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COMBINED EFFECTS OF GENTAMICIN AND NOISE ON AUDITORY BRAINSTEM RESPONSE IN RATS

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Two series of animal experiments were carried out to clarify the combined effect of gentamicin (GM) and noise on the auditory brainstem response. In the first experiment, 64 rats were divided into eight groups: six groups for GM treatment (0, 20, 30, 40, 50 and 100 mg/kg, respectively), one group for exposure to 110 dB SPL white noise and one for control. After four weeks of injections, no prolonged latency of peak 1 was found in the rats injected with less than 30 mg/kg GM. Prolongation was observed in rats injected with 50 mg/kg GM. When the rats were exposed to the noise for three hours a day in the third week, the latency of peak 1 was temporarily prolonged in response to a four-kHz tone burst. The prolonged latency of peak 1 returned gradually to the initial level. In the second experiment, the obvious and irreversible prolonged latencies in response to four- and 16-kHz tone bursts were found in the rats given 50 or 30 mg/kg GM for four weeks, combined with a one-week exposure to 110 dB SPL white noise. The combined effects of GM and noise typically appeared in the latency of peak 1 in response to four kHz and then 16 kHz. The combined effects were synergistic with a GM effect and a noise effect. The results suggest that hearing damage is easily caused by noise during GM therapy.

1. INTRODUCTION

Many researchers have reported that aminoglycoside antibiotics alone or noise alone cause a histological alteration and an electrophysiological change in the hearing organ [1–3]. However, few researchers have examined the combined effects of aminoglycoside antibiotics and noise on hearing [4–7]. Brummett *et al*. [6] demonstrated the interaction between kanamycin and puretone. In contrast, some research on puretone exposure in combination with kanamycin administration failed to demonstrate an interaction. The inconsistent result may be related to the dose of kanamycin used in the experiments. It is still unclear as to what is the combined effect of GM and noise when they are given simultaneously [5]. Thus, in this study, two experiments were carried out. The first experiment was designed to clarify that the effect of gentamicin (GM) is dose-dependent, and to confirm the effect of noise on the auditory brainstem response (ABR). After considering the results of the first experiment, the second experiment was designed to determine the combined effects of GM and noise on the ABR when given simultaneously.

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2. MATERIAL AND METHODS

2.1. FIRST EXPERIMENT: EFFECTS OF GM ALONE OR NOISE ALONE ON THE ABR

The materials used were 15 week old Wistar male rats showing a normal Preyer reflex. They were caged in a sound-attenuating chamber with a controlled room temperature of $23 \pm 2^{\circ}C$ and a timed light cycle of 12 h a day (09:00 to 21:00). The background noise was below 45 dB(A). They were allowed to take food and water *ad libitum* for eight weeks of the experiment.

Sixty-four rats were divided into eight equal groups: six groups for GM treatment, one group for exposure to noise and one for control. In the GM groups, rats were intraperitoneally injected with GM in quantities of 0, 20, 30, 40, 50 and 100 mg/kg. In the GM 0 mg/kg group, rats were injected with 2·5 ml/kg saline as a control group for GM treatment. The injection was continued, six days a week for four weeks.

In the noise group, rats were exposed to 110 dB SPL white noise in an exposure chamber for three hours a day, but only six days in the third week of the experiment. The exposure chamber has a 0·59 m^2 floor area and 0·59 m^3 of effective air capacity. To generate white noise, the output of a function generator (Brüel & Kjaer, 1024) was filtered by a high-pass filter (NF Electronic Instrument, FV-665) with a high pass 500 Hz and then amplified by a power amplifier (Technics, SE-A5MK2). Finally, it was fed to a loudspeaker (BOSE, 301AVM) placed in the exposure chamber. The sound pressure level (SPL) in the exposure chamber was monitored directly by a one-inch condenser microphone (Brüel & Kjaer, 4144) connected to a sound level meter (Brüel & Kjaer, 2606) placed outside the exposure chamber. The control group was housed in a sound-attenuating chamber.

To evaluate the hearing of the rats, the ABR activity was recorded by an evoked response recording system (Nihon Koden, MEB-5304) before the experiment, and on the first, second, third, fourth, fifth, sixth and eighth weeks of the experiment. The rats were anesthetized through an intraperitoneal injection of 60 mg/kg sodium pentobarbital. This dose has no effect on the latency and amplitude of ABR [8]. The active electrode was inserted at the middle of both ears, the reference electrode at the nasal vertex and the ground electrode at the left hind foot. The stimuli of click or two-, four- or eight-kHz tone bursts, generated by the evoked response recording system, and the stimuli of 16-kHz tone burst, generated by an acoustic stimulator system (Nihon Koden, SSS-3200), were fed to a loudspeaker (BOSE, 301AVM). The click had rise and fall times of 0·1 ms. The tone burst had rise and fall times of 0·2 ms and a plateau time of 1·0 ms. The stimuli were repeated ten times a second. The intensities of stimuli were sampled descending to 90, 80, 70 and 60 dB SPL. The ABR activities collected with 2000 responses were averaged.

