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„SENSORINEURAL" HEARING LOSS DUE TO INJURIES OF CILIO-TECTORIAL JUNCION

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Abstract: *The paper reviews some studies on the connection between tectorial membrane and stereocilia – part of acoustic receptor. Genetical defects of proteins OTOGELIN, α-, β-TECTORIN, PLASTIN-1, COCHLIN, POLYCISTIN-1 and others are expessed with pure profound sensorineural hearing loss on experimental selected mouses-mutants and isolated human families. All the investigators report that only visible morphological defects are in tectorial membrane and stereocilia bundles, not in other cochlear structures. Resting cochlear potentials manifest normal values. On the other side, ABR and DPOAE are negative – evidence for profound sensoryneural hearing loss. This constellation of results is logically related to loss of connection between tectorial membrane and stereocilia. Thus mechano-electrical transducer (MET) of hair cells can not be activated.*

Keywords: *Sensoryneural hearing loss, Tectorial membrane, Stereocilia.*

JEL Codes: *I10, I20*

INTRODUCTION

External, middle ear and membranous labyrinth and fluids of inner ear conduct mechanically sound energy to hair cells. The deflection of the hair-cell stereocilia, by tectorial membrane (TM) opens mechanically gated ion channels and then cascade of reactions leading to tonic releasing of neurotransmitters to the sinapses. Using bone transducer we reach sound energy directly to membranous labyrinth and suggest that any decrease of perception is due to sensoryneural hearing loss (SNHL).

EXPOSITION

The TM is a gel-like structure containing 97% water. Its dry weight is composed of collagen (50%), non-collagenous glycoproteins (25%) and proteoglycans (25%). Specific glycoproteins are expressed in the TM - α-tectorin, β-tectorin, otolin, ceacam-16 and otogelin. Of these proteins α-tectorin and β-tectorin form the striated sheet matrix that regularly organises the collagen fibres.

Radially the TM is divided into three zones, the limbal, middle and marginal zones. Of these the limbal zone is the thinnest (transversally) and overlies the auditory teeth of Huschke with its inside edge attached to the spiral limbus. The marginal zone is the thickest (transversally) and is divided from the middle zone by Hensen's Stripe.

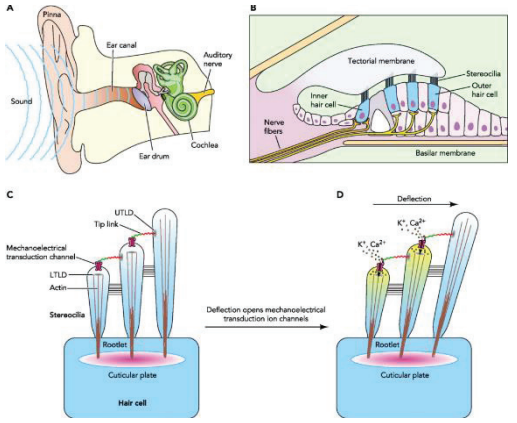

Fig. 1. MET By Lifeng Pan, Mingije Zhang

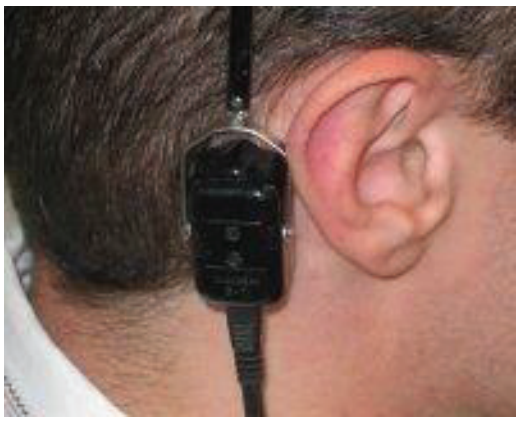

Fig. 2. Bone transducer

Current theoretical conceptions state that sensorineural hearing loss is associated only with injuries of hair cells and neurons of auditory nerve. In the last years there is accumulated data, that defected extracellular proteins of TM like α-tectorin, otogelin, otolin, ceacam-16, or hair cells (plastin-1, polycystin-1, cochlin), cause disability of junction between (TM) and hair cell, without visible morphological or functional injuries of hair cells itself. Intact mechano-electric transducer (MET) in hair cell can't be activated by bone transducer. Thus the results of a number of tests, like threshold audiometry, DPOAE and ABR, can be interpretated like SNHL without real injuries of auditory nerve.

