ORIGINAL ARTICLE

Functional and Histopathological Investigation of the Remote Organ Injury in Cochlea after Ischemia and Reperfusion Caused by Aortic Occlusion

Ayse Iriz, Hakan Ozgul, Erkan Iriz, Gulnur Take, Yildirim Bayazit, Adil Eryilmaz, Serpil Allusoglu

Numune Educational and Research Hospital, Clinic of Third Otorhinolaryngology, Ankara, Turkey (AI, AE, SA)
Gazi University Medical School, Department of Cardiovascular Surgery, Ankara, Turkey (HO)
Gazi University Medical School, Department of Histology, Ankara, Turkey (GT)
Gazi University Medical School, Department of Otorhinolaryngology, Ankara, Turkey (YB)

Objective: To assess the toxic remote organ damage in the cochlea due to ischemia–reperfusion injury following descending aorta surgery, and to investigate the protective effect of iloprost and ascorbic acid.

Materials and Methods: Twenty-one New Zealand rabbits were divided in to 3 groups. In group 1 and 2, a remote injury was created in the cochlea after ischemia-reperfusion injury in spinal cord, renal and lower extremities caused by clamping the descending aorta. In group 1, no additional medications were given. In group 2, iloprost and ascorbic acid were infused 30 minutes before and two hours after the surgical procedure. A sham operation was performed in group 3. The cochlear function was tested using Otoacoustic Emission (OAE) testing before and after the operation. In addition, the cochlea was examined using electron microscopy.

Results: OAE amplitudes decreased postoperatively in group 1 ($p<0.05$). In group 2, pre- and postoperative OAE results were not significantly different ($p>0.05$). In group 3, a postoperative decrease was encountered at only 4 kHz after the operation ($p<0.05$). The morphological changes were more prominent in group 1 when compared to group 2.

Conclusion: Cochlear remote organ damage can occur after ischemia-reperfusion injury in spinal cord, renal and extremities. Iloprost and ascorbic acid has a protective effect against this injury.

Submitted : 15 July 2011 *Accepted : 16 December 2011*

Introduction

Various studies have shown that remote organ damage may develop after ischemia and reperfusion (IR).[1] Aortic cross-clamping is usually performed in aortic surgery. Morbidity and mortality rate is related with the duration and degree of ischemia and mass of tissue involved. Medulla spinalis and renal IR injury is the most frequent complication of thorocoabdominal aorta aneurysms and surgery. At the same time, various systemic complications can arise due to the ischemia – reperfusion and volume loading on heart and brain after these operations. [2]

A number of studies investigated the impact of local IR injury on the cochlea and organ of Corti. [1,3,4,5] However, the impact of remote IR injury on the cochlea and organ of Corti has not been addressed up to date. This study was performed to address this question.

IR injury induces an inflammatory response, which results in the formation of reactive oxygen species (ROS) that augments local tissue damage or affects organs remote from the site of IR. [6] For this reason, we investigated the protective effects of iloprost and ascorbic acid on remote ischemia-reperfusion injury of the cochlea in this study.

Materials and Methods

Twenty-one New Zealand rabbits weighing between 2300 and 2700 grams were used in the study after obtaining Ethic Committee approval. The rabbits were taken to the laboratory 10 days before the experiment, and fed on a standard diet.

Corresponding address:
Ayse Iriz,
Ankara Numune Egitim ve Arastırma Hastanesi, 3. Kulak Burun Bogaz Hastalıkları Kliniği, Samanpazarı, Ankara, Turkey
Phone: 90 312 508 52 20
Mobil Phone: 0505 858 03 70
E-mail: ayseiriz@gmail.com

The rabbits were dived into 3 groups; there were 7 rabbits in each group. For the experimental procedure, they were anesthetized using ketamine and xylazine. Their arterial blood pressures were monitored from the femoral artery. To create ischemia and reperfusion, a 8–9 cm abdominal incision was performed. A cross-clamp was placed on the descending aorta in diaphragm level of the rabbits in groups 1 and 2. In group 3, a sham operation was performed and only an incision was made under anesthesia induced with the same agents. In group 1, aortic clamping was applied to the rabbits for 30 minutes. In group 2, 5 ng/kg/min iloprost and 10 mg/kg ascorbic acid were infused intravenously 20 minutes before the ischemia. The dose of iloprost was increased to 5 ng/kg/min during aortic occlusion, and ascorbic acid was administered at the same dose until postoperative hour 2.

