Protective Effect of Calcium Channel Blocker Verapamil on Morphological and Functional State of Hair Cells of the Organ of Corti in Experimental Kanamycin-Induced Ototoxicity

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The protective effect of verapamil against acute ototoxic sensorineural damage produced by intraperitoneal injections of kanamycin (50 mg/kg daily for 14 day) was studied on rats. The functional (otoacoustic emission), histological, and physiological methods proved the protective effect of daily injections of calcium channel blocker verapamil (2 mg/kg) on the state of hair cells of the organ of Corti.

Key Words: *cochlear hair cells; ototoxicity; kanamycin; verapamil; otoprotection*

The absence of regenerative cell proliferation in the inner ear of higher mammals determines low efficiency of therapeutic interventions in case of loss of sensorineural elements such as hair cells (HC), neurons of the spiral ganglion, and auditory nerve fibers. This necessitates the search and comprehensive studies of efficient otoprotectors and their introduction into clinical practice. These preparations should prevent the formation and progression of degenerative changes in the epithelium of the organ of Corti under the effect of adverse factors (acoustic trauma, trophic disturbances, presbyacusis, and iatrogenic effects of ototoxic aminoglycoside antibiotics and antitumor preparations).

Ototoxicity of aminoglycoside antibiotics has common pathologic features with their nephrotoxicity; these drugs disturb mitochondrial bioenergetics, phospholipid metabolism, and ionic transport and stimulate production of free radicals and reactive oxygen species [6]. Therefore it is logical to consider common ways of prevention of these effects [5,10]. It was established that calcium channel blockers act as pharmacological protectors of aminoglycoside nephrotoxicity [7]. Our aim was to study the protective properties of calcium channel blocker verapamil under conditions of experimental sensorineural deafness.

MATERIALS AND METHODS

Chronic experiments were carried out on 20 randombred albino male rats (250-380 g) with ideal otoscopic characteristics. The sensorineural damage was produced using the widely known model of ototoxic antibiotic-induced deafness [1]. During 14 days, group 1 rats received intraperitoneal injections of 0.9% NaCl (controls) and group 2 rats received 2 mg/kg verapamil hydrochloride (ICN Oktyabr') for 14 days. In group 3 and 4 rats, deafness was produced by intraperitoneal injection of kanamycin sulfate (daily dose of 50 mg/kg for 14 days). Group 3 rats were simultaneously injected with verapamil hydrochloride (2 mg/kg), group 4 rats received no correction therapy.

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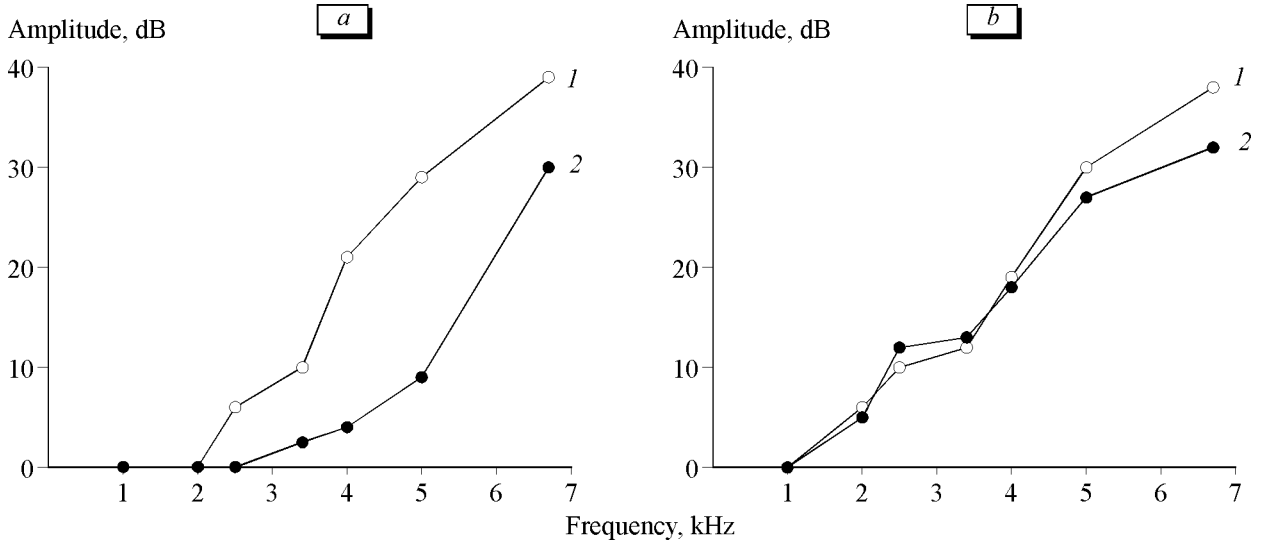

Fig. 1. Otoacoustic emission during kanamycin-provoked ototoxicosis not corrected (*a*) and corrected with verapamil (*b*). *1*) before the experiment; *2*) on day 14.