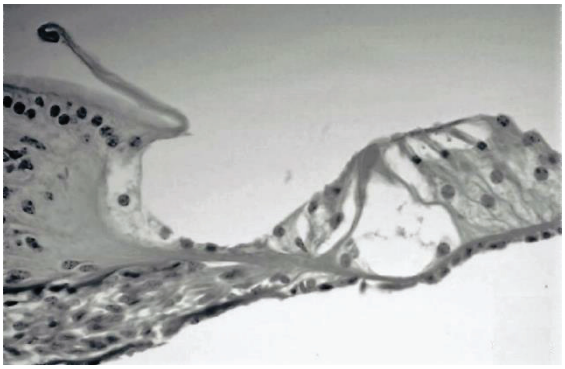

Fig. 3. Luxation of TM (D. Stavrev)

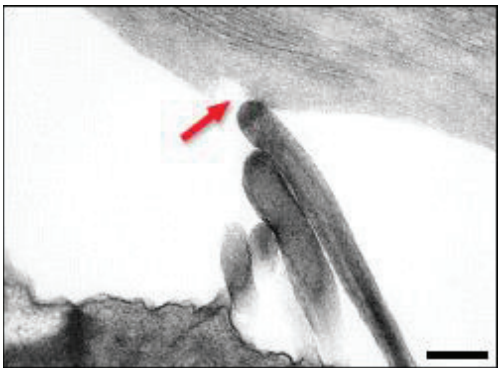

Fig. 4. OHC is detached from TM(R. Pujol)

Part of syndromic and nonsyndromic hearing loss are inherited and caused by a disturbance of building of TM and cilia of hair cells. There are families with hereditary neurosensory hearing loss with definitive genetic abnormalities. Thus laboratory tests were conducted with induced mutations in lab animals and it was proved that there is a relationship between the deficiencies in the synthesis of the structural proteins of m. tectoria and the stereocilia containing proteins and the hearing loss.

At least 12 mutations in the TECTA zone are found in people (locus 11q23.3). These are topically in the sound transducing part of the hearing analizator, but are classified as neurosensory hearing loss: 3 of the mutations lead to recessive NSHL DFNB 21: IVS9DS G-A+1; 1-BP INS 649C; 1-BP DEL 6037G; the remaining 9 lead to dominant NSHL type DFNA12: TECTA LEU1820THR AND GLY1824ASP, TYR1870CYS, CYS1619SER, CYS1837GLY, ARG2021HIS, ARG1890CYS, CYS1837ARG, 5331G-A (WWW.OMIM.ORG).

The mutation Y1870C, same TECTA, induces hearing loss of 50-80 dB in mice, as the adhesive area with the cilia is reduced, and the matrix of the membrane is destroyed (Legan 2005). Studying mice with targetly destroyed entactin G1-like domain in TECTA (Legan 2000), establishes a luxation and deformation of m. tectoria, as well as thinning of the membranes of utriculus and sacculus – these are other places in the labyrinth where α-tectorin is produced. In m. tectoria the other 2 glucoproteins - β-tectorin and otogelin, are not expressed. Morphologically the stereocilia and the cupula in the labyrinth are not changed, and the endocochlear potentials resemble those of the controls (+94.11 ± 8.67 mV as compared to 97.92 ± 5.26 mV). No DPOAE are established, even at acoustic stimuli of 60 dB.