Before and 48 hours after the operation, the otoacoustic emission (OAE) measurements were performed under anesthesia. Forty-eight hours after the operation, the rabbits were euthanized and their temporal bones were removed.

OAE investigation:

Both distortion product and transient evoked OAEs were measured using Bio-logic System equipment (Audx Scout Spot; Bio-logic Systems, Chicago, Illinois, USA) with Scout Acoustic Emmisions System (version 3.45.00) software installed on the computer (which used a 2 GHz Pentium IV processor) in a sound-proof room. Forty-two ears of 21 rabbits were assessed. Once the probe was placed with a good seal in the ear canal, the levels of two frequencies were set according to our protocol. Equilevel primary tones f1 and f2 were fixed at f1/f2 =1.22, and f2 frequencies ranged from 1kHz to 8kHz. The stimulus intensities used for distortion product OAEs were 65 dB for f1 and 55 dB for f2. For transient evoked OAE testing, the recording bandwidth was set between 1 and 4 kHz, with a repetition rate of 40. The stimulus intensity was 80±4 dB. The measurements were performed pre- and postoperatively.

Histopathological Investigation:

The temporal bones of the rabbits in all groups were fixed in 1M phosphate-buffer containing 2.5% glutaraldehyde (Sigma-Aldrich Co.) for 2–3 hours, and decalcified with formic acid. The organ of Corti was dissected under microscope after decalcification for 15-30 days. The tissues were post-fixed in 1% osmium tetroxide (Sigma-Aldrich Co.) and dehydrated in a series of graded alcohols (50, 60, 70, 80, 90, 96 and 100% ethanol). After passing through propylene oxide (Sigma-Aldrich Co.), the specimens were embedded in Araldite CY 212 (Ciba-Geigy), (2-dodecen-1-yl) succinic anhydride (Sigma-Aldrich Co.), benzyldimethyl amine (Poly Sciences Inc.) and dibutylphthalat (Sigma-Aldrich Co.). The semi-thin sections were stained with toluidine blue (Sigma-Aldrich Co.) and examined under a photomicroscope (Leica DM4000, Germany). After selection of the appropriate specimens, thin sections were cut and stained with uranyl acetate (Pro Sci Tech) and lead citrate (Sigma-Aldrich Co.). They were examined by means of an electron microscope (Carl Zeiss EM 900, Germany).

Statistical analysis:

SPSS-15.0 software program was used for the statistical analysis. Wilcoxon nonparametric test was used to compare preoperative and postoperative DPOAE and TEOAE measurements of all rabbits.

Results

OAE testing:

DPOAE amplitudes postoperatively decreased at 1500, 2000 and 8000 Hz, and TEOAE amplitudes decreased at 3000 Hz in group 1, in which IR was made without administration of preventive medications. In group 2, in which IR was performed with administration of the preventive medications (iloprost and ascorbic acid), no difference was found between the preoperative and postoperative OAE test results. In group 3 (sham), DPOAE amplitudes decreased only at 4000 Hz after the operation (Tables 1,2,3).

Electron microscopy:

In group 3 (sham), the round shape of the nuclei of the outer hair cells and their chromatins were prominent. The mitochondria and the other organelles scattered in their cytoplasm were normal. Outer phalangeal cells were normal except for a small amount of vacuolization (Figure 1).

Table 1. Comparison of the results of DPOAE and TEOAE in Group 1 rabbits, preoperative and postoperative

OAE amplitudes (µv)					
	Preoperative		Postoperative		Statistics
TEOAE (Hz)	**Mean**	**SD**	**Mean**	**SD**	**P**
1000	0,1	1,4	-0,1	1,3	0,1
1500	0,02	1,4	-0,3	1,3	0,2
2000	1	2,3	0,7	1,3	0,3
3000	0,5	1,3	0,3	0,8	0,03
4000	1,6	1,7	1,2	1,7	0,1
DPOAE (Hz)					
1000	2,4	6,8	4	12	0,1
1500	9	2,1	6	5,6	0,001
2000	16	7	12	7	0,001
3000	23	11	19,8	9	0,09
4000	37	18	31,7	10	0,1
5000	37,7	8	36	5,7	0,1
6000	43	5,4	40	10,9	0,4
8000	45,8	5,8	43	4,3	0,02

Table 2. Comparison of the results of DPOAE and TEOAE in Group 2 rabbits, preoperative and postoperative

