DOI 10.1007/s10517-015-2979-5
Bulletin of Experimental Biology and Medicine, Vol. 159, No. 4, August, 2015

PHYSIOLOGY

Auditory Function in Immature Animals after Two Consecutive Courses of Ototoxic Antibiotics

I. N. D'yakonova*, O. V. Kamkina, Yu. S. Ishanova, I. V. Rakhmanova, and D. S. Burmistrova*****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 159, No. 4, pp. 400-405, April, 2015
Original article submitted January 12, 2015

In chronic experiments on immature rabbits receiving therapeutic courses of vancomycin, gentamicin, and consecutive administration of vancomicin and gentamicin by the scheme used in neonatology, hearing function was evaluated by the methods of auditory evoked potentials (auditory brainstem response, ABR) and distortion product otoacoustic emission (DPOAE). Comparison with the control group revealed ototoxic effects of all studied antibiotics that manifested in increased sound tolerance and more rapid shortening of latencies in 30-100 dB range. Higher thresholds were found only after gentamicin administration. Vancomycin administration significantly reduced the responses at 4 kHz. Subsequent gentamicin course did not potentiate this effect.

Key Words: *gentamicin; vancomycin; immature rabbits; auditory brainstem response (ABR); distortion product otoacoustic emission (DPOAE)*

Premature infants have immature auditory structures and their maturation is going on over the first year of life. That is why the auditory analyzer is susceptible to various damaging influences.

The use of ototoxic antibiotics in intensive care units is associated with high risk of damage and can lead to hearing and speech disorders [6].

Aminoglycoside antibiotics are prescribed to 95.7% premature infants and the incidence of hearing disorders in these children is 41.3% [9]. Experimental study of the influence of therapeutic doses of vancomycin and gentamicin on the auditory organ during its maturation allowed localizing the damage and evaluation of the negative effect of the drugs [2].

Research Laboratory of Clinical and Experimental Pediatric Otorhinolaryngology, *Department of Physiology, Medico-Biological Faculty, **Department of Pharmacology, ***Department of Pediatric Otorhinolaryngology, N. I. Pirogov Russian National Research Medical University, Ministry of Health of the Russian Federation, Moscow, Russia. ***Address for correspondence:*** ishanova@hotmail.com. Yu. S. Ishanova

Gentamicin treatment affected cochlear outer hair cells (OHC) with predominant damage to the basal coil [4]. After intratympanic injection of gentamicin (0.1 ml 40% solution) to guinea pigs, the absence of OHC in basal coil and changes in inner hair cells (IHC) in the middle coil were observed. In the apical coil, all types of receptor cells were present with certain deformations of OHC and ultrastructural changes in IHC [7].

Ototoxic effects of vancomycin was demonstrated in clinical and experimental studies. In experiments, intratympanic injection of vancomycin increased the thresholds demonstrated by auditory brainstem response (ARB) recording [11]. Vancomycin administration to premature infants was associated with test fail results in 22% cases [10]. Vancomycin treatment in older children in combination with diuretics led to ototoxic side effects only in 10% cases [8]. Clinical data on the absence of distortion product otoacoustic emission (DPOAE) in 14% cases of gentamicin administration followed by vancomycin treatment were published [10].

Retro- and prospective analysis of 212 clinical records of premature neonates successively treated with vancomycin and gentamicin showed that these drugs were prescribed in 20% cases to infants with gestation age <28 weeks, in 21% cases to infants born at 29-32 weeks gestation, and in 12% cases to infants with 33-38 weeks gestation [5].

Here we studied the influence of therapeutic doses of gentamicin on immature auditory analyzer after vancomycin course.

MATERIALS AND METHODS

The experiments were carried out on 44 rabbits. Group 1 comprised 12 intact animals. Group 2 animals (n=7) received vancomycin (15 mg/kg per day) for 7 days starting from day 12 of life, group 3 rabbits (n=10) received 5 mg/kg gentamicin per day for 7 days, and group 4 rabbits (n=15) received 15 mg/kg per day vancomycin for 7 days and then 5 mg/kg per day gentamicin during the next week.