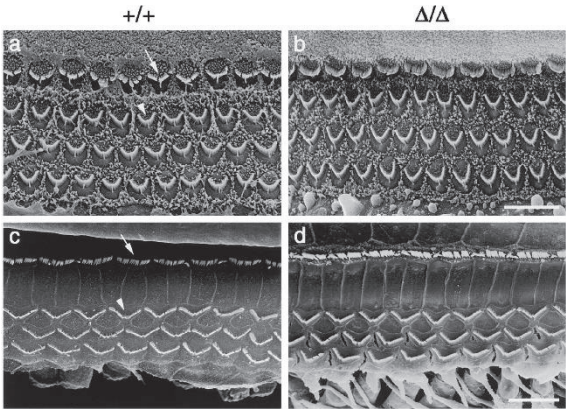

Fig. 5. Intact cilia in mutants

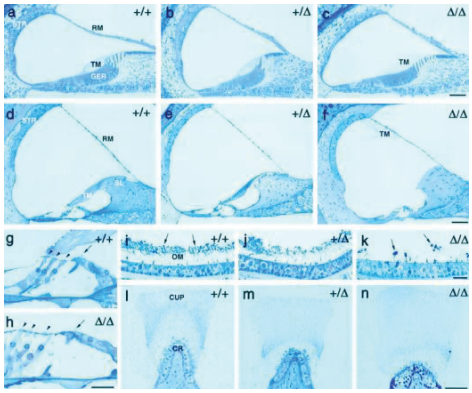

Fig. 6. Morphological changes in control group (Legan) m. tectoria (Legan)

Otogelin is an N-glycosylated protein that is present in the acellular membranes. 3 mutations were discovered in locus 11p15: OTOG 1-BP DEL, 5508C, OTOG PRO2116LEU, OTOG ARG2187TER. Affected individuals had moderate hearing impairment DFN18B (WWW.OMIM.ORG), that was sometimes associated with vestibular dysfunction.

CEACAM-16 is a secreted glycoprotein with no transmembrane domain or glycosylphosphatidylinositol anchor stated by Zheng et al. to chromosome 19q13.31-q13.32. They proposed that CEACAM16 may have a role in connecting stereocilia with the tectorial membrane. There are 2 mutations of gene: THR140PRO, GLY169ARG and affected persons suffer of deafness type DFNA4B.

There are many examples for concomitant injury of hair cells and cochlear membranes. Song et al (2015) investigated regulation of the protein prestin which is important for the hearing. Using mice mutants TECTA C1509G, wich malformative m. tectoria do not contact with all OHC, Song demonstrates local autofeedback, as in isolated from m. tectoria hair cells level of prestin decrease, but **secondary**.

In 1986 Uziel published a paper about the relation between hypothyroidism and malformations of m. tectoria. Forest (1996) reported that injuries of receptors of the thyroid hormone (TH) or jodine deficit cause deafness both in people and mice. Encoding gene TR_{α} и TR_{β} expression continue pre- and postnastal. In mice THRb mutation, THRb(PV) leads to the loss of binding of thyroid hormone (T3) to the TRb protein. Orthologous human THRB(PV) mutation and other similar mutations in human THRB cause resistance to thyroid hormone (RTH), which is occasionally associated with sensorineural hearing impairment and malformations of m. basillaris, stria vascularis and m. tectoria. (Griffith 2002). Having in mind that in Pendred syndrome (1896, mutation of PDS gene 7q31), except deafness and hypothyroidism, there are hydrops of aqueductus vestibule and s. endolymfaticus. Forest, as others did not found cochlear malformations but in 2009 a big team lead by Harold Winter described increased autoimmune reaction against α – and β – tectorin and abnormal m. tectoria in $TR\beta^{-/-}$ mice, as in apical region of cochlea changes are stronger. Winter write: "abnormal structure of the tectorial membrane and

enhanced tectorin levels suggest that disturbed mechanical performance is the primary cause of deafness resulting from TRβ deficiency".