OAE amplitudes (µv)					
	Preoperative		Postoperative		Statistics
TEOAE (Hz)	**Mean**	**SD**	**Mean**	**SD**	**P**
1000	-0,3	2,5	-1,2	2,2	0,3
1500	0,6	1,8	0,5	4,7	0,7
2000	0,1	2,2	1,6	2,3	0,1
3000	0,02	0,8	0,9	2,5	0,1
4000	0,1	2,6	1,9	2,5	0,07
DPOAE (Hz)					
1000	-1,6	5,9	0,01	5,9	0,7
1500	2,1	8,3	6,6	6,8	0,1
2000	6,9	8,8	6	7,5	0,3
3000	7,1	14,6	6,6	13	0,8
4000	11,7	17,8	13	16	0,9
5000	13,3	15,3	16,5	23,9	0,5
6000	18,9	18,5	28	19	0,6
8000	22,7	20	22	27,4	0,9

Table 3. Comparison of the results of DPOAE and TEOAE in Group 3 rabbits, preoperative and postoperative.

OAE amplitudes (µv)					
	Preoperative		Postoperative		Statistics
TEOAE (Hz)	**Mean**	**SD**	**Mean**	**SD**	**P**
1000	0,9	3,2	-1	2,2	0,1
1500	1,3	2,6	0,8	2,4	0,6
2000	2	2,5	0,5	3,4	0,05
3000	0,4	1,8	0,9	2,1	0,4
4000	0,8	1,9	1,1	1,6	0,7
DPOAE (Hz)					
1000	5,9	5	5	4,7	0,9
1500	9,6	4,3	9,4	4,5	0,2
2000	18	6,5	18	6,4	0,3
3000	23	7,8	23	12	0,5
4000	36,9	9,9	29,8	7	0,01
5000	32,5	10	29	12	0,4
6000	42,5	7,7	39	11	0,1
8000	48,4	5	47	6,2	0,7

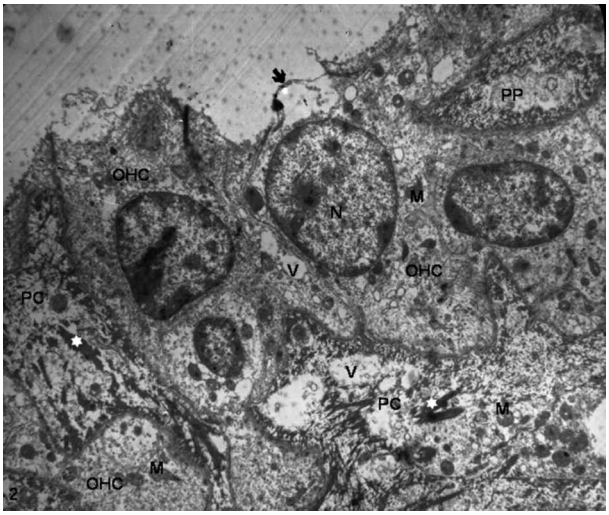

Figure 1. Electron microscopic evaluation of control group. OHC: Outer hair cell, N: nucleus of outer hair cell, PC: Phalangial cell, PP: Phalangial process, M: mitochondria of outher hair cells an phalangial cells are seen in their normal structure, V: vacuolar formation in outer hair cells and phalangial cells,↓: apical evagination like cilia in outer hair cells, ★: electron dense filamentous structure in phalangial cells (Uranyl acetate – lead citrate X3000).

In group 1 (IR group), there was widespread intracellular edema in the organ of Corti, and mitochondria with dense matrices were located underneath the cell membrane. The nuclei were swollen and chromatin network was less dense. Diffuse vacuolar formation was observed in the phalangeal cells (Figure 2).

