Utility of Drug Loaded Nanoparticles in the Treatment of Inner Ear Pathology

CRISTIAN MARTU[1], MADALINA GABRIELA GEORGESCU[2]*, IOANA MARTU[3], CORINA BUTNARU[1], VLAD PORUMB[4], LUMINITA RADULESCU[1]

[1] University of Medicine and Pharmacy Gr. T. Popa, ENT Department, 16 Universitatii Str., 700115, Iasi, Romania

[2] Carol Davila University of Medicine and Pharmacy, ENT Department, 8 Eroii Sanitari Blvd., 050474, Bucharest, Romania

[3] University of Medicine and Pharmacy Gr. T. Popa, Department of Implantology, 16 Universitatii Str.,700115, Romania

[4] Surgery Department, University of Medicine and Pharmacy Gr. T. Popa, 16 Universitatii Str., 700115, Iasi, Romania

The lack of drug specificity for inner ear structures and the side effects of systemic administration have determined the treatment strategies to evolve from systemic administration to local application of drugs. The disadvantages of systemic or local administration of drugs for the inner ear, have led to the development of nanoparticles with specific activity. The study presents current research advances regarding the use of nanoparticles (liposomes, PLGA, dendrimers, silica or magnetic nanoparticles, etc.) in inner ear pathology treatment.

Kewords: nanoparticle, inner ear, deafness, drug administration

On demand drug delivery at disease-specific site with minimum side effects represents a great challenge for nanomedicine, hence the efforts are now focused on designing multi-functional nanocarriers to release drugs (nanoparticles) at the particular location.

In case of congenital hearing loss the speech development isis affected with an important impact on academicachievements and further on in adulthood when might play a role on restricted career choice and possible lower earnings[1]. Furthermore, in the adult, the hearing loss leads to social isolation and behavioral disturbance up to depression. Thus the consequences of deafness may be devastating.

The prevalence of hearing loss was first assessed in 1985. At that time it was appreciated that approximately 42 million (0,9%) of people of the world population have disabling hearing impairment[2].Disabling Hearing Loss (DHL) is defined as the loss of hearing greater than 40dB in the better hearing ear in adults and greater than 30dB in the better ear in children. The estimation made 25 years later shows that 360 million (5,3%) people worldwide have DHL[3]. Of these, approximately 32 million (9%) were children, 7,5 millions being younger than 5 years[4]. The escalation of DHL prevalence starting from 1985 until 2011 has several explanations. Starting from the year 1978 the problem of hearing loss screening was emerged at a large scale. The Saskatoon Conference on Early Diagnosis of Hearing Loss recommended in 1978 the registration of neonates as being at high risk for hearing impairment, a resolution requesting provincial and local governments to make registration mandatory was passed with that occasion[5]. On the other end are the elderly. The rise of mean life expectancy from many countries leads to increased prevalence of presbyacusis and not at least the increasing exposure to the. The occupational and environmental noise might be also an important cause of hearing impairment. To these 3 factors that explain the rise of prevalence of hearing loss there has to be added the fact that none of the previously known factors to cause hearing loss has been removed, these factors still contributing in hearing loss. These classic factors that lead to hearing loss development are: ototoxic drugs (more important the aminoglicosid antibiotics and citostatic drugs) – so useful in treatment of serious illnesses, the infectious – especially viral ones – but not only: urlian virus – responsible for mumps, rubella virus, etc. In this field, important progress has been made regarding the prophylaxis. Genetic factors are responsible for more than 50% of congenital deafness. Although real progress has been made in detecting gene mutations responsible for deafness, the development of a prophylactic and/or curative treatment is still on the wish list. Sensorineural hearing loss is nowadays one of the most common diseases accounting for about 50% of all disabling diseases[6].