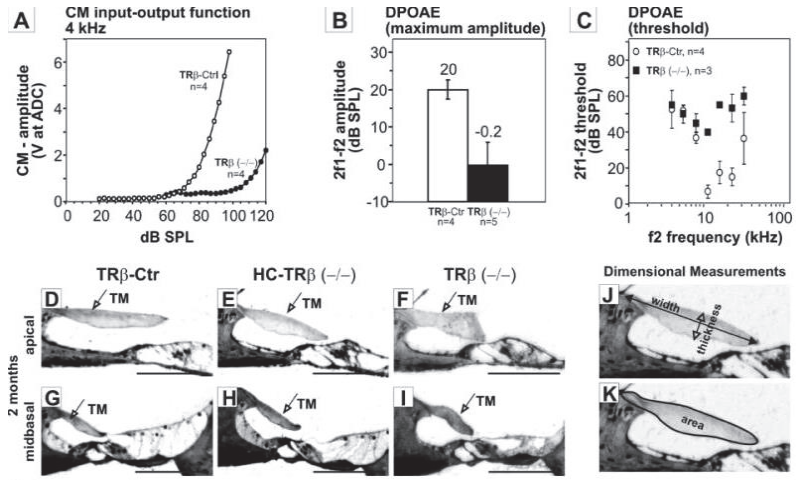

Fig. 7. Cochlear microphonics (CM), DPOAE and morphology of TRβ-Ctr and TRβ−/−mouses

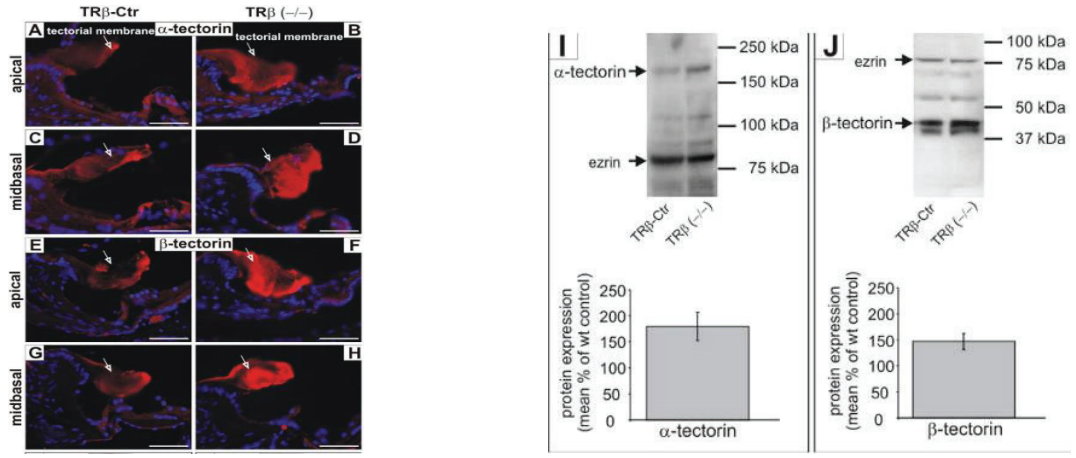

Fig. 8. α- and β-tectorin in TRβ-Ctr and TRβ−/−mice by Winter. A-D - α-tectorin; E-H - β-tectorin; I-J Western blots and densitometric quantification of α-tectorin (*I*) and β-tectorin expression levels (*J*) in TRβ−/− mice compared to TRβ-Ctr animals. Ezrin keeped for normalization of tests

What will happen in cases of injured proteins with preserved connection between cilia and m. tectoria? Study of Jones et al. (2015) on mice with mutations $Tecta^{Y1870C/+}$, $Tectb^{-/-}$, and $Otoa^{EGFP/EGFP}$ reports, that injuries of proteins lead to changes in amplitude-frequency characteristic of m. tektoria, and torment process of TRANSMITION of sound energy to cilia. Result of bone transducer examination will show decrease of PERCEPTION.

The other side of tecto-ciliar junction – cilia of hair cells bounded with a many extracellular proteins like a sailing boat tackle. A number of them take part in transmembrane ion transport, but others have only support function and if they are injured or missing, this should not worsen electrophysiological function of hair cells. Steigelman et al. (2011) published study on Polycistin-1, reported to function as a fluid flow-sensor in the kidney. Mutations on this protein cause polycistyc kidney disease and SNHL in involved people. Based on 3 type mice – mutants they first proved binding of Polycistin-1 with F-actin in hair cell. Then using mutants PC-1 KI and PC-1 CKO observed significant differences in ABR и DPOAE thresholds (фиг.7) between mutants and wild-type (WT) controls. In MET channels of mutants they did not find any abnormalities (Fig.10).

