Letter

Changes in auditory brainstem response (ABR) in Kanamycin-induced auditory disturbance model rats

Hiroshi Kuse, Tatsuya Ogawa, Norihiko Nakamura, Yoshihiro Nakayama,
Ayumi Nakakarumai, Chiaki Komori, Yuichi Tsuda, Keita Matsushima, Atsushi Nakamura
and Kazutoshi Tamura

Kannami Laboratory, Bozo Research Center Inc, Japan, 1308-125, Kuwahara-Sanbonmatsu, Kannami-cho,
Tagata-gun, Shizuoka 419-0101, Japan

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ABSTRACT — This study was designed to evaluate changes in auditory brainstem response (ABR) in the course of auditory disturbance in rats induced by Kanamycin (KM). KM was administered subcutaneously to 12 CD (SD) male rats aged 6 weeks for 10 days at a dose of 800 mg/kg. Death was observed in one male on day 8 and 2 males on day 10. It was thought that kidney damage was the cause of death from histopathological findings. ABR was recorded before KM treatment and on days 4, 8, 10 and 11 after KM treatment. The ABR changes after KM treatment in rats were as follows. On day 4, 6 rats showed an increase in amplitude of waves I and/or II and on day 8, among those, 4 rats still showed a high amplitude of waves I and/or II. On day 8, 2 rats showed an elevation of ABR threshold (15-40 dB SPL) and a decrease in amplitude of wave I and increase in amplitude of wave II at the same time. On day 11, 7 rats showed a decrease in amplitude of wave I. In addition, ABR threshold shifts (10-70 dB SPL) were observed in those rats. In ABR recording, KM-induced auditory disturbance model rats showed an increase in amplitude of waves I and/or II earlier than an ABR threshold shift. By analyzing temporal alteration of amplitude of the ABR components, we could detect precursory phenomenon of the auditory disturbance at an early phase of treatment. By following the pathway of click-ABR and tone pip-ABR examination, the auditory disturbance of low- frequency to high-frequency range could be analyzed at an early date in detail.

Key words: Kanamycin, ABR, Auditory disturbance, Rat

INTRODUCTION

There are well-known drugs which cause auditory disturbance, including amino glycoside antibiotic (Russel *et al.*, 1979; Ohtani *et al.*, 1981), loop diuretic (Rybak, 1993) and anti cancer drug (May *et al.*, 2004; Rybak *et al.*, 2009). There are many reports showing an auditory disturbance by repeated doses of Kanamycin (KM), which is an aminoglycoside antibiotic, and it has been reported in the rat (Astbury and Read 1982), guinea pig (Yagi and Tokimoto, 1987), rabbit (Sakatsume, 1980), dog (Yamamura *et al.*, 1994; Uzuka *et al.*, 1996), human (Brummett and Fox, 1989) and others. There are some reports of KM ototoxicity which calculate auditory brainstem response (ABR) threshold and analyze changes of amplitude or latency. But there are

few reports that record the time course of the ABR (Yagi and Tokimoto, 1987; Kuse *et al.*, 1989) and the electrocochleogram (Sakatsume, 1980).

In the guinea pig ABR, a transient increase in the amplitude of wave I after repeated dosing of KM was reported (Yagi and Tokimoto, 1987). In the rabbit electrocochleogram, a transient increase in the amplitude and delay of latency of wave I with repeated dosing of KM was reported (Sakatsume, 1980). Also, a similar result was reported in the dog (Kuse *et al.*, 1989).

On the other hand, we have not found any record of ABR over time in induced auditory disturbance in rats by the repeated dosing of KM, or any report of ABR recorded over days. Therefore, we administered KM to rats in a repeated dose study and recorded ABR with the passage of time.

Correspondence: Hiroshi Kuse (E-mail: kuse@bozo.co.jp)

In the rat study, four to five ABR components were recorded; however, only the waves I and II of ABR components were analyzed, because waves I and II are derived from the cochlea nerve and cochlea nucleus.

