.932 mg/kg and 60.27 mg/kg, respectively (Fig. 1A–B).

Amikacin and furosemide, when administered systemically (*ip*) and separately at 30 min. prior to the hearing threshold evaluation, impaired (dose-dependently) the hearing threshold in mice. Doses of amikacin and furosemide were linearly related to their decreases in the hearing thresholds in mice and the experimentally-derived doses of amikacin and furosemide that reduced the hearing threshold by 50% (TDD_{50}) in mice were 1.006 mg/kg and 63.24 mg/kg, respectively (Fig. 2A–B). Similarly, amikacin combined with vitamin C (500 mg/kg) and furosemide combined with vitamin C (500 mg/kg), reduced dose-dependently the hearing threshold in experimental animals. In this case, doses of amikacin and furosemide were also linearly related to their decreases in the hearing thresholds in mice and the experimentally denoted TDD_{50} values for the combination of amikacin with vitamin C and furosemide with vitamin C were 1.189 mg/kg and 66.22 mg/kg, respectively (Fig. 2A–B).

Evaluation of hearing threshold in mice received the combination of amikacin with furosemide at the fixed-ratio of 1:1 either alone or in combination with vitamin C

The experimental animals that received a mixture of amikacin and furosemide (at doses corresponding to the isobolographically-derived fixed-ratio of 1:1 for TDD_{20} and at 15 min. prior to the hearing threshold evaluation) had the hearing threshold reduced by 20.32% (Fig. 3A). Vitamin C administered alone *ip* at a dose of 500 mg/kg exerted no significant effect on the hearing threshold in the animals, although the threshold was impaired in 0.41% (Fig. 3A). However, the combined application of vitamin C (500 mg/kg) to animals receiving a mixture of amikacin and furosemide reduced only the hearing threshold in experimental animals by 13.65% (Fig. 3A). Comparison of TDD_{20} values for mixtures of amikacin with furosemide alone or with vitamin C revealed no statistical significance with one-way ANOVA followed by the *post-hoc* Sidak's multiple comparisons test (Fig. 3A).

In contrast, the mice receiving the mixture of amikacin and furosemide (at doses corresponding to the isobolographically-derived fixed-ratio of 1:1 for TDD_{50} and at 30 min. before the hearing threshold evaluation) had reduced the hearing threshold by 49.82% (Fig. 3B). Vitamin C administered singly *ip* at a dose of 500 mg/kg exerted no significant effect on the hearing threshold in the animals and the impairment in hearing threshold amounted to 1.05% (Fig. 3B). However, the co-application of vitamin C (500 mg/kg) with the mixture of amikacin and furosemide reduced the hearing threshold in experimental animals by 21.56% (Fig. 3B), which was statistically significant at $p < 0.01$ (one-way ANOVA followed by the *post-hoc* Sidak's multiple comparisons test; Fig. 3B).