Treatment for sensorineural hearing loss (SNHL) has evolved from systemic administration of drugs (ex. Ototoxic drugs for intractable MD) to local application of pharmaceutical agents. The systemic administration of medications was started in the middle of the 20th century when Fowler 1948 gave streptomycin to his patients for control of vertigo[7]. After more than half of a century systemic administration of ototoxic drugs (streptomycin) continues to be possible in the treatment of bilateral Meniere's disease by intramuscular drug delivery and the dose calculated by the clinical effect[8]. Among the systemic use drugs there are: steroids as standard treatment in sudden deafness, alone or combined with antiviral drugs [9-12] – although there are authors that question the efficacy of systemic steroid use [13,14] – diuretics used in Meniere's disease treatment [15], biphosponates in otosclerosis [16, 17], vasodilators intravenous in sudden deafness and tinnitus treatment [18], Vitamin E and vitamin C in the treatment of idiopathic sudden sensorineural hearing loss [19, 20] and Betahistine [21,22] (H1 receptor agonist and H3 receptor antagonist) or Arlevert [22 - 24] (combination of cinnaryzine -and a calcium channel blocker and dimenhydrinate –an inverse agonist of the histamine a H1 receptor) given oraly are used in the chronic treatment of Meniere's disease, in vertebrobasilar insufficiency or vestibular neuritis.

* *email: madalina.georgescu@gecad.com; Tel: (+40)722544115*

All above mentioned therapies are in current clinical use although they have significant limitations. The main concern regarding systemic administration is represented by the potential undesirable side effects that may range from minor to life threatening effects – like in, but not limited to, systemic steroid therapy[25,26]. The second important limitation of systemic route for inner ear delivery of medication is the highly variable pharmacokinetic profiles that result in variability in concentrations of the drug at the level of the cochlea[27]. This variability is determined by the patient (age, renal and liver problems, genetic predisposition). Regarding the anatomical condition of the targeted organ – inner ear in our case – it is important to emphasis the anatomic inaccessibility of the cochlea due to the blood-cochlear barrier [28].

To overcome this obstacle, the round window (RW) approach was proposed. Although adverse effects associated with systemic administration are eliminated, the transtympanic administration raises some problems. The most important seems to be the necessity of repeated administration – even in the same day [29,30] because of drug loss through the Eustachian Tube (ET) and different diffusion rate of the drug through the RW membrane according to the individual structure and thickness [28,30]. Added to this is the slow diffusion towards the cochlear apex, with the risk of failure to reach therapeutic concentration.

Administration of different drugs like steroids [31] or insulin like grow factor type I (IGF I)[32] through RW to treat sudden hearing loss or neurotrophic factors to restore synaptic connection and to promote neuron outgrowth after noise trauma show promise[33], but specific delivery methods of drug delivery towards the inner ear are necessary.

One other problem consists in the lack of the specificity of the drug for the sensorineural structures. In this regard, the European Union Consortium (NanoEar) developed some nanoparticles (Np) for specific targeting the sensorineural structures from the inner ear. The specific organ targeting is possible using the tropomyosin receptor kinase B (TRK B) which is a specific receptor for brain-derived neurotrophic factor (BDNF)[34].

Nps are particles of very small size, with nanometric range dimensions. Generally the term of Np is referring to particles with the size ranging from 1 to 100 nm. The structure of a Np has to be constructed according to the function that they have to accomplish. To fulfill a certain function, the size and the shape of the Np is of major importance.

Nps have different properties from the bulk solid of the same material. The changes of the characteristics are due to the small size of the Np – and this phenomenon is known as *size effect*. There are two reasons for these differences. Firstly the molecules located at the surface of the Np are more active because of the free hand and thus they can bond easier with different materials. Secondly, the specific surface area in a Np is increasing in reversal proportion to the particle size and eventually they can load and carry more active substance to the diseased target. In order to use Np for medical purpose they have to disassemble in the components that will be utilized by thehuman body.

The most studies regarding the use of Nps in otorhinolaringology are related to the inner ear diseases. The interest in the use of Np in the pathology of the cochlea derives from the necessity of a targeted therapy at this level. Np might improve drug delivery to the inner ear facilitating crossing the blood labyrinth barrier and targeting more specifically the inner ear. There are Nps described to cross the RW membrane[35] to reach the sensory cells in the cochlea. Tipically the size of Nps used for drug delivery to the inner ear is in the range of 200nm or less[36].