MATERIALS AND METHODS

Fifteen CD(SD) male rats aged 6 weeks were used in this study. The animals were maintained individually in metallic gauze cages in animal room under controlled conditions (temperature: $23 \pm 3^\circ\text{C}$, relative humidity: $50 \pm 20\%$, air ventilation: 12 to 17 times per hour, artificial lighting: 12 hr per day), and were allowed free access to diet (CR-LRF, Oriental Yeast Co., Ltd., Tokyo, Japan) and to water via an automatic water supply system (Gotemba Water Union, Shizuoka, Japan). The rats were randomly divided into the KM treated group ($n = 12$) and the non-treated group ($n = 3$). Body weights were recorded every 4 days to determine the dose of KM.

Kanamycin (sulfuric acid kanamycin injection, Meiji, Tokyo, Japan, 4 ml per ampule as 1 g titer) was injected under the back skin of 12 rats once a day for 10 days at a dose of 800 mg/kg (0.2 ml/kg) using disposable syringes- (Terumo Corp., Tokyo, Japan)

The experimental procedures were conducted according to the Animal Welfare Guidelines of Bozo Research Center Inc., Shizuoka, Japan.

ABR recording

ABR was recorded before KM treatment and on days 4, 8, 10 and 11 after KM treatment.

In the ABR recording, a pole paste (Elefix, Nihon Kohden Corp., Tokyo, Japan) for the brain waves was applied to the plate pole (Nihon Kohden Corp.), and the active recording electrode [+] was attached to the vertex and the reference electrode [-] to the ear lobes. Ground electrode was attached to the dorsal part of the neck.

Recording of ABR was carried out under quiet and electrically shielded conditions using evoked potential inspection equipment MEB-9102 (Nihon Kohden Corp.). Clicks were delivered through an inner-type ear phone, 800 responses to repetitive stimuli were averaged at a rate of 20 Hz and analyzed for 10 msec. The signals were filtered with a bandpass of 0.3 Hz to 3 kHz. The click stimuli lowered sound pressure alternately at 10 or 20 dB from 90 dB to 10 dB on either side. Incidentally, the ear opposite the stimulation ear was stimulated by white noise of below 30 dB.

Auditory threshold was determined in 10 dB steps of decreasing stimulus intensity until waveforms lost reproducible morphology. When a rise of the auditory thresh-

old was observed by the click stimuli, it was examined by the tone-pip stimuli with the frequency changed to 8, 4, 2 and 1 kHz.

Four hundred tone-pip stimuli were averaged at a rate of 20 Hz and analyzed for 10 msec. Each rise and fall time for tone-pip stimuli was set at 0.3 msec and stimulation at 90 or 70 dB. To avoid hypothermia under the gas anesthesia isoflururane, body temperature was maintained at 36.5 to 38.5 degrees Celsius with an electric warming pad.

Blood chemical examination

Blood was collected from the jugular vein without anesthesia on the day after last dosing. Blood samples were collected into blood collection tubes containing heparin sodium and plasma was obtained by centrifugation. Blood chemical examination was conducted for the enzyme system associated with kidney and liver, measured by Clinical Laboratory System TBA-120FR (Toshiba Corporation, Tokyo, Japan).

Pathological examination

For all surviving animals, histopathological examination was conducted 4 days after 10 administrations of KM, after death from exsanguination under gas anesthesia. Kidneys were fixed in phosphate buffered 10 vol% formalin for all individuals. The cochlea was fixed with Wittmaack liquid, and the next day with formalin solution of 10 vol% phosphate buffer and decalcified for 1 week with formic acid formalin. For the kidney and the cochlea of all the surviving rats of the KM dosing group, paraffin-embedded thin sections were prepared, stained with hematoxylin-eosin and examined microscopically.

RESULTS

Death was observed in one male on day 8 and 2 males on day 10. It was considered that kidney damage was the cause of death from histopathology findings.

ABR recording

Changes in ABR threshold and amplitude of each animal are shown in Table 1.

Typical ABR patterns are shown in Figs. 1 to 4.