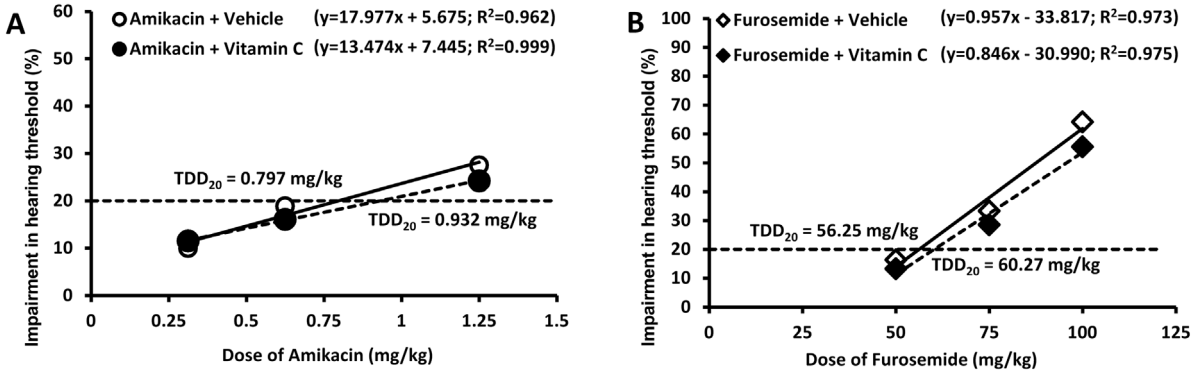

Fig. 1. A–B. Linear regression analysis of doses of amikacin, furosemide alone and in combination with vitamin C along with their impairments in hearing threshold by 20% in experimental animals. Linear regression equations for amikacin and furosemide alone and in combination with vitamin C are presented on the graph; where y – is the impairment in hearing threshold (in %) as compared to the control animals; x – is the dose of the tested drug; R^2 – is the coefficient of determination. TDD_{20} – is the hearing threshold decreasing dose by 20%. Each point consists of 6–10 animals.

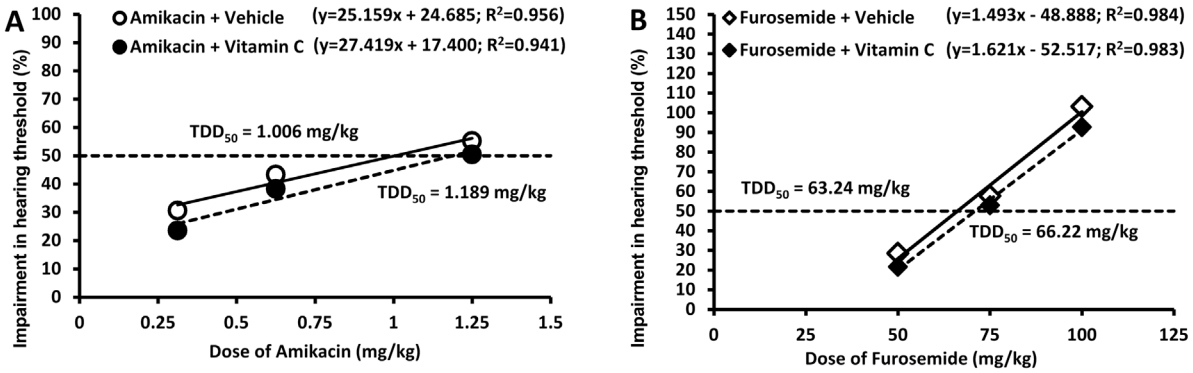

Fig. 2. A–B. Linear regression analysis of doses of amikacin, furosemide alone and in combination with vitamin C along with their impairments in hearing threshold by 50% in experimental animals. Linear regression equations for amikacin and furosemide alone and in combination with vitamin C are displayed on the graph; where y – is the impairment hearing threshold (in %) as compared to the control animals; x – is the dose of the tested drug; R^2 – is the coefficient of determination. TDD_{50} – is the hearing threshold decreasing dose by 50%. Each point consists of 6–10 animals.