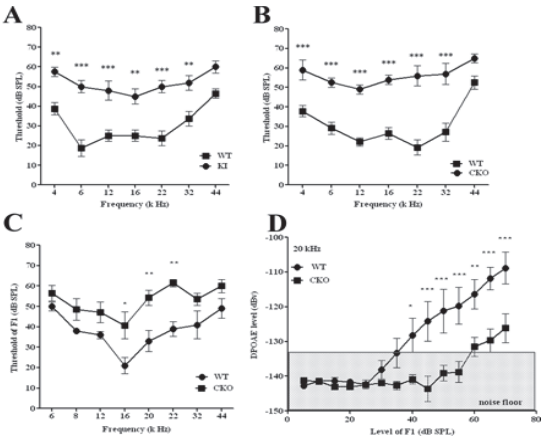

Fig. 9. Increased thresholds of ABR

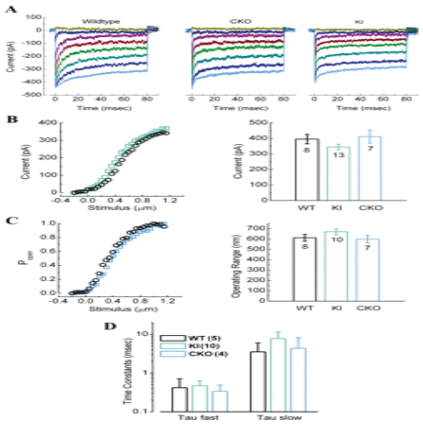

Fig. 10. Normal MET function in WT and mutants

In cochlear structures Steigelman did not ascertain morphological changes except in stereocilia, where both the stereocilia number per bundle and the height of individual stereocilia were significantly different in the mutants compared with controls (Fig. 11)

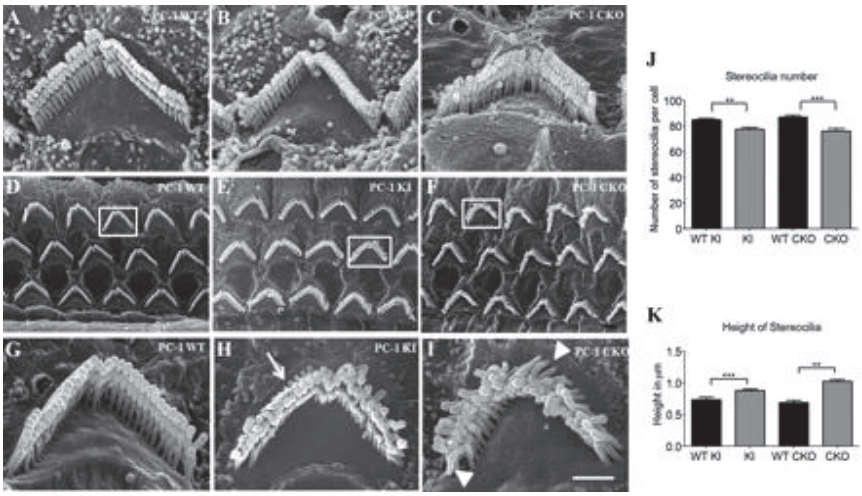

Fig. 11. Sterocilia in WT and mutants mouses

In PLS KickOut mice, withouth Plastin1 Taylor et al.(2015) observed analogical to Legan and Steigelman profound hearing loss with morphological change only in cilia, without any other structural and electrophysiological injuries:

Resting potential (mV) - 74.2 ± 0.7 in het mouses −71.5 ± 1.7 in PLS KO- mouses;
I_K at 0 mV (nA) - 20.4 ± 2.3 7 in het mouses 20.0 ± 0.8 in PLS KO- mouses;
$I_{K,f}$ at −25 mV (nA) - 3.5 ± 0.4 in het mouses 3.0 ± 0.4 in PLS KO- mouses;
$I_{K,n}$ at −124 mV (pA) - 291 ± 37 in het mouses 231 ± 32 (4) in PLS KO- mouses;

Mechanoelectrical transducer currents exhibit minor changes in their adaptation properties in Pls1 KO mice. The maximum MET current was found to be similar between het (−937 ± 46 pA at −81 mV, n = 7) and KO cells (−843 ± 34 pA at −81 mV, n = 12).