In such recordings, five positive waves were usually obtained. Following Stockard *et al*. [9], the waves were named as peak 1, peak 2, peak 3, peak 4 and peak 5. As parameters of hearing, the latency of peak 1 (the time elapsing between the tone stimulation and the peak), the amplitude of peak 1 and 1–5 interpeak latency (the time elapsing between 1 and peak 5) were computed.

In this experiment, the completely randomized design of the analysis of variance was used for the analyses among the groups, and the randomized block design was used for the analyses between pre-exposure and post-exposure.

2.2. SECOND EXPERIMENT: COMBINED EFECTS OF GM AND NOISE

Rats of the same kind in the same surrounding conditions as the first experiment were subjected to this experiment. Forty-eight rats were divided into six equal groups. Doses of 50 and 30 mg/kg of GM were used. The 50 mg/kg dose significantly prolonged the latency of peak 1, but the 30 mg/kg dose induced little prolongation. In the two GM

groups, rats were injected with 50 or 30 mg/kg GM, respectively, for four weeks. In a noise group, rats were injected with 2·5 ml/kg saline for four weeks, with exposure to 110 dB SPL white noise in the third week. In two combined groups, rats were injected with 50 or 30 mg/kg GM, respectively, for four weeks combined with exposure to 110 dB SPL white noise in the third week. In a control group, rats were injected with 2·5 ml/kg saline alone for four weeks.

The ABR activity was measured by using the same methods as described for the first experiment. In this experiment, two-, four-, eight- and 16-kHz tone bursts were selected as the stimuli for ABR measurement.

The same statistical methods were used as in the first experiment.

3. RESULTS

3.1. FIRST EXPERIMENT: EFFECTS OF GM ALONE OR NOISE ALONE ON THE ABR

In all groups, only the latency of peak 1 showed significant changes in response to the stimuli. Thus, the latency of peak 1 was used as a parameter of ABR on hearing.

The latency of peak 1 in response to click stimuli in the GM 0 mg/kg group showed no significant prolongation throughout the experiment when compared with the latency of peak 1 before the experiment. In the GM 100 mg/kg group, the latency of peak 1 in response to 60 dB or 90 dB click stimuli was remarkably prolonged after one week of injections. In the second week, all rats died. In the GM 50 mg/kg group, the latency of peak 1 in response to 60 dB click stimuli was prolonged after one week of injections and lasting to the end of the experiment. It reached its maximum after two weeks of injections. In the GM 40 mg/kg group, the latency of peak 1 was prolonged after two weeks of injections, but the prolongation was less than that in the GM 50 mg/kg group. There was no significant prolongation of the latency of peak 1 in the GM 30 mg/kg group and 20 mg/kg group.

The correlation between the doses of GM and the latency of peak 1 was examined after four weeks of injections. When compared with the GM 0 mg/kg group, no prolongation of the latency of peak 1 in response to 60 dB click stimuli was observed in the rats injected with less than 40 mg/kg GM. In the rats injected with 50 mg/kg GM, the latency of peak 1 was prolonged. The latencies of peak 1 in response to 70 dB click stimuli were significantly prolonged from 30 mg/kg and above. The latencies of peak 1 in response to 80 dB or 90 dB click stimuli were significantly prolonged from 20 mg/kg and above.

In the noise group, the rate of latency of peak 1 was calculated at each frequency with the following equation: rate of the latency of peak 1 (latency of peak 1 in the noise group/average of the latency of peak 1 in the control group).

The latency of peak 1 in response to a four-kHz tone burst was significantly prolonged 24 hours after a one-week exposure. The latency of peak 1 in response to a two-, eight- or 16-kHz tone burst showed no significant prolongation.

The latency of peak 1 in response to a four-kHz tone burst at 60 dB was remarkably prolonged in the noise group after a one-week exposure to noise. The prolongation remained until the end of the experiment. The latency of peak 1 in response to a four-kHz tone burst at 70, 80 and 90 dB was remarkably prolonged after a one-week exposure. The latency of peak 1 then returned to the initial level by the end of the experiment.