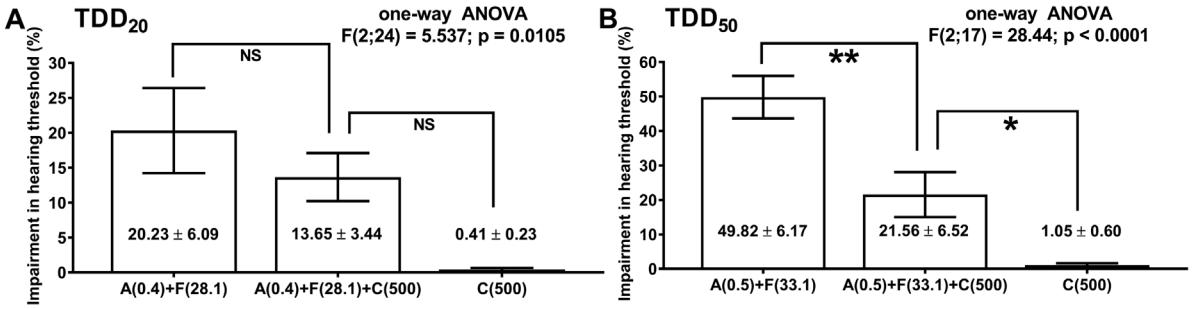

Fig. 3. A–B. Influence of vitamin C on the impairment in hearing threshold in animals receiving the combination of amikacin (A) and furosemide (F) at doses corresponding to the threshold decreasing doses by 20% and 50% (TDD_{20} and TDD_{50} values). Results are presented as % of impairment in hearing threshold (± S.E.M.) in mice receiving the combination of amikacin (A) and furosemide (F) at doses corresponding to the threshold decreasing doses by 20% (TDD_{20}). TDD_{20} (A + F) and TDD_{50} (A + F) – threshold decreasing doses of mixtures of amikacin and furosemide at the fixed-ratio of 1:1 that reduced hearing threshold by 20% and 50%, respectively. TDD_{20} (A + F+C) and TDD_{50} (A + F+C) – threshold decreasing doses of mixtures of amikacin, furosemide at the fixed-ratio of 1:1 and vitamin C that experimentally reduced hearing threshold in the mice by 20% and 50%, respectively. Impairment in hearing threshold was calculated by comparing the hearing threshold values denoted experimentally for the combination of amikacin and furosemide with those determined in the same animals before administration of the drugs (pretreatment hearing threshold). Subsequently, vitamin C (500 mg/kg) was co-administered with the combination of doses of amikacin and furosemide. The impairment in hearing threshold value for the combination of amikacin + furosemide with vitamin C was statistically compared to that determined for the combination of amikacin + furosemide by using one-way ANOVA followed by the Sidak's multiple comparisons test. $^*p < 0.05$ and $^{**}p < 0.01$ *vs.* the respective group. NS – not significant. Doses of particular drugs (in mg/kg) are presented in parentheses.

Discussion

Our experiments clearly demonstrate a potent protective action of vitamin C against the ototoxic effects exerted by the combination of amikacin and furosemide.

To the best of our knowledge, we used in this study for the first time the isobolographic analysis to determine the experimental mixture of amikacin and furosemide that produced a fixed impairment in hearing threshold in animals. Generally, the isobolographic analysis is based on presumptions that the final effects are evoked by a mixture of 2 drugs that are equi-effective in terms of impairment of hearing threshold in animals. The isobolographic analysis was designed to denote doses of amikacin and furosemide that shift the hearing threshold by 20% and 50%, respectively, as compared to the control. Results presented herein indicate that the combination of amikacin and furosemide produced synergistic interaction in terms of ototoxicity. However, in this study two independent evaluations of data were performed. The first one was associated with dose-response analysis for the combination of amikacin and furosemide, while the second evaluation allowed for comparison of the results obtained from the combination of amikacin, furosemide and vitamin C.

It is important to note that in this study we evaluated the TDD_{20} and TDD_{50} values in two various treatment times. The TDD_{20} was determined in animals receiving the treatment at 15 min before the testing of hearing, whereas the TDD_{50} was evaluated in animals that received the respective treatment at 30 min before the hearing threshold determination. Although both values were determined experimentally, the TDD_{50} value seems to be superior to the TDD_{20} value in experimental animals.

In this study, the mixture of amikacin and furosemide produced ototoxic effects that manifested as the impairment in hearing threshold in experimental animals. In another set of experiments, the influence of vitamin C on the hearing threshold in mice receiving the respective treatments (amikacin with furosemide), was determined. It should be stressed that doses of amikacin and furosemide were isobolographically selected allowing for the evaluation of hearing threshold in experimental animals. There is no doubt that amikacin and furosemide exert synergistic interaction in terms of ototoxicity and hearing loss. Thus, based on this interaction, the influence of vitamin C was determined experimentally and it has been proved that vitamin C alleviated the impairment in hearing threshold evoked by the combination of furosemide and amikacin. Another fact deserves more attention while explaining the observed effects in experimental animals. It was found that vitamin C significantly alleviated the effects exerted by the two-drug mixture administered *ip* at 30 min after vitamin C application. The pretreatment time for vitamin C in this experiment was set up at 30 min. In contrast, a shorter pretreatment time (i.e., 15 min.) for vitamin C did not significantly affected the

impairment in hearing threshold. It was evidently observed that only vitamin C injected *ip* at 30 min. before the application of furosemide and amikacin produced the best protective effects in animals, as compared to the effect observed after *ip* injection at 15 min. prior to the ototoxic drugs application.