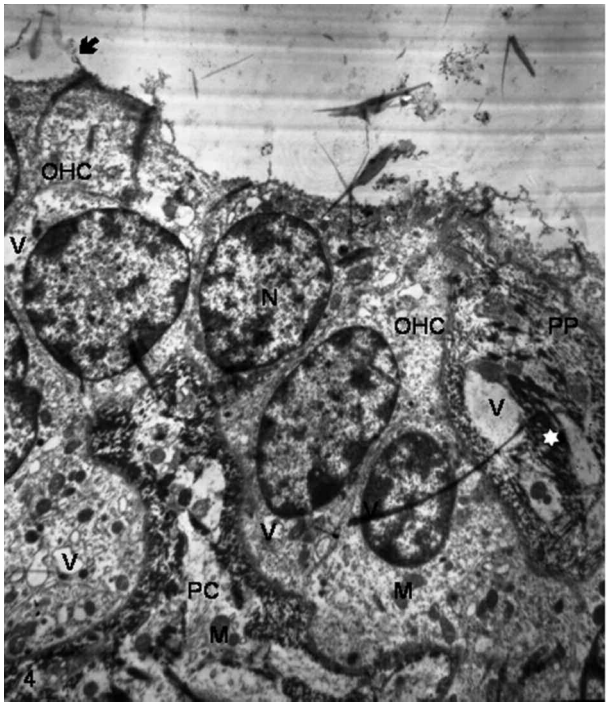

Figure 2. Electron microscopic evaluation of ischemia-reperfusion group. OHC: Outer hair cell, N: Outer hair cell nuclei with heterochromatin, PC: Phalangial cell, PP: phalangial process, M: mitochondria of outer hair cells an phalangial cells are seen in their normal structure, V: increased vacuolar formation in outer hair cells and phalangial cells, ↓: apical evagination like cilia in outer hair cells, ★: electron dense filamentous structure in phalangial cells (Uranyl acetate – lead citrate X3000).

In group 2 (IR group treated with protective agents), although there were edematous cells in the organ of Corti, the organelles of the outer hair cells were usually preserved. Their mitochondria with their dense crista were scattered in the cytoplasm, and their euchromatic nuclei could be observed. However, vacuolization and disruption of the cell membrane was observed in patches (Figure 3).

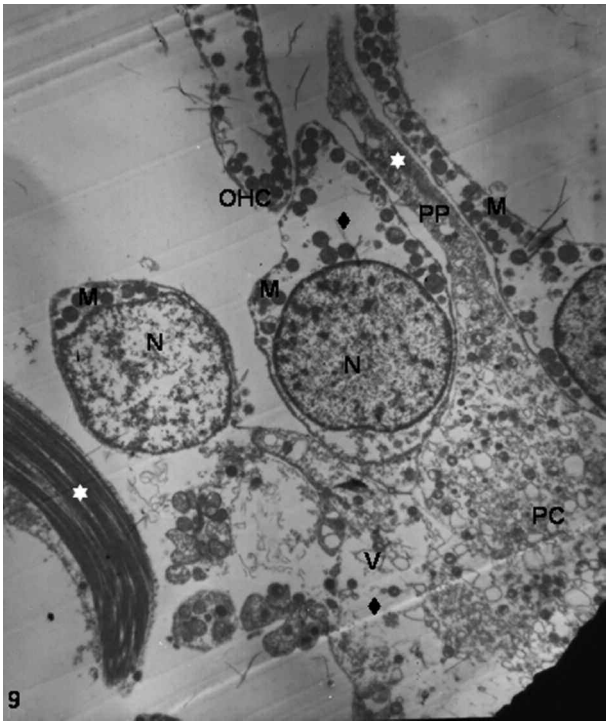

Figure 3. An electron microscopic evaluation after ischemia-reperfusion procedure in iloprost treatment group. OHC: Outer hair cell, N: nuclei of outer hair cells, M: mitochondria with electron dense cristae are seen in peripheral region of cells, PC: Phalangial cell, PP: phalangial process, V: vacuolar formation in phalangial cells, ♦: intracellular edema is seen outer hair cells and phalangial cells, ★: increased and compact electron dense filamentous structure are seen in phalangial cells (Uranyl acetate – lead citrate X3000).

Discussion

The IR injury is seen when the anoxic organ is reperfused by blood supply. IR creates large amount of free oxygen radicals released into the blood circulation and results in generalized inflammatory response and systemic damage. Especially, reperfusion period generates toxic oxygen radicals which have deleterious effect on cellular membranes. [7,8]

Recently, local tissue damage and negative systemic effects that depend on some toxic agents emerging from remote-organ ischemia-reperfusion injury has been investigated. [9] One of the most frequent complications of thoracoabdominal aorta surgery are spinal cord and renal IR. Renal IR injury occurs in many clinical situations, such as transplantation, partial nephrectomy, sepsis, hydronephrosis, or elective urological operations. Although most research in this area has focused on the renal response to this injury, recent work has suggested that renal injury affects and is also regulated by the extra-renal organs including the liver. [10]

The cochlea and organ of Corti can be affected by numerous systemic disorders as well as local ischemia-reperfusion injury.[3] Hearing loss can also result from cerebral ischemia, spinal cord disease and toxic materials such as glutamate. [11] An increase in the perilymphatic glutamate level can be encountered in cases of cochlear ischemia. Glutamate is also responsible for the neuronal damage in ischemia–reperfusion injury. Glutamate antagonists have been used in the treatment of acute stroke. [4] Cochlear damage after remote organ ischemia–reperfusion has been investigated for the first time in this study in the literature.