The effect of the test drugs of the auditory function was evaluated by ABR on days 26, 35, and 45 of life and then at the age of 2 and 3 months. The parameters evaluated by this method were thresholds of the first peak appearance and the rate of first peak latency decrease depending on stimulus intensity.

For functional evaluation of OHC activity after administration of ototoxic drugs, DPOAE was recorded at the same terms as ABR. OHC activity was measured as the mean response amplitude (intensity) and response amplitudes at 1, 2, 4, and 6 kHz. Moreover, the ratio of response amplitude at each frequency to the total cochlear response was calculated.

Inclusion criteria, experimental methods, and technical characteristics, and drug dosing were the same as described in the previous studies [1,3].

The data were processed by methods of variation statistics using Statgraphics Centurion XV software. The differences were significant at p<0.05.

RESULTS

The general toxic effect of antibiotics on the body in immature animals manifests in changes in weight gain dynamics. The decrease in weight gain in comparison with the control was found in all experimental groups, but at different terms. In vancomycin group, weight gaining was reduced during the period from day 12 through 26 (p<0.05), in gentamycin group it was found later: 35-45 days and 2-3 months (p<0.05). In the group receiving vancomycin and gentamycin, weight gain was reduced in comparison with the control during the periods of 26-35 days and 45 days-2 months (p<0.05). Thus, the general toxic effect in animals receiving gentamicin was more pronounced than in animals receiving vancomycin alone.

Analysis of the auditory function revealed significant elevation of ABR peak I thresholds in rabbits of all experimental groups in comparison with the control (Fig. 1). The highest thresholds were found in rabbits receiving only gentamicin alone from day 26 to the end of observation. In rabbits treated with vancomycin and then gentamycin, the thresholds significantly surpassed the control values at all terms of observation, but did not differ significantly from thresholds of in rabbits receiving vancomycin alone, except day 26.

In the group treated with gentamycin, bilateral deafness was found in 2 rabbits to the age of three months; in one of these animals, significant hearing loss remained after 1.5 months and in the other, no improvement was found. In animals receiving vancomycin alone, no such effects were found. In one rabbit receiving vancomycin followed by gentamicin,

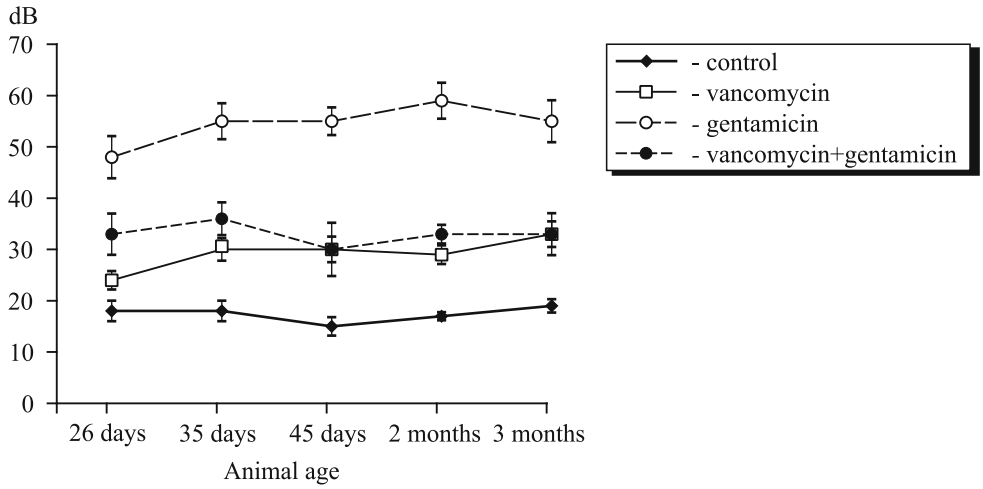

Fig. 1. Thresholds of the first ABR peak appearance in control rabbits (group 1) and animals receiving combined therapy (group 4) groups throughout the observation period.