It should be stressed that vitamin C administered *ip* at a dose of 500 mg/kg did not significantly change the hearing threshold in mice. A slight shift in hearing threshold ranged from 0.41% to 1.05% was observed for TDD_{20} and TDD_{50}, respectively. This demonstrates that the hearing threshold did not considerably change in the animals in the absence of ototoxic treatment. On the other hand, one could confirm that both, amikacin and furosemide produced ototoxicity in Albino Swiss mice. It should be also mentioned that both amikacin and furosemide when combined together exerted synergistic effects in terms of ototoxicity in experimental animals. However, owing to isobolographic analysis we have calculated doses of both drugs that shifted the hearing threshold exactly by 20% and 50%, respectively. Application of isobolographic analysis in this study was a new approach in this type of experimental research and an innovative strategy allowing for evaluation of the strength of impairment in hearing threshold in animals without manipulating the intensities of ABR signals. In other words, due to isobolographic analysis we selected doses of amikacin and furosemide that synergistically interacted together in terms of ototoxicity and simultaneously precisely impaired hearing threshold by 20% and 50%. This new methodological approach allow researchers to exactly determine the strength of interaction by programming the received results. In other words, despite synergistic interaction of both drugs, doses of amikacin and furosemide were precisely chosen to clearly reach and determine endpoints, which were TDD_{20} and TDD_{50} in this study. The classical application of isobolography is related with determination of the type of interactions occurring between drugs [34]. However, this method (due to mathematical transformation of isobolographic equations) allowed to calculate doses of drugs that exert a specific pre-defined effect that was based on the evaluation of TDD_{20} and TDD_{50} values in this study.

From a practical viewpoint, the impairment in hearing threshold by 20% should be evaluated by an experimenter who has experience in this type of evaluation in animals because the changes in hearing threshold and ABRs were subtle (20%). On the other hand, when considering doses of particular drugs evoking impairment in hearing threshold by 20% and 50%, one can observe a slight difference between TDD_{20} and TDD_{50} values in animals. As documented herein, the TDD_{20} for amikacin was 0.852 mg/kg and the TDD_{50} for this drug was 1.006 mg/kg. Similarly the TDD_{20} value for furosemide was 56.25 and TDD_{50} was 66.22 mg/kg.

Of note, in this study the animals aged 14–21-days were used because only young mice were sensitive to the induction of ABR [29]. Generally, the onset of hearing in mice is around postnatal day 12, so the hearing evaluation should be performed on mouse older than 12 days [29]. On the other hand, some strains of the mice exhibit a severe hearing loss before 8 weeks of age (i.e., in 56-day old) [30]. Therefore, to avoid a genetically-programmed hearing loss in mice we used Albino Swiss strain of outbred mice at the age of 14–21-days, as recommended elsewhere [29]. In our opinion, the Albino Swiss mice at the age of 14–21-days were sensitive enough for the investigation of deficits in hearing threshold.

Another fact needs short explanation while interpreting the results from this study, especially, those for the combination of amikacin, furosemide and vitamin C. In our experiments, all three drugs were administered as three independent injections. Vitamin C was administered 30 min. prior to the application of amikacin and furosemide, which were injected at 15 and 30 min. before the hearing threshold evaluation. This mode of separate *ip* administration eliminated any potential pharmaceutical interactions of vitamin C with amikacin and/or furosemide. There was no doubt that vitamin C could chemically interact with amikacin and/or furosemide if the drugs would be mixed in one syringe during their administration. Because of separate *ip* administration of the drugs, no pharmaceutical inactivation occurred in this study.