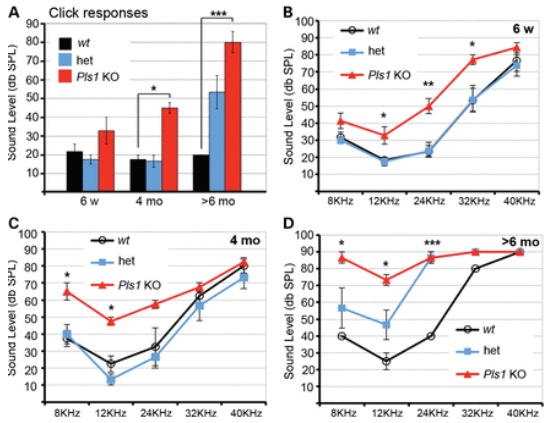

Fig. 12. Increased thresholds of ABR

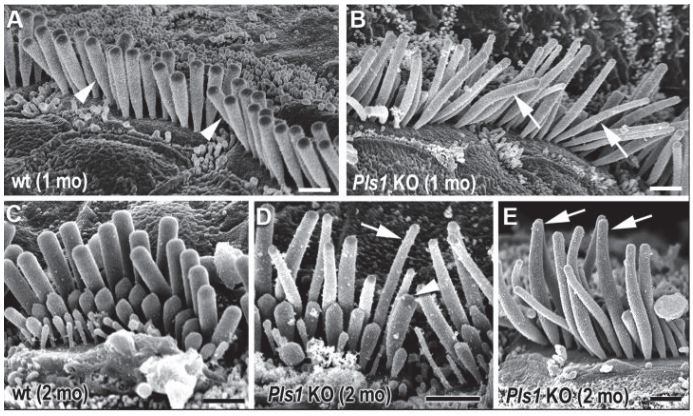

Fig. 13. IHC in Pls1 KO and WT mouses (Taylor)

Autoimmune deaffness, constitute about 1% of all cases, and as often as not are associated with antibodies against HSP-70, tectorin и cochlin proteins. Cochlin is large widespread in cochlea and in case of mutation patients suffer SNHL type DFNA9 and vestibular disfunctions, and up to now are described 9 mutations (WWW.OMIM.ORG): VAL66GLY, GLY88GLU, TRP117ARG PRO51SER, ILE109ASN, ALA119THR CYS542PHE, CYS542TYR, MET512THR.
Solares et al (10) in 2004 investigated murines with induced autoimmune reaction against cohlin and β-tectorin and reported significant increase of CD4+ T-cells and increased ABR treshold. This paper shows the possibility to acquire autoimmune hearing loss, and good treatment results with glucocorticoides.

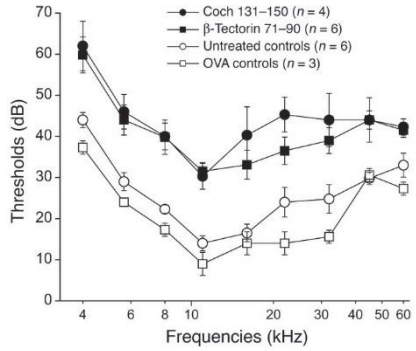

Fig. 14. Increased thresholds of ABR

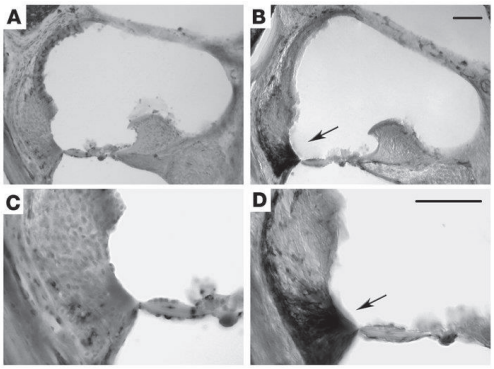

Fig. 15. Inner ear inflammatory infiltrates (Solares)