The RW membrane in humans consists of three main layers (epithelial, connective tissue and cellular), with a variable thickness across different species[37-39] that behaves as a semi permeable membrane, protecting against substances washing out from the inner ear towards the middle ear[40,41]. Furthermore the permeability of the RW membrane is also variable, depending on what vehicle or method is used to cross it[41]. For example the permeability is increased by local anesthetics like tetracaine[42] or by histamine[43] or by lowering the humidity at the level of the RW niche[44]. Once the drugs are passing the RW membrane, the presence of a basal-apical gradient in the scala tympany was reported by numerous studies, with a higher concentration in the basal turn than in the apex[45-47]. These differences are due to the slow rate of the perilymph and endolymph flow inside the cochlea and also to the loss of drugs to the adjacent compartments[48].

Crossing the RWM might be done by passive diffusion and also by active targeting mechanisms using some proprieties of the Np (ex. magnetically guided NPs in drug delivery for the inner ear application shows promise[49,50,51] or ligands directed against receptors of the surface of targeted cells[52-54].

On demand drug delivery at disease-specific site with minimum side effects represents a great challenge for nanomedicine, hence the efforts are now focused on designing multi-functional nanocarriers to release drugs at the particular location – referred as NanoCure[55].

To facilitate enhanced drug delivery from the Np, some methods of stimulation have been proposed: thermal, optical, electrical or acoustic activation[56].

Biodegradable as well as non-biodegradable Nps were investigated for inner ear drug delivery purposes. The best known biodegradable particles are those generated from poly-lactic glycolic acid (PLGA). PLGA was approved for human use by the US FDA and has been established as the best NP drug carrier[57]. PLGA administered at the level of the RW was found in high concentration in the cochlea of chinchilla showing good capabilities for delivery of drugs to the inner ear[58]. The next step was to demonstrate that Dex-Ac has a good loading in PLGA[59].

In 2015 Sun C have demonstrated that PEG-PLA Np loaded with Dexamethasone has a hearing protection effect against cisplatin induced deafness[60]. The authors have applied PEG-PLA-Np (labeled with coumarin 6 – a fluorescent labeling dye) and also free cumarin into RWM in guinea pigs through a bulla opening to determine the distribution of Nps in the cochleea after RWM delivery. They found that in guinea pigs treated with Nps labeled with dye the fluorescence was strong in all cochlear turns and also in modiolus compared with free administration of the dye when the fluorescence in the cochleea was weak. In the second experiment they have compared the concentration of drug in the cochlea after free Dexamethasone vs Dexamethasone Nps administration through the RWM of the guinea pigs. They found that the concentration of free Dexamethasone was significantly lower than that of Dexamethasone Nps. When otoprotective effects of Dexamethasone Nps were evaluated, a significant attenuation of cisplatin-induced hearing loss in guinea pigs was found, proven by both functional and histological evaluation[60].

The final aim was to prove that targeted delivery of a drug (dexamethasone) into the inner ear through RWM is

possible in mammalian using PLGA magnetite nanopaticles under an external magnetic field. It was demonstrated that in association with PLGA and magnetite and in the presence of an external magnetic field Dexamethasone Acetate reaches significant higher concentrations in cochlea when compared to passive diffusion[51].

Some other particles that have shown promise for inner ear delivery are silica Nps. Amorphous silica based Np are a new class of mesoporous materials with a large surface area and pour volume for high drug loading. The particles are biodegradable and biocompatible. Placed against RWM of mice, silica Nps were found in the cochlea without any toxic effect on inner hair cells or vestibular hair cells[61]. Silica particles can be functionalized with different surface modification. Glueckert et al. had tested silica Nps with surface modification by peptides mimicking tropomyosin receptor kinase (TrkB) activating antibodies. They found that agonistic antibodies linked to silica nanoparticles showed TrKB activation and enhanced binding to inner ear cells[34].