Conclusions

Despite the severity of the ototoxic effects evoked by the combined administration of aminoglycosides and loop diuretics, no therapeutic strategy has been proposed to prevent this effect. In our experiments we found that ascorbic acid (vitamin C) was very effective in reducing ototoxic effects of aminoglycosides and loop diuretics used simultaneously. Vitamin C as non-toxic agent gives hope for its possible use in clinical practice as a preventive drug. Application of isobolographic analysis in the evaluation of protective properties of ascorbic acid and against ototoxicity caused by co-administration of amikacin and furosemide is a novel approach that allows to precisely estimate the interaction between these drugs.

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Disclosure of conflicts of interest

The authors have no conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.pharep.2019.01.002.

References

[1] Campbell KC, Meech RP, Klemens JJ, Gerberi MT, Dyrstad SS, Larsen DL, et al. Prevention of noise and drug-induced hearing loss with D-methionine. Hear Res 2007;226(1-2):92–103.
[2] Jiang H, Sha SH, Schacht J. NF-kappaB pathway protects cochlear hair cells from aminoglycoside-induced ototoxicity. J Neurosci Res 2005;79(5):644–51.
[3] Kawamoto K, Sha SH, Minoda R, Izumikawa M, Kuriyama H, Schacht J, et al. Antioxidant gene therapy can protect hearing and hair cells from ototoxicity. Mol Ther 2004;9(2):173–81.
[4] Rybak LP, Whitworth CA. Ototoxicity: therapeutic opportunities. Drug Discov Today 2005;10(19):1313–21.
[5] Schacht J. Biochemical basis of aminoglycoside ototoxicity. Otolaryngol Clin North Am 1993;26(5):845–56.
[6] Perletti G, Vral A, Patrosso MC, Marras E, Ceriani I, Willems P, et al. Prevention and modulation of aminoglycoside ototoxicity. Mol Med Rep 2008;1(1):3–13.
[7] Desa DE, Nichols MG, Smith HJ. Aminoglycosides rapidly inhibit NAD(P)H metabolism increasing reactive oxygen species and cochlear cell demise. J Biomed Opt 2018;24(5):1–14.
[8] Jiang M, Taghizadeh F, Steyger PS. Potential mechanisms underlying inflammation-enhanced aminoglycoside-induced cochleotoxicity. Front Cell Neurosci 2017;11:362.
[9] O'Sullivan ME, Perez A, Lin R, Sajjadi A, Ricci AJ, Cheng AG. Towards the prevention of aminoglycoside-related hearing loss. Front Cell Neurosci 2017;11:325.
[10] Jiang M, Karasawa T, Steyger PS. Aminoglycoside-induced cochleotoxicity: a review. Front Cell Neurosci 2017;11:308.
[11] Lanvers-Kaminsky C, Zehnhoff-Dinnesen AA, Parfitt R, Ciarimboli G. Drug-induced ototoxicity: mechanisms, pharmacogenetics, and protective strategies. Clin Pharmacol Ther 2017;101(4):491–500.
[12] Gratacap B, Charachon R, Stoebner P. Results of an ultrastructural study comparing stria vascularis with organ of Corti in guinea pigs treated with kanamycin. Acta Otolaryngol 1985;99(3-4):339–42.
[13] Maru D, Malky GA. Current practice of ototoxicity management across the United Kingdom (UK). Int J Audiol 2018;20:1–13.
[14] Ralli M, Rolesi R, Anzivino R, Turchetta R, Fetoni AR. Acquired sensorineural hearing loss in children: current research and therapeutic perspectives. Acta Otorhinolaryngol Ital 2017;37(6):500–8.