On day 4, the amplitude of waves I and/or II increased in 6 cases, but elevation of the ABR threshold was not observed in any cases (Fig.1, #2002). While a few animals showed a slight decrease in wave I and/or II, threshold changes was not observed in those animals. On day 8, 2 animals (#2009, #2011) showed elevation of the ABR threshold and a decrease in amplitude of wave I and

Table 1. Changes in ABR threshold values after treatment with Kanamycin 800 mg/kg for 10 days

	DAY	0		4		8		10		11	
No.		L	R	L	R	L	R	L	R	L	R
2001		30	30	20	30↓	30	30↓	-	-	30↓↑	60↓↑
2002		20	30	20↑	20↑	30↑	20↑	30↓↑	30↓↑	60↓	50↓
2003		30	30	20↑	20↑	30↑	30↑	-	-	40↓↑	60↓↑
2004		30	30	20↑	20↓	Anesthetic death					
2005		20	20	20↓	20↓	20↓	20	Found dead			
2006		30	20	20↑	10↑	20↑	20↑	90 <	90↓	Moribund sacrifice	
2007		20	20	20↑	20↑	20↓↑	20↑	Found dead			
2008		30	30	20↑	20↑	-	-	-	-	30↑	30↑
2009		20	30	20↓	20↑	60↓↑	45↓	-	-	90 <	80↓
2010		20	20	20↓	20↑	20↓	20↓	30↓	30↓	80↓	80↓
2011		20	20	20↑	20↑	55↓↑	55↓↑	-	-	70↓	80↓
2012		30	30	30↓	30↓	20↓↑	20↓	20↓↑	20	30↓↑	40↓

↑: Increase in amplitude of wave I and/or II, ↓: Decrease in amplitude of wave I and/or II, ↓↑: Decrease in amplitude of wave I and increase in amplitude of wave II. The increases and decreases in the amplitude of waves I and II that exceeded the range of $\pm 20\%$ of the pre-dosing value of each animal are marked. On day 4, increases in amplitude of waves I and/or II were observed in 6 animals on both ear sides, while decreases in amplitude of waves I and/or II were observed in 2 animals. On day 8, 2 animals showed elevation of ABR threshold. On days 10 to 11, a total of 7 animals showed ABR threshold shifts. The highest elevated ABR threshold values were 90 dB SPL. Most of the animals showed decrease in amplitude of waves I and/or II on days 10 to 11.

Fig. 1. Changes in click ABR at different sound pressure levels and tone pip ABR at different frequencies (Animal No.2002). On days 4 and 8, increases in amplitude of waves I and II were observed on both sides at 90 dB SPL. However, ABR threshold shift was not observed on either side. On day 10, decreases in amplitude of wave I were observed on both sides. On day 11, ABR threshold shift was observed on both sides, elevation of ABR threshold values were 40 dB (left) and 20 dB (right). On day 11, at the intensity of 70 dB by tone-pip ABR, ABR amplitude was observed only at 2kHz frequency (left) and 4, 2 and 1kHz frequency (right).

Fig. 2. Changes in click ABR at different times and tone pip ABR at different frequencies (Animal No.2009). On day 11, at the intensity of 90 dB, flattening of the click ABR component was observed on the left side, while two small wave peaks were observed on the right side. Tone pip ABR showed some peaks between 1 and 4 kHz frequency at 90 dB, on the right side. However, no wave was observed at 8 kHz frequency. Elevation of the ABR threshold value was 70 dB (left) and 50 dB (right).

increase in amplitude of wave II at the same time.