The functional state of HC was assessed by distortion product otoacoustic emission (DPOAE). The data were recorded on an ILO 88/92 analyzer (Otodynamics Ltd) coupled with a computer. DPOAE was recorded on both ears under Nembutal narcosis (0.2 ml/100 g, 2%, intraperitoneally) with preliminary injection of 0.1% atropine sulfate (0.01 ml/100 g subcutaneously).

The temporal bones and the cochlea were isolated for morphological examination; the preparations of the organ of Corti were made as described previously [2]. The state of HC was evaluated by the reaction of their nuclei [1]. Nucleic acids in HC nuclei were visualized by staining according to the method of Einarson [4].

The karyometric data were processed statistically. We calculated the volume of nuclei of 30 outer and 30 inner HC in each row at the beginning and end of the basal cochlear turn. The data were processed statistically using Student's *t* test.

RESULTS

In kanamycin-treated rats, DPOAE was absent at frequencies 1.5-2.5 kHz and was characterized by decreased amplitude at higher frequencies (Fig. 1, *a*). All nuclei of outer HC in the basal cochlear turn located in the proximal two-thirds looked pyknotic (Fig. 2, *b*). The structure of karyoplasm was not differentiated. Outer HC located in the distal portion of the basal cochlear turn had polymorphic nuclei in comparison with the corresponding cells in control rats (Fig. 2, *a*). Apart from pyknotic nuclei, slightly deformed, enlarged (compared to control), and normal nuclei were seen. These findings suggest that this region was less affected than the proximal portion of the basal turn. The mean volume of HC nuclei in experimental rats significantly decreased compared to the control (Table 1). There were no degenerative changes in other turns of the organ of Corti. In the row of inner HC, the cells with intact and reduced nuclei were seen.

In group 3 rats (kanamycin+verapamil) DPOAE in the range of 2-5 kHz did not differ from the control. A significant decrease in DPOAE amplitude was observed only at 6.7 kHz ($p<0.05$; Fig. 1, *b*). Morphological analysis of the organ of Corti revealed polymorphism of outer HC nuclei and the absence of pyknosis (Fig. 2, *c*). The karyoplasm of most nuclei in group 3 rats was more intensively stained compared

TABLE 1. Volume of Hair Cell Nuclei (μ^3) in the Organ of Corti of Basal Cochlear Turn during Kanamycin-Induced Ototoxicosis ($M\pm m$)

HC type	Control (0.9% NaCl)	Verapamil, 2 mg/kg	Kanamycin, 50 mg/kg	
			without correction	+verapamil, 2 mg/kg
Inner	116.59±1.04	122.76±3.83	106.94±2.10*	112.59±4.13
Outer	68.21±0.68	70.97±1.13	55.78±1.70*	67.74±2.06

Note. *$p<0.05$ compared with the control.