[15] Richardson GP, Russell IJ. Cochlear cultures as a model system for studying aminoglycoside induced ototoxicity. Hear Res 1991;53(2):293–311.
[16] Stone JS, Leano SG, Baker LP, Rubel EW. Hair cell differentiation in chick cochlear epithelium after aminoglycoside toxicity: in vivo and in vitro observations. J Neurosci 1996;16(19):6157–74.
[17] Weisleder P, Rubel EW. Hair cell regeneration after streptomycin toxicity in the avian vestibular epithelium. J Comp Neurol 1993;331(1):97–110.
[18] García-Alcántara F, Murillo-Cuesta S, Pulido S, Bermúdez-Muñoz JM, Martínez-Vega R, Milo M, et al. The expression of oxidative stress response genes is modulated by a combination of resveratrol and N-acetylcysteine to ameliorate ototoxicity in the rat cochlea. Hear Res 2017;358:10–21.
[19] Le Prell CG, Ojano-Dirain C, Rudnick EW, Nelson MA, DeRemer SJ, Prieskorn DM, et al. Assessment of nutrient supplement to reduce gentamicin-induced ototoxicity. J Assoc Res Otolaryngol 2014;15(3):375–93.
[20] Wu CY, Lee HJ, Liu CF, Korivi M, Chen HH, Chan MH. Protective role of L-ascorbic acid, N-acetylcysteine and apocynin on neomycin-induced hair cell loss in zebrafish. J Appl Toxicol 2015;35(3):273–9.
[21] Klemens JJ, Meech RP, Hughes LF, Somani S, Campbell KC. Antioxidant enzyme levels inversely covary with hearing loss after amikacin treatment. J Am Acad Audiol 2003;14(3):134–43.
[22] Rybak LP. Ototoxicity of loop diuretics. Otolaryngol Clin North Am 1993;26(5):829–44.
[23] Ozaki H, Kurata K, Horinouchi A, Ando T. Auditory brainstem response (ABR) and effects of furosemide on ABR in conscious F344 rats. J Toxicol Sci 1996;21(3):167–75.
[24] Ding D, Liu H, Qi W, Jiang H, Li Y, Wu X, et al. Ototoxic effects and mechanisms of loop diuretics. J Otol 2016;11(4):145–56.
[25] Rybak LP. Furosemide ototoxicity: clinical and experimental aspects. Laryngoscope 1985;95(9 Pt 2 Suppl. 38):1–14.
[26] Hirose K, Sato E. Comparative analysis of combination kanamycin-furosemide versus kanamycin alone in the mouse cochlea. Hear Res 2011;272(1-2):108–16.
[27] Brummett RE, Traynor J, Brown R, Himes D. Cochlear damage resulting from kanamycin and furosemide. Acta Otolaryngol 1975;80(1-2):86–92.
[28] Ohtani I, Ohtsuki K, Omata T, Ouchi J, Saito T. Potentiation and its mechanism of cochlear damage resulting from furosemide and aminoglycoside antibiotics. ORL J Otorhinolaryngol Relat Spec 1978;40(1):53–63.
[29] Akil O, Oursler AE, Fan K, Lustig LR. Mouse auditory brainstem response testing. Bio Protoc 2016;6(6):e1768, doi:http://dx.doi.org/10.21769/BioProtoc.1768.
[30] Zheng QY, Johnson KR, Erway LC. Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. Hear Res 1999;130(1-2):94–107.
[31] Kocharov SL, Panosyan HA, Marzęda P, Wróblewska-Łuczka P, Kochman E, Wlaź A, et al. Anticonvulsant potency of 10 various p-isopropoxyphenyl-succinimide derivatives in the maximal electroshock-induced seizure threshold model in mice. Health Probl Civil 2017;11:195–201.
[32] Borowicz KK, Zadrozniak M, Luszczki JJ, Czuczwar SJ. Interactions between tiagabine and conventional antiepileptic drugs in the rat model of complex partial seizures. J Neural Transm 2008;115(5):661–7.
[33] Kondrat-Wróbel MW, Załuska K, Walczak A, Panasiuk-Poterek AN, Gut-Lepiech A, Wróblewska-Łuczka P, et al. Antagonistic interaction of lacosamide with carbamazepine and valproate in the tonic-clonic seizure model in Health Probl Civil. , doi:http://dx.doi.org/10.5114/hpc.2019.81105 in press.
[34] Gessner PK. Isobolographic analysis of interactions: an update on applications and utility. Toxicology 1995;105(2-3):161–79.