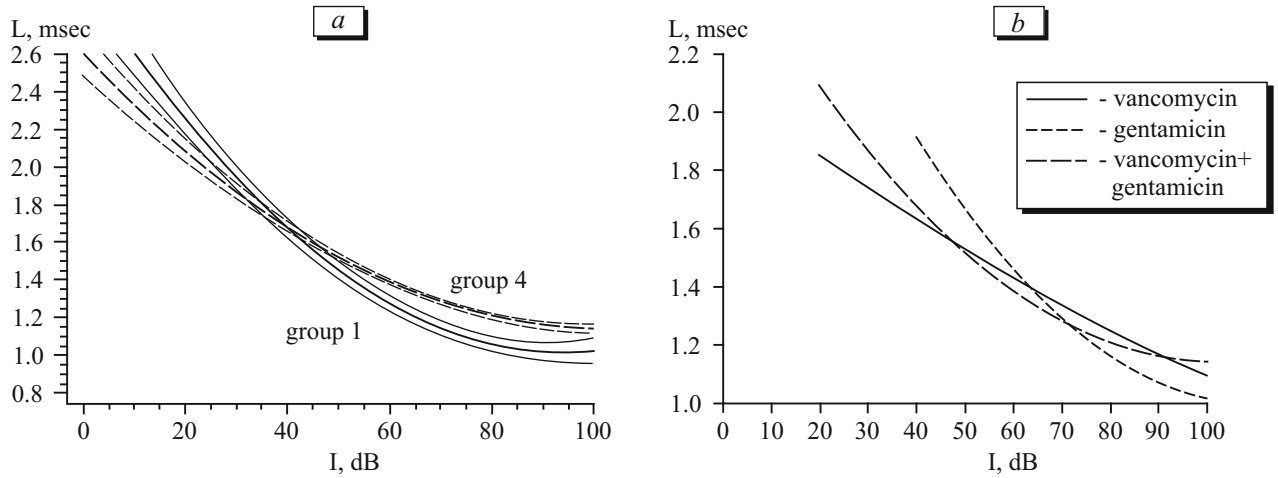

Fig. 2. Latency of ABR peak I as a function of stimulation intensity in rabbits of groups 1 and 4 (*a*) and experimental groups (*b*). Polynomic approximation curves. Ordinate: peak I latency; abscissa: stimulation intensity.

we found reversible deafness in the left ear and severe hearing impairment in the right. By the 2nd month, the first ABR peak threshold in the left and right ears decreased to 50 and 30 dB, respectively.

These data suggest that gentamicin produced more pronounced ototoxic effect. Successive administration of vancomycin and gentamicin did not potentiate the ototoxic effects of vancomycin course.

The correlation between general toxicity (weight gain dynamics) and increase in ABR peak I thresholds at different stages of the experiment was insignificant (r<0.2).

For each term of observation, the curves of polynomic approximation showing lengthening of the latencies of first ABR peak with decreasing acoustic stimulus intensity were constructed. These curves for control group showed quadratic dependency ($L1=a+bI+cI^2$), where L is latency, I is stimulus intensity, and a, b, and c are coefficients. At the age of 3 months, the coefficients were as follows: a=3.03 msec, b=4.3×10^{-2} msec/dB, and c=2×10^{-4} msec/dB^2; the significance the approximating curve was R^2=0.91.

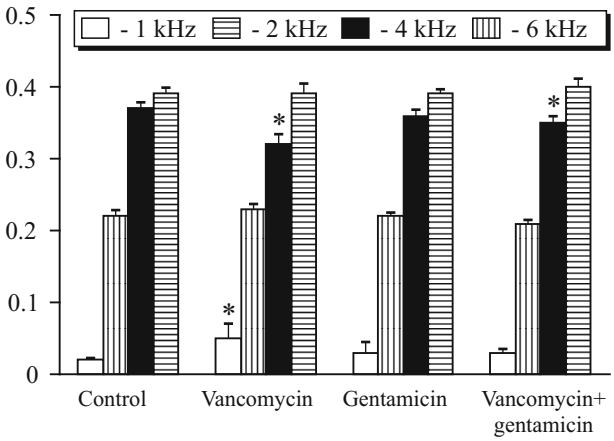

Fig. 3. Frequency ratio (1:2:4:6 kHz) in rabbits of experimental groups.