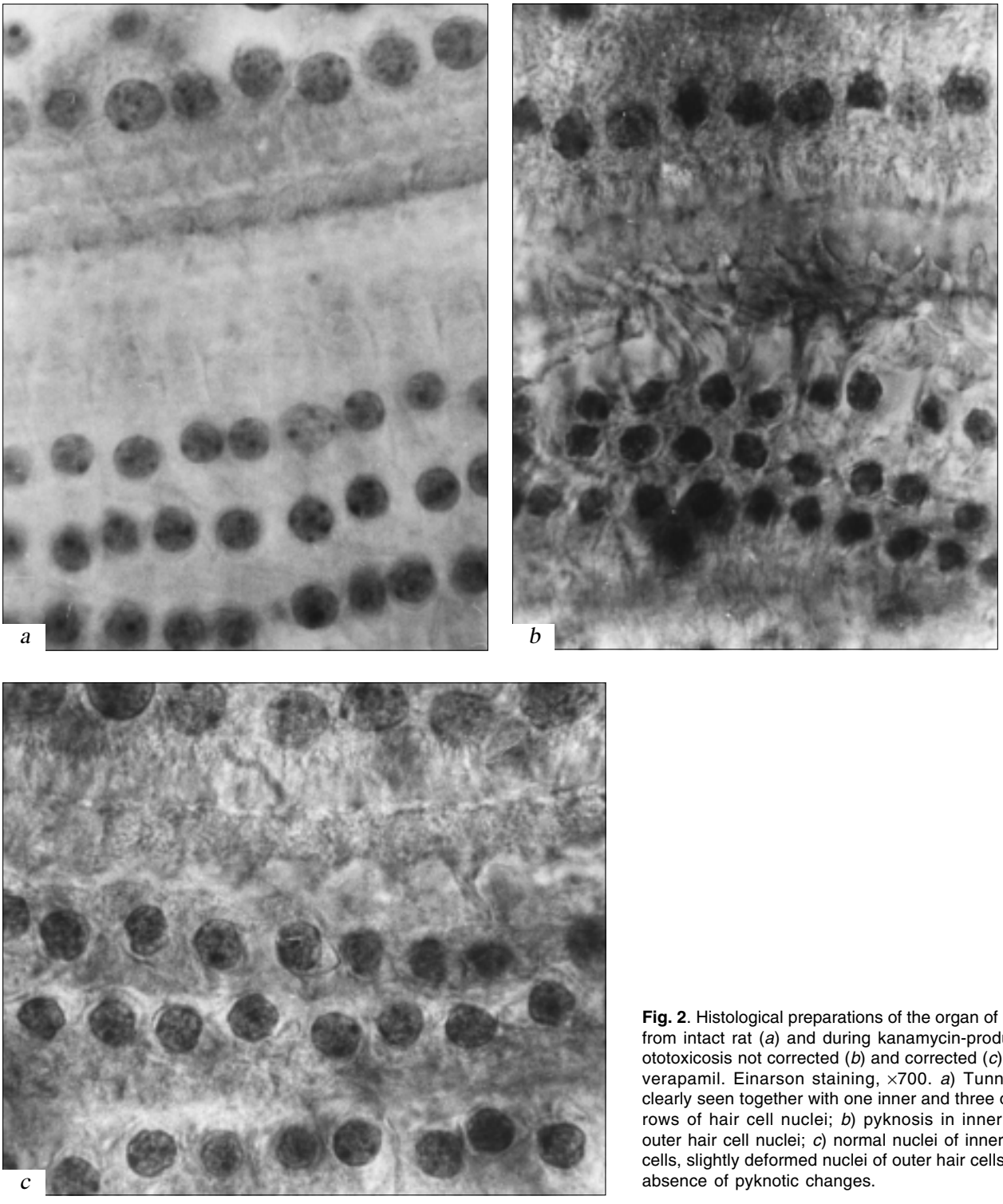

Fig. 2. Histological preparations of the organ of Corti from intact rat (*a*) and during kanamycin-produced ototoxicosis not corrected (*b*) and corrected (*c*) with verapamil. Einarson staining, ×700. *a*) Tunnel is clearly seen together with one inner and three outer rows of hair cell nuclei; *b*) pyknosis in inner and outer hair cell nuclei; *c*) normal nuclei of inner hair cells, slightly deformed nuclei of outer hair cells, the absence of pyknotic changes.

to that in controls and chromatin lumpiness was less pronounced. Large and hyperchromatic nuclei were predominantly located eccentrically and ectopically. There were individual cells with swollen nuclei characteristic of adaptation to physiological and functional load [2]. In this group, there was only a tendency to a decrease in the mean nucleus volume of outer HC. The inner HC had preserved nuclei. The mean volume of these nuclei did not significantly differ from the control (Table 1).

Less pronounced degradation of HC of the basal cochlear turn (the absence of pyknosis, morphological sign of functional activity of the organ of Corti — swollen nuclei of HC) confirmed the protective effect

of verapamil against ototoxic activity of aminoglycoside antibiotics. Our findings agree with clinical data on stability of the audibility threshold in patients treated for a long time with calcium channel blockers for concomitant pathology [8].

Our findings can be explained by vascular effects of calcium blockers. These drugs specifically block the effects of Ca^{2+} ions on smooth muscle cells, thereby promoting dilation of constricted blood vessels. Experiments showed that calcium channel blockers improved microcirculation in the stria vascularis of the cochlear duct even against the background of decreased systemic blood pressure [11]. The protective effect of verapamil is probably associated with elimination of arteriolar spasm in the stria vascularis induced by aminoglycoside antibiotics [1], which improves its metabolic and electrochemical functions. Moreover, verapamil can antagonize the catalyzing effect of intracellular Ca^{2+} on DNA-endonucleolysis, thus inhibiting the initial stages of apoptosis [9,12]. This effect can take place in our experiments, because verapamil was found to inhibit nuclear Ca^{2+}-dependent endonucleases in neurons and neural secretory cells of hypothalamus [3].

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