Liposomes are the most common used Nps[62]. They are phospholipid two layer vesicles with the size in rage of 50 to 200nm. They can be composed of Sph, egg PC, DSPE-PEG-2000, peptide – PEG-lipidic conjugate and DPPRho. The main advantage of liposomes derives from their amphiphilic nature that allows them to carry both hydrophobic and hydrophilic molecules. Other important advantages of liposomes are: greater stability of large compounds, extended circulation lifetime when coated with PEG with prolonged systemic drug exposure and high cellular penetration[63]. New generations of liposomes are represented by: *p*H sensitive liposomes (used to deliver the drugs to intracellular target site), thermo sensitive liposomes – that require the addition of an external stimuli – hence their use might be limited to local treatment, magnetic liposmes (lipid coated nanomagnetic particles) – that also require external application of an magnetic field to attract the drug carrier to the diseased site. Multifunctional liposomes – combine several of all possible properties in a single nanosystem to finally facilitate the accumulation of drug to target site and to minimize side effects. Various side effects were reported for liposomes such as: different types of skin reactions – most common being hand-foot syndrome[64] and hypersensitivity reaction like hypotension or hypertension, dyspnea, flushing, rash and feeling of chocking were found in 30.8% of patients treated with doxorubicin[65,66]. Liposome reactions in such patients can be life threatening[67] Jing Zou et al in 2015 validate a delivery system of liposomes for inner ear therapy. They found that liposome nanocarriers containing gadolinium tetra-azacyclo-dodecane-tetra-acetic acid enters in the cochlea through both oval and round window reaching therapeutic doses[68]. In 2013 Okada et al found that liposome encapsulated hemoglobin (an artificial oxygen carrier) might be utilized in preventing hearing loss due to cochlear ischemia[69]. In 2016 Kechai shows that local administration of corticosteroid embodied in hyaluronic acid liposomal gel is efficient for sustained drug delivery to the inner ear[70]. Delivery of DNA in the inner ear using liposome was also subject of evaluation. One study conducted by Wareing demonstrates the potential of liposome as drug carriers for gene therapy[71].

Dendrimers are water soluble tree-like branched polymers. At the end of their branches are located functional groups that can have positive, negative and neutral charge. Poliamidoamine (PAMAM) dendrimers have ideal properties for gene delivery: high positive charge, a high flexible structure, an internal cavity surface shell, fast biodegradation[72].

Conclusions

The high incidence of deafness and the consequences on the pacient at an individual level but also socialy represents a significant impact in socieconomic matters.

Clasic used drugs do not offer a satisfactory resolution at this moment. The discovery of different types of drug carrying Np translates in a better dose control for the active component, higher drug concentrations in the target organ, the possibility of releasing the drug on a desired schedule, no side effects from sistemic administration. This can be acheived by designing Np that can be activated by different kind of stimuli in order to guide the drug administration process.

Experimental research on animals provide proof of their efficiency in the treatment of deafness but further studies on human patients still need to be performed to acheive results towards better treatment options for inner ear pathology.

References

1.SCHROEDER L, PETROU S, KENNEDY C, MCCANN D, LAW C, WATKIN PM, WORSFOLD S, YUEN HM. The economic costs of congenital bilateral permanent childhood hearing impairment. Pediatrics. 2006 Apr;117(4):1101-12.

2.SMITH,A., Preventing deafness—an achievable challenge. The WHO perspective, International Congress Series, Volume 1240, October 2003, Pages 183-191.

3.***http://www.who.int/mediacentre/factsheets/fs300/en /

4.***http://www.who.int/pbd/deafness/estimates/en/

5.GERBER SE, MENCHER GT (Eds):Early Diagnosis Of Hearing Loss,Grune, New York, 1978

6.LI, L., CHAO, T., BRANT, J., O'MALLEY, B. JR, TSOURKAS, A., LI, D., Advances in Nano-based inner ear delivery systems for the treatment of sensorineural hearing loss..Adv Drug Deliv Rev. 2016 Jan 12.

7FOWLER, E.P. Jr., Streptomycin treatment of vertigo.Trans Am Acad Ophthalmol Otolaryngol. 1948 Mar-Apr;52:293-301.

8.BERRYHILL, W.E., GRAHAM, M.D., Chemical and physical labyrinthectomy for Meniere's disease. Otolaryngol Clin North Am. 2002 Jun;35(3):675-82.