3.2. SECOND EXPERIMENT: COMBINED EFFECTS OF GM AND NOISE

In the control group, no prolongation of the latency of peak 1 was observed throughout the experiment period. In Figures 1 and 2, the range between the two dotted lines shows the interquartile range of the latency of peak 1 in the control group.

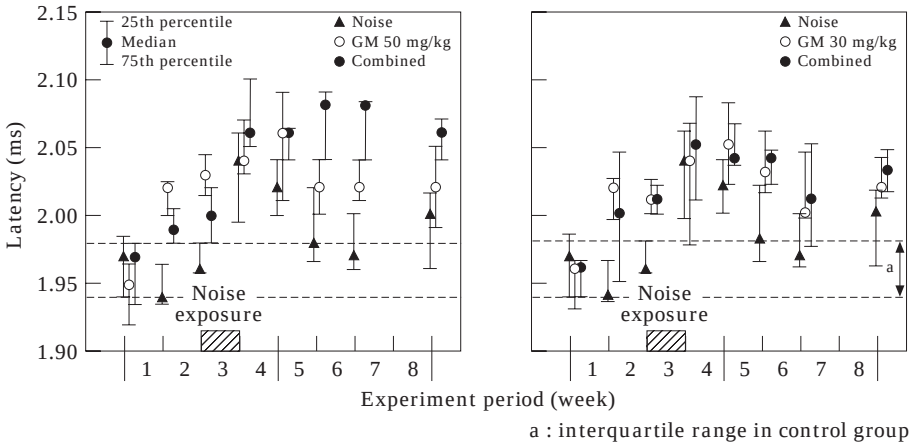

Figure 1. Change of latency of peak 1 in response to a four-kHz tone burst at 60 dB. The left panel shows the results of the rats injected with 50 mg/kg GM, the right panel those of the rats injected with 30 mg/kg GM.

Figure 1 shows the change of latency of peak 1 in response to a four-kHz tone burst at 60 dB. In the combined groups, the latency of peak 1 was gradually prolonged during the injection period. It was remarkably prolonged after a one-week exposure to noise. In the left panel (50 mg/kg GM), no recovery from the prolongation was observed until the end of the experiment. In the right panel (30 mg/kg GM), a slight recovery from the prolongation was found. In the GM group, the prolongation appeared after one-week of injections and continued throughout the injection period. The prolongation decreased gradually by the end of the experiment. In the noise group, the latency of peak 1 was prolonged after exposure to noise and then returned to the initial level.

Figure 2 shows the change of latency of peak 1 in response to a 16-kHz tone burst at 60 dB. In the combined group the latency of peak 1 was gradually prolonged during the injection period. The prolongation became most obvious after a one-week exposure to noise. In the left panel (50 mg/kg GM), no recovery from the prolongation was observed until the end of the experiment. In the right panel (30 mg/kg GM), recovery from the

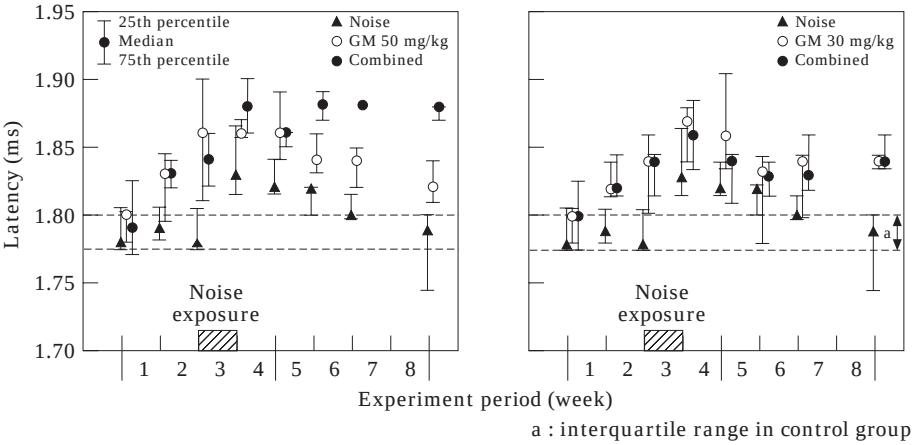

Figure 2. Change of latency of peak 1 in response to a 16-kHz tone burst at 60 dB. The left panel shows the results of the rats injected with 50 mg/kg GM, the right panel those of the rats injected with 30 mg/kg GM.