Physical factors also can harm junction between m. тectoria and stereocillia. Using sound source 105 dB SPL for 72 hours, Canlon (1988) ascertains in vitro alteration, both in tectorial membrane and stereocillia. Topically those injuries correspond to frequencies with hearing loss. Thus can be explained part of cases of acquisite hearing loss. Flock et al (1999) investigated cochlea which have been acoustically traumatized of pure tone 140 Hz with 112 dB SPL. They found temporary and convertible displacement of Deiters and Hensen's cells, with adequate decreasing of microfonic potentials of cochlea. Restitution of cells and microfonic potentials finished for an hour. Similar were the results of Cotanche (1987) in mature avian, but he described in addition cells regeneration.

Assertion of Mattox (1977), that 65% of cases of SNHL recovered fully spontaneously, and independent of treatment in the first 14 days of accident sounds so extreme. But in these cases we can ask question – **is there generally injury of nervous tissue, despite we diagnoze SNHL? Is it possible that in some cases of longstanding deafness, primary injuries in structural proteins of cochlea end secondary degeneration of hair cells and auditory nerve?** There are some of our suspected patients:

9 y.o. girl, underwent emergency hospitalization for absolute deafness of left ear on 19.Apr.2013. Treated with HBO, Cavinton, Betaserc, Milgamma. Discharged on 30.Apr.2013 – small area between 500-1000 Hz with sensivity about 100 dB. After 40 days with home therapy Memo-plant, Betaserc, Milgama – full recovery.

19 y.o. student hospitalized 6 days after full losing of hearing of left ear. Intolerated pure tones with 110 dB SPL. Treated with HBO, Ca^{++} antagonist, Nootropil, Milgamma. On discharge – average hearing loss was 72.5 dB. Rehospitalizated after 8 months with average hearing loss 45 dB, with strong recruitment. One month after second treatment – full recovery up to 4 kHz

65 y.o. man with mild presbyacusis, suddenly lost left ear hearing with small areas of hearing between 250-500 Hz with sensivity about 100 dB. Intolerated pure tones with 110 dB SPL. Treated with HBO, Cavinton, Betaserc, Milgamma. One month after treatment hearing was on level before accident (about 40 dB average).

Medical representative, female, 46 y.o. fully lost hearing of right ear. Treated with HBO, Cavinton, Betaserc, Milgamma. Therapy at home - Tebokan, Nootropil, Milgama. Discharged on V day without improvement. After 15 days – full recovery. Announced that on XII day hearing recovered in 5 minute interval with concomitant strong noices.

CONCLUSIONS

Using results of aforesaid papers, of injures of tectorial membrane and some cell-junction proteins we can expect the following results on examination:

- Profound hearing loss measured by bone transducer, low amplitude of ABR;
- Increase of DPOAE treshold. SOAE should be normal;(no enough evidence)
- All resting cochlear potentials are normal;
- Absent ipsilateral and normal contralateral stapedius reflex at unilateral hearing loss;
- Probably intolerance of extremely loud sounds. (no enough evidence)

Audiologists interprete and treat these results at the present time as sensoryneural hearing loss. Especially similar are results in cases with auditory neuropathy, where injuries are retrocochlear, but in that cases DPOAE is normal, and contralateral stapedius reflex and speech perception are worsen. Investigations of Legan and Solares show us that injuries of tectorin and cochlin can do harm to both auditory and vestibular function.

Succesful treatment of SNHL with glucocorticoids and hyperbaric oxygenation doesn't contradict to aforesaid, because both therapies have antioedema and membrane stabilistation effects. Steroids have significant effect in all autoimmune deseases, thus in these cases are proper therapy.

If we concentrate our clinical thinking on the fact, that real conductive injurie of hearing sense, may be interpretated like SNHL, we can be more effective with our treatment.

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