Rabbits treated with vancomycin followed by gentamicin showed increased latencies in response to high intensity stimulation and short latencies in response to low-intensity stimulation: a=2.61 msec, b=-2.9×10^{-2} msec/dB, and c=1×10^{-4} msec/dB^2 (R^2=0.89) (Fig. 2, *a*). These findings attests to changes in acoustic stimulus transformation after antibiotic therapy. The curves obtained in experimental rabbits at the age of 3 months were different. In rabbits receiving vancomycin alone (Fig. 2, *b*), the curve became a linear dependence with the coefficients a=2.05 msec and b=-1.1×10^{-2} msec/dB (R^2=0.84).

In rabbits receiving gentamycin alone, the approximation curve was described by a dependency with the coefficients a=3.27 msec, b=-4.1×10^{-2} msec/dB, and c=2×10^{-4} msec/dB^2 and showed reduced latencies of the response to high-intensity stimulation.

These curves allowed us to conclude that the studied antibiotics produce different effects on the auditory function. Changes in lengthening of the latencies after antibiotics treatment confirm their effect on processes in either hair cells and/or in neuroreceptors on cochlear synapses, and/or in neurocytes of the spiral ganglion.

The comparison of the auditory function approximation curves in experimental groups showed that successive administration of vancomycin and gentamicin changed, but not potentiated the toxic effect on the processes underlying perception and transmission of the acoustic stimuli to neurons of the spiral ganglion.

To evaluate the functional state of the central structures of the ear, we calculated the central conduction time (difference between the latencies of II and IV peaks at 60 dB stimulation intensity) after antibiotic

TABLE 1. Time of ABR Peak I Latencies Decrease with Increasing Stimulus Intensity in the Range of 30-100 dB

Age	Control	Vancomycin	Gentamicin	Vancomycin+gentamicin
26 days	1.10±0.04	0.72±0.03*	–	0.67±0.09*
35 days	1.10±0.05	0.64±0.03*		0.72±0.09*
45 days	1.11±0.05	0.69±0.03*		0.74±0.1*
2 months	1.12±0.04	0.63±0.02*		0.72±0.11*
3 months	1.11±0.05	0.66±0.03*		0.74±0.11*

Note. "–" calculation impossible due to high peak I thresholds. Here and in Table 2: *$p<0.05$ in comparison with the control.

administration. Significant differences at the age of 3 months were found between the control and animals treated with gentamicin alone ($p<0.05$). This suggests that gentamicin alone in therapeutic doses (group 2) produced more pronounced delayed side effects on the central conducting structures of the ear in comparison with combined treatment (vancomycin followed by gentamicin; group 4).

The intensity curves in 30-100 dB range demonstrated more rapid decrease in response latencies with increasing stimulation intensity in all experimental groups in comparison with the control (Table 1). This fact is known to be an electrophysiological signature of hearing function impairment in sensoneural pathology and confirms adverse effects of the studied aminoglycoside antibiotics on auditory function of the immature auditory analyzer.

Then we used DPOAE test for the analysis of the functional state of OHC, the most susceptible structures to the side effects of aminoglycosides. DPOAE was recorded in all rabbits of both control and experimental groups at all stages of observation.

To estimate functional activity of OHC, we calculated the mean amplitude of evoked acoustic potential (intensity). Considerably lower values in comparison with the control were found in animals receiving combined treatment on day 35 and at the age of 2 months (Table 2). In 2-month-old rabbits, the intensity of the response in the group receiving combined therapy was significantly lower than in the group receiving vancomycin alone ($p=0.004$) or gentamicin alone ($p=0.002$). On day 45, higher intensities of the response were found in vancomycin group in comparison with the control. By the moment of sexual maturity, the cochlear response intensity was similar in animals of all groups. Thus, antibiotic treatment did not change the intensity of cochlear response to the moment of maturity in general, but the effect fluctuated during the growth and development of rabbits.

To reveal possible differentiated local impairments of OHC affecting sound perception in different parts of the cochlear basilar membrane in a frequency-specific way we analyzed the data for each stimulation frequency. Antibiotic therapy produced different changes in response amplitude at different frequencies: we found decrease and increase in the amplitude of the evoked auditory response (Table 3).

Thus, significant differences from the control were found at 4 Hz frequency in rabbits receiving vancomycin alone or drug combination ($p<0.05$), while the amplitudes in these groups did not differ significantly ($p=0.78$). No cumulative toxic effect after successive administration of these drugs was found.