9.CONLIN, A.E., PARNES, L.S., Treatment of sudden sensorineural hearing loss: I. A systematic review. Arch Otolaryngol Head Neck Surg. 2007 Jun;133(6):573-81.

10.PLONTKE, S.K., GIRNDT, M., MEISNER,C., PROBST, R., OERLECKE, I., RICHTER, M., STEIGHARDT, J., DREIER, G., WEBER, A., BAUMANN, I., PLOBL, S., LÖHLER, J., LASZIG, R., WERNER, J.A., RAHNE, T., Multicenter trial for sudden hearing loss therapy - planning and concept. HNO. 2016 Apr;64(4):227-36.

11.SWACHIA, K., SHARMA, D., SINGH, J.,Efficacy of oral vs. intratympanic corticosteroids in sudden sensorineural hearing loss.J Basic Clin Physiol Pharmacol. 2016 Jan

12.TUCCI, D.L., FARMER, J.C. JR., KITCH, R.D., WITSELL, D.L.,Treatment of sudden sensorineural hearing loss with systemic steroids and valacyclovir. Otol Neurotol. 2002 May;23(3):301-8.

13.WEI, B.P., MUBIRU, S., O'LEARY, S.,Steroids for idiopathic sudden sensorineural hearing loss.Cochrane Database Syst Rev. 2006 Jan 25;(1):CD003998

14.CONLIN, A.E., PARNES, L.S., Treatment of sudden sensorineural hearing loss: II. A Meta-analysis.Arch Otolaryngol Head Neck Surg. 2007 Jun;133(6):582-6.

15.COELHO, D.H., LALWANI, A.K., Medical management of Ménière's disease. Laryngoscope. 2008 Jun;118(6):1099-108.

16.QUESNEL, A.M., SETON, M., MERCHANT, S.N., HALPIN, C., McKENNA, M.J., Third-generation bisphosphonates for treatment of sensorineural hearing loss in otosclerosis..Otol Neurotol. 2012 Oct;33(8):1308-14.