A number of studies that aimed to explain this topic in the literature focused on reactive oxygen species that usually play an important role in ischemia–reperfusion injury, and superoxide anion, hydrogen peroxide and hydroxyl radicals that could be to be responsible for the damage in cochlea. [3,12] Recurrent oxidative stress causes cell death through apoptosis and by damaging the proteins, lipids, nucleic acids and carbonhydrates directly.[13,14]

Several approaches have been suggested to ameliorate both local and remote organ oxidation in IR injury and some antioxidant agents have been successfully used in experimental animal models. [15,16] Local radical scavengers are shown to decrease the damage caused by ischemia-reperfusion injury. [1] Recently, free radical suppressants, immune system modulators and adenosine have been used to manage the reperfusion

damage. In the past, ascorbic acid (Vitamin C) was used as an antioxidant to scavenger the free radicals in the treatment of myocardial ischemia–reperfusion injury. [17,18]

Iloprost inhibits thrombocyte adhesion and aggregation by binding prostacyclin receptors. It causes vasodilatation, decreases superoxide anions, increases cholesterol circulation, decreases free radicals and regulates microcirculation. [19] The protective effect of iloprost was shown in spinal cord ischemia induced by aortic occlusion for 15 minutes. [20] Antioxidants and circulation regulators have also been used in the treatment of cochlear damage caused by ototoxicity, sudden hearing loss and acoustic trauma. [21,22] Our study has shown the protective effect of iloprost and ascorbic acid in the cochlea after remote IR.

OAEs are generated by the outer hair cells in the cochlea, and can be recorded non-invasively from the external auditory canal. [23] The OAE testing can be used to determine cochlear damage after IR injury. [3,4,24] In a study, a sudden decrease was recorded in DPOAE amplitudes at 4 Hz, 8 Hz and 12 Hz after ischemia and reperfusion. [3] According to our results, as far as OAE results are concerned, iloprost and ascorbic acid protects the cochlea against IR injury.

Previously, immunohistopathological investigations were performed after cochlear local ischemia. [25] However, none of them investigated remote organ damage in the cochlea. We histopathologically investigated cochlear damage in three groups of rabbits after ischemia-reperfusion injury. In group 1 in which ischemia–reperfusion was performed without administration of any preventive medications, a more remarkable cellular degeneration was found when compared to group 2, in which ischemia–reperfusion was created and preventive medications (iloprost and ascorbic acid) were administered.

Conclusion

Cochlear remote organ damage may occur after ischemia-reperfusion injury caused by aortic clamping. Iloprost and ascorbic acid have protective effects against this injury.

References

1. Teke Z, Kabay B, Ozden A, Yenisey C, Bir F, Demirkan NC, et al. Effect of Tempol, a membrane-permeable radical scavenger, on local and remote organ injuries caused by intestinal ischemia/reperfusion in rats. J Surg Res 2008; 149: 259-71

2. Svensson LG, Crawford ES, Hess KR, Coselli JS, Safi HJ. Experience with 1509 patients undergoing thoracoabdominal aortic operations. J Vasc Surg 1993; 17: 357-70.

3. Morawski K, Telischi FF, Niemczyk K. A model of real time monitoring of the cochlear function during an induced local ischemia. Hearing Research 2006; 212: 117-27.

4. Hakuba N, Matsubara A, Hyodo J, Taniguchi M, Maetani T, Shimizu Y, et al. AMPA/ kainite-type glutamate receptor antagonist reduces progressive inner hair cell loss after transient cochlear ischemia. Brain Research 2003; 979: 194-202.

5. Jacobs TP, Kempski O, McKinley D, Dutka AJ, Hallenbeck JM, Feuerstein G. Blood flow and vascular permeability during motor dysfunction in a rabbit model of spinal cord ischemia. Stroke 1992; 23: 367-73.

6. Remick DG, Villarete L. Regulation of cytokine gene expression by reactive oxygen and reactive nitrogen intermediates. J Leukoc Biol 1996; 59:471–475

7. The ische Waisman D, Brod V, Wolff R, Sabo E, Chernin M, Weintraub Z, Rotschild A, Bitterman H. Effects of hyperoxia on local and remote microcirculatory inflammatory response after splanchnic ischemia and reperfusion. Am J Physiol Heart Circ Physiol 2003; 285: 643-52.