On day 11, one animal (Fig.1, #2002) showed decrease in waves I and II on the left ear side by click-ABR, and also showed disappearance of ABR components on the right ear side by tone-pip-ABR of 6 and 8 kHz frequency

at 70 dB SPL. As a result, the elevation of the threshold at 6 and 8 kHz frequency was observed in the right ear. The other animal (Fig. 2, #2009) showed flat wave on the left ear side by click-ABR and tone-pip-ABR. In this case, no response was recorded at 8 kHz frequency (70 dB SPL)

Fig. 3. Changes in click ABR at different sound pressure levels after treatment with Kanamycin 800 mg/kg for 10 days (Animal No.2011). On day 4, increases in amplitude of waves I and II were observed, but ABR threshold shift was not observed on either side. On day 8, in addition to decrease in amplitude of wave I and increase in amplitude of wave II, ABR threshold shift was observed, and those values were both 35 dB (left and right). On day 11, sudden decreases in amplitude of waves I and II were observed. The ABR threshold shift was 50 dB (left) and 60 dB (right). In this case, increase in amplitude and the ABR threshold shift was observed at the same time.

on the right side by tone pip ABR.

On day 11, in all 7 remaining animals, elevation of the ABR threshold was recorded to a varying degree. The ABR threshold shifts were observed in 7 rats in both ears, but the timing of expression was different on each side. The behavior changes of amplitude between waves I and II were different in some animals. The amplitude of waves I and II increased around the same time at first. Subsequently, the amplitude of wave I decreased, but that of wave II continued high.

On day 8, one animal (#2011) showed an increase in amplitude of wave I and decrease in amplitude of wave II at the intensity of 90 dB SPL (Fig. 3). The ABR threshold shift of 35 dB was observed on both sides. Input-output curve of amplitude of waves I and II are shown in Fig. 4. The amplitude of waves I and II rapidly decreased, falling with the intensity of sound pressure level.

On day 11, one animal (#2006) was moribund sacrificed in humane manner. In this animal, on day 4 to 8, increases in amplitude of waves I and II were recorded. On day 10, all the waves almost disappeared and showed 60-70 dB threshold elevation suddenly.

Blood chemical examination

Blood chemical examination values are shown in Table 2.

In blood chemical examination, all KM-treated animals showed elevated levels of Aspartate aminotransferase

Amplitude of I wave

Amplitude of II wave

Fig. 4. Changes in amplitude of waves I and II of click ABR at different sound pressure levels (Animal No.2011). Wave I amplitude: On day 4, increase in amplitude of wave I at 90 dB was observed on both sides, but ABR threshold shift was not observed. On day 8, a slight fall in amplitude of wave I and elevation of the ABR threshold was observed. Wave II amplitude: On day 4, increase in wave II amplitude was observed at 90 dB. At 10 dB, wave II amplitude disappeared as pre data. On day 8, high amplitude of wave II was observed on both sides at 90 dB. But wave II amplitude disappeared at 50 (left) and 40 (right) dB. ABR threshold shift was induced on day 8. On day 8, input-output curve of wave I and II amplitude indicated rapid ascent.

Auditory brainstem response changes induced by Kanamycin in rat

Table 2. Blood chemical examination after treatment with Kanamycin 800 mg/kg for 10 days

Animal No.	AST ^{a)}	ALT ^{a)}	LDH ^{a)}	CPK ^{a)}	ALP ^{a)}	T-CHO ^{b)}	GLU ^{b)}	BUN ^{b)}	CRN ^{b)}	TP ^{c)}	ALB ^{c)}
1001	60	37	100	662	2294	61	134	12	0.23	5.7	3.1
1002	59	36	82	438	1830	81	128	19	0.22	5.6	3.3
1003	55	31	99	255	1961	54	128	14	0.24	5.8	3.1
2001	147	28	309	818	1102	81	141	98	1.37	6	3.3
2002	235	36	614	471	921	104	135	121	2.44	5.9	3.3
2003	178	27	374	410	1374	92	134	88	1.65	5.7	3.2
2006	220	24	925	824	1262	96	705	263	4.74	4.9	2.3
2008	135	24	314	992	1157	118	145	101	2.49	5.8	3.0
2009	65	32	106	404	1125	115	132	53	0.84	5.8	3.1
2010	151	33	360	495	1309	87	149	188	2.84	5.7	3.2
2011	94	22	182	260	1015	141	144	89	1.12	5.7	3.1
2012	118	27	268	311	1706	95	131	126	1.97	5.7	3.1