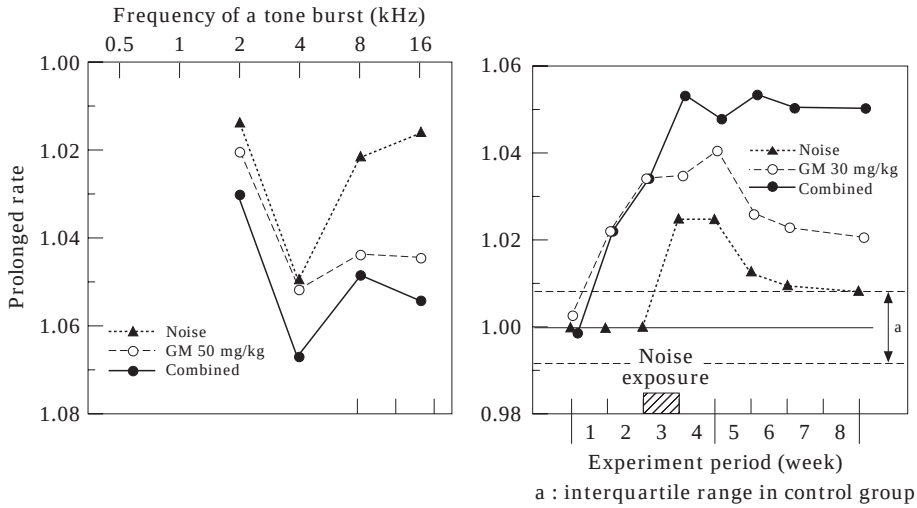

Figure 3. The latency of peak 1 in response to the tone bursts at 60 dB in rats injected with 50 mg/kg GM. The rate was calculated from the median of each group divided by the median of the control group. The left panel shows the characteristics of the averaged rates of the latency of peak 1 in response to frequencies of tone bursts at the third and fourth week. The right panel shows the arithmetic average with rates of the latency of peak 1 in response to two-, four-, eight- and 16-kHz tone bursts.

prolongation was observed. The prolongation in the GM group appeared after two weeks of injections and continued throughout the injection period. Recovery from prolongation was observed.

A temporary prolongation of the latency of peak 1 in response to a two- or eight-kHz tone burst appeared in each group during the experiment, but was not significant.

Figure 3 summarizes the latency of peak 1 in response to the stimuli at 60 dB in rats injected with 50 mg/kg GM. The combined effects of GM and noise typically appeared in the latency of peak 1 in response to four kHz and then 16 kHz. The combined effects were synergistic with a GM effect and a noise effect.

In the GM group, the latency of peak 1 was gradually prolonged during the injection period. In the combined group, the latency of peak 1 was gradually prolonged, but became remarkably prolonged after a one-week exposure to noise. The prolongation in the combined group remained until the end of the experiment. This shows that the combined effects of GM and noise on the ABR are irreversible.

4. DISCUSSION

The latency of peak 1 was more obviously prolonged in the combined group than in the GM or the noise group. This shows the interaction between GM and noise. The combined effect is synergism of a GM effect and a noise effect. Moreover, the prolongation of the latency of peak 1 in the combined group of 50 mg/kg GM had little recovery, showing that the combined effect on auditory function is irreversibly ototoxic.

GM dose-dependently prolonged the latency of peak 1. After four weeks of injections, the prolongation of the latency of peak 1 appeared when the rats were injected with GM 50 mg/kg and above. The results were consistent with the results of Parravicini *et al*. [10]. They reported that no significantly increased threshold of ABR in response to click stimuli was observed in guinea pigs injected with 44 mg/kg GM with a single subcutaneous injection daily for three weeks, but that a significantly increased threshold was observed

in those injected with 67 mg/kg GM. Thus, the dose of 50 mg/kg of GM was used as an ototoxic dose in the second experiment. The dose of 30 mg/kg GM was also selected as a subclinical dose to demonstrate a possible combined effect of noise.

As to the effect of noise, the significantly prolonged latency of peak 1 was found in response to a four-kHz tone burst. The finding was similar to a four-kHz dip on an audiogram. It lessened gradually after the exposure, indicating that the phenomenon is temporary. Gao *et al*. [11] reported the same results of reversible threshold shift in response to a four-kHz tone burst when rats were exposed to 110 dB SPL white noise.

In conclusion, the combined exposure of GM and noise led to the augmentation of a prolonged latency of peak 1 to levels exceeding that latency caused by either GM alone or noise alone. This fact suggests that hearing damage is easily caused by noise during GM therapy. Brown *et al*. [4] also pointed out that persons receiving treatment with an aminoglycoside antibiotic may be an extremely high risk population with respect to acoustic trauma. When subjects are injected-treated with GM, special consideration should be given to their history of exposure to noise.

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