8. Cetinus, E., Kilinc, M., Inanc, F., Kurutas, E.B. & Buzkan, N. The role of urtica dioica (urticaceae) in the prevention of oxidative stress caused by tourniquet application in rats. Tohoku J Exp Med 2005; 205: 215-221.

9. Hall E.D, Braughler J.M. Central nervous system trauma and stroke. Biochemical considerations for oxygen radical formation and lipid peroxidation. Free Radic Biol Med 1989; 6: 303-13.

10. Grace P.A. Ischemia-reperfusion injury. Br J Surg 1994; 81: 637-47.

11. Kelly KJ. Distant effects of experimental renal ischemia reperfusion injury. J Am Soc Nephrol 2003; 14: 1549-58.

12. Vlajkovic SM, Hausley GD, Thorne PR. Adenosine and the auditory system. Current Neuropharmacol 2009; 7: 246-56.

13. Billett TE, Thorne PR, Gavin JB. The nature and progression of injury in the organ of corti during ischemia. Hear Res 1989; 41: 189-97.

14. Morawski K, Telischi FF, Merchant F, Abiy LW, Lisowska G, Namyslowski G. Role of mannitol in reducing postischemic changes in distortion- product otoacoustic emission (DPOAE): A Rabbit Model. Laryngoscope 2003; 113: 1615-22

15. Lautermann J, Crann SA, McLaren J, Schacht J. Glutathion dependent antioxidant system in the mammalian inner ear: effect of aging, ototoxic drugs and noise. Hear Res 1997; 114: 75-82.

16. Calikoglu M, Tamer L, Sucu N, Coskun B, Ercan B, Gul A, Calikoglu I, Kanik, A. The effects of caffeic acid phenethyl ester on tissue damage in lung after hindlimb ischemia-reperfusion. Pharmacol. Res 2003; 48: 397-403.

17. Sakaguchi S, Iizuka Y, Furusawa S, Ishikawa M, Satoh, S, Takayanagi M. Role of Zn2+ in oxidative stress caused by endotoxin challenge. Eur J Pharmacol 2002; 451: 309-316.

18. Saidman MD, Quirk WS, Nuttall AL, Schweitzer VG. The protective effect of Allopurinol and superoxide dismutase-polyethyleno glycol on ischemic and reperfusion- induced cochlear damage. Otolaryngol Head Neck Surg 1991; 105: 457-63.

19. Shadid M, Moison R, Steendijk P, Hiltermann L, Berger HM, van Bel F. The effect of antioxidative combination therapy on post hypoxic-ischemic perfusion, metabolism and electrical activity of the newborn brain. Pediatr Res 1999; 44: 119-24.

20. Riva CM, Morganroth ML, Ljungman AG, Schoeneich SO, Marks RM, Todd RF 3rd, et al. İloprost inhibits neutrophil induced lung injury and neutrophil adherence to monolayers. Am J Respir Cell Mol Biol 1990; 3: 301-9.

21. Coskun K, Attar A, Tuna H, Sargon MF, Yüceer N, Türker RK, et al. Early protective effects of iloprost after experimental spinal cord ischemia in rabbits. Acta Neurochir 2000; 142: 1143-50.

22. Sergi B, Fetoni AR, Ferraresi A, Troiani D, Azzena GB, Paludetti G, et al. The role of the antioxidans in protection from ototoxic drugs. Acta Otolaryngol Suppl 2004; 552: 42-5.

23. Cassandro E, Secuino L, Mondola P, Attanasio G, Barbara M, Filipo R. Effect of superoxide dismutase and allopurinol on impulse noise-exposed guinea pigs-electrophysiological and biochemical study. Acta Otolaryngol 2003; 123: 802-7.

24. Morawski K, Namyslowski G, Lisowska G, Bazowski P, Kwiek S, Telischi FF. Intraoperative monitoring of the cochlear function using distortion product emission (DPOAEs) in patients with cerebellopontine angle tumors. Otol Neurotol 2004; 25: 818-25.

25. Olszewski J, Chudzik W, Miłoński J, Kuśmierczyk K. Qualitative and quantitative studies in electron microscopy on influence of experimental ischemia of the vertebral arteries on the outer hair cells function in guinea pigs. Acta Otorhinolaryngol Belg 2003; 57: 151