#1001~1003: Non-treated control group

^{a)}: IU/l ^{b)}: mg/dl ^{c)}: g/dl

#2001~2012: Kanamycin treated group

#2006: Moribund condition

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactic dehydrogenase, CPK: Creatine phosphokinase, ALP: Alkaline phosphate, T-CHO: Total cholesterol, GLU: Glucose, BUN: Blood urea nitrogen, CRN: Creatine, TP: Total protein, ALB: Albumin

Fig. 5. Figures a (low magnitude) and b (high magnitude) show the cochlear duct and the organ of Corti of non treated control rat (Animal No.1002, right), while figures c and d show that of Kanamycin-treated rat (Animal No.2003, right). The rat showed 30 (right) dB ABR threshold shift on day 11. Scale bars = 50 μm.

(AST), Blood urea nitrogen (BUN) and Creatine (CRN) compared with non treated groups. Kidney damage was suspected from those values.

Pathological findings

Pathological specimen of the cochlea is shown in Fig. 5.

In autopsy findings, the 3 animals that died showed enlarged kidneys, and in histopathology necrosis of tubular epithelium, dilatation of renal tubule lumen and enhanced cell infiltration of interstitial were observed in KM-treated animals.

In agreement with the animals showing ABR threshold shifts, disappearance or degeneration of outer hair cells of the Corti organ from the basal turn to the apex turn was observed. However, abnormal findings were not observed in the stria vascularis or the spiral ganglion of cochlea.

DISCUSSION

KM-induced auditory disturbance model rats were examined in detail by the click and tone-pip stimuli combination with ABR recording.

Increase and decrease in amplitude of ABR components and that of electrocochleogram has been reported in KM-treated animals such as guinea pigs (Yagi and Tokimoto, 1987), rabbits (Sakatsume *et al.*, 1980) and dogs (Kuse *et al.*, 1989; Yamamura *et al.*, 1994). Yagi and Tokimoto (1987) reported ABR threshold shift and increase in amplitude of wave I in repeated dose study of KM in guinea pigs. Those changes began at the same time.

In this study, we observed increases in amplitude of waves I and/or II in a number of animals at an early date, but the elevation of the ABR threshold was not observed at the same time. A few animals showed decreases in those of waves I and/or II. These changes may indicate a precursory phenomenon of the auditory disturbance. However, the increases in those amplitudes were considered to be a precursory phenomenon of the auditory disturbance. High amplitude of waves I and II dropped to lower amplitude with increased dosage and the ABR threshold shift progressed.

When the ABR threshold shift was observed by click ABR, in addition to tone-pip ABR recording, we could identify the damaged frequency in this study.

Yagi and Tokimoto (1987) observed recovery after the load of 10 minutes of pure tone and compared recovery of the ABR amplitude between normal guinea pigs and guinea pigs treated with 400 mg/kg of KM for 9 days intramuscularly. It was reported that the guinea pigs treated with KM showing high ABR amplitude were slower in

the recovery of the ABR amplitude than the normal guinea pigs. Therefore, it was supposed that auditory functional decline occurs in the guinea pig showing high ABR amplitude by repeated dosing of KM.

In this rat study, 4 days after dosing of KM, amplitude of waves I and/or II increased, but elevation of the ABR threshold was not observed, and after an increase in amplitude, the ABR threshold rose. Also, the ABR threshold rose rapidly with the decline of the amplitude. There was a difference in the animals used, but it is thought that increase in amplitude in rats was based on the decline of the auditory function like the case of the guinea pig.

Recently, Hong *et al.* (2008) administered Sildenafil, a type 5 phosphodiesterase inhibitor, to mice repeatedly. He reported increase in amplitude of wave I, and subsequent decrease in amplitude of that after Sildenafil treatment, by following the ABR pathway.

By ABR recording over time to compare the amplitude of the ABR components before dosing and after dosing of KM, and in addition by the combination of the click and the tone-pip stimuli, at the frequency range where hearing difficulty happens, we could predict auditory disturbance pathogenesis at an early date.

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Auditory brainstem response changes induced by Kanamycin in rat

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