Analysis of DPAOE amplitude as a function of frequency (DP-gram) in control and the experimental groups revealed peculiar distribution of the amplitudes at chosen frequencies related to the sum of amplitudes recorded at all frequencies (Fig. 3). DP-gram configuration remained the same and had an ascending trend with a dominant peak at 6 kHz. The proportion

TABLE 2. Intensity of the Response in Rabbits of Control and Experimental Groups at Different Observation Terms ($M±m$)

Age	Control	Vancomycin	Gentamicin	Vancomycin+gentamicin
26 days	20.8±1.4	18.7±0.8	19.2±1.3	21.9±0.7
35 days	23.1±0.8	24.1±0.9*	22.0±1.3	21.1±0.7*
45 days	21.7±0.8	23.7±0.9*	21.3±1.3	20.5±0.5
2 months	21.7±1.6	22.8±0.9	21.5±2.0	18.9±0.5*
3 months	20.9±1.3	21.4±1.1	21.1±2.0	19.7±1.3

TABLE 3. Test Frequencies (kHz) Demonstrating Significant Change in Response Amplitude in Comparison with the Control Group

Age	Vancomycin	Gentamicin	Vancomycin+ gentamicin
26 days	4↓	4↓	–
35 days	4↓	4↓	4↓
45 days	–	1↑,4↓	4↓,6↓
2 months	–	4↓,6↑	2↓,4↓
3 months	4↓	–	4↓

Note. Arrows show the direction of amplitude changes at specified frequencies.

between some frequencies (4 and 1 kHz) changed in animals receiving vancomycin. These data attest to specific sensitivity of OHC perceiving 4 kHz stimuli to the ototoxic effects of vancomycin.

Thus, gentamicin alone produced most pronounced toxic effects in comparison with combined therapy or vancomycin alone.

Our experimental findings suggest that we should be aware of possible isolated hearing impairment at 4 kHz after vancomycin therapy. Moreover, hearing function testing in children receiving ototoxic antibiotic therapy should not be limited by only DPOAE recording. Control of hearing function by ABR or auditory steady state response (ASSR) methods should be recommended to these children during the first year of life.

REFERENCES

1. I. N. D'yakonova, Yu. S. Ishanova, I. V. Rakhmanova, *et al., Pediatr. Farmakol.*, **8**, No. 5, 70-75 (2011).
2. I. N. D'yakonova, I. V. Rakhmanova, D. S. Burmistrova, *et al*., *Vestn. Ross. Gos. Med. Univer.*, No. 4, 75-79 (2013).
3. I. N. D'yakonova, I. V. Rakhmanova, A. M. Tikhomirov, *et al*., *Bull. Exp. Biol. Med*., **151**, No. 5, 601-603 (2011).
4. E. V. Il'inskaya, K. D. Yusifov, V. E. Kuzovkov, *et al*., *Ross. Otorinolaringol*., No. 3, 61-67 (2007).
5. I. N. D'yakonova, I. V. Rakhmanova, D. S. Burmistrova, *et al*., *Pediatriya. Zh. in G. N. Speranskogo*, **92**, No. 2, 174-175 (2013).
6. A. Berardi, L. Lugli, C. Rossi, *et al*., *Minerva Pediatr.*, **62**, No. 3, Suppl. 1, 51-54 (2010).
7. E. N. Maudonnet, J. A. de Oliveira, M. Rossato, *et al*., *Drug Chem. Toxicol*., **31**, No. 1, 11-25 (2008).
8. C. M. Robertson, J. M. Tyebkhan, A. Peliowski, *et al*., *Acta Paediatr*., **95**, No. 2, 214-223 (2006).
9. J. H. Sun, J. Li, P. Huang, *et al*., *Zhonghua Er Ke Za Zhi*, **41**, No. 5, 357-359 (2003).
10. J. W. Vella-Brincat, E. J. Begg, B. J. Robertshawe, *et al*., *Neonatology,* **100**, No. 2, 186-193 (2011).
11. K. Xoinis, Y. Weirather, H. Mavoori, *et al*., *J. Perinatol*., **27**, No. 11, 718-723 (2007).