17.UPPAL, S., BAJAJ, Y., Otosclerosis 2: the medical management of otosclerosis., Coatesworth AP.Int J Clin Pract. 2010 Jan;64(2):256-65.
18. PROBST, R., TSCHOPP, K., LUDIN, E., KELLERHALS, B., PODVINEC, M., PFALTZ, C.R.,A randomized, double-blind, placebo-controlled study of dextran/pentoxifylline medication in acute acoustic trauma and sudden hearing loss. Acta Otolaryngol. 1992;112(3):435-43.
19.HATANO, M., URAMOTO, N., OKABE, Y., FURUKAWA, M., ITO, M.,.Vitamin E and vitamin C in the treatment of idiopathic sudden sensorineural hearing loss.Acta Otolaryngol. 2008 Feb;128(2):116-21.
20.KAYA, H., KOÇ, A.K., SAYYN, Y., GUNES, S., ALTYNTAÞ, A., YEÐIN, Y., KAYHAN, F.T.,Vitamins A, C, and E and selenium in the treatment of idiopathic sudden sensorineural hearingloss.Eur Arch Otorhinolaryngol. 2015 May;272(5):1119-25.
21.FRAYSSE, B., BEBEAR, J.P., DUBREUIL, C., BERGES, C., DAUMAN, R., Betahistine dihydrochloride versus flunarizine. A double-blind study on recurrent vertigo with or without cochlear syndrome typical of Menière's disease.Acta Otolaryngol Suppl. 1991;490:1-10.
22.NOVOTNÝ, M., KOSTRICA, R., Fixed combination of cinnarizine and dimenhydrinate versus betahistine dimesylate in the treatment of Ménière's disease: a randomized, double-blind, parallel group clinical study. Int Tinnitus J. 2002;8(2):115-23.
22.NOVOTNY, M., KOSTRICA, R., Fixed combination of cinnarizine and dimenhydrinate versus betahistine dimesylate in the treatment of Méniere's disease: a randomized, double-blind, parallel group clinical study. Int Tinnitus J. 2002;8(2):115-23.
23.OTTO, V., FISCHER, B., SCHWARZ, M., BAUMANN, W., PREIBISCH-EFFENBERGER, R., Treatment of vertebrobasilar insufficiency—associated vertigo with a fixed combination of cinnarizine and dimenhydrinate. Int Tinnitus J. 2008;14(1):57-67.
24.SCHOLTZ, A.W., STEINDL, R., BURCHARDI, N., BOGNAR-STEINBERG, I., BAUMANN, W.,Comparison of the therapeutic efficacy of a fixed low-dose combination of cinnarizine and dimenhydrinate with betahistine in vestibular neuritis: a randomized, double-blind, non-inferiority study. Clin Drug Investig. 2012 Jun 1;32(6):387-99.
25.CHROUSOS, G.P., KINO, T.,Glucocorticoid action networks and complex psychiatric and/or somatic disorders.Stress. 2007 Jun;10(2):213-9.
26.FAUCHER, K., AAS-HANSEN, O., DAMSGARD, B., STENKLEV, N.C., Effects of systemic versus local gentamicin on the inner ear in the Atlantic cod, Gadus morhua (L.), relevance for fish hearing investigations. Hear Res. 2008 Jun;240(1-2):12-21.
27.PLONTKE, S.K., WOOD, A.W., SALT, A.N., Analysis of gentamicin kinetics in fluids of the inner ear with round window administration.Otol Neurotol. 2002 Nov;23(6):967-74.
28.SWAN, E., MESCHER, M., SEWELL, W., TAO, S., BORENSTEIN, J., Inner Ear Drug Delivery for Auditory ApplicationsAdv Drug Deliv Rev. 2008 Dec 14; 60(15): 1583–1599.
29.CHIA, S.H., GAMST, A.C., ANDERSON, J.P., HARRIS, J.P.,Intratympanic gentamicin therapy for Ménière's disease: a meta-analysis.Otol Neurotol. 2004 Jul;25(4):544-52.
30.SALT, A.N., PLONTKE, S.K., Principles of local drug delivery to the inner ear.Audiol Neurootol. 2009;14(6):350-60.
28.SWAN, E., MESCHER, M., SEWELL, W., TAO, S., BORENSTEIN, J., Inner Ear Drug Delivery for Auditory ApplicationsAdv Drug Deliv Rev. 2008 Dec 14; 60(15): 1583–1599.
30.SALT, A.N., PLONTKE, S.K., Principles of local drug delivery to the inner ear.Audiol Neurootol. 2009;14(6):350-60.
31.NG, J.H., HO, R.C., CHEONG, C.S., NG, A., YUEN, H.W., NGO, R.Y., Intratympanic steroids as a salvage treatment for sudden sensorineural hearing loss? A meta-analysis.Eur Arch Otorhinolaryngol. 2015 Oct;272(10):2777-82.
32.NAKAGAWA, T., KUMAKAWA, K., USAMI, S., HATO, N., TABUCHI, K., TAKAHASHI, M., FUJIWARA, K., SASAKI, A., KOMUNE, S., SAKAMOTO, T., HIRAUMI, H., YAMAMOTO, N., TANAKA, S., TADA, H., YAMAMOTO, M., YONEZAWA, A., ITO-IHARA, T., IKEDA, T., SHIMIZU, A., TABATA, Y., ITO, J., A randomized controlled clinical trial of topical insulin-like growth factor-1 therapy for sudden deafness refractory to systemic corticosteroid treatment.BMC Med. 2014 Nov 19;12:219.
33.GLUECKERT, R., BITSCHE, M., MILLER, J.M., ZHU, Y., PRIESKORN, D.M., ALTSCHULER, R.A., SCHROTT-FISCHER, A., Deafferentation-associated changes in afferent and efferent processes in the guinea pig cochlea and afferent regeneration with chronic intrascalar brain-derived neurotrophic factor and acidic fibroblast growth factor. J Comp Neurol. 2008 Apr 1;507(4):1602-21.
34.GLUECKERT, R., PRITZ, C.O., ROY, S., DUDAS, J., SCHROTT-FISCHER, A.,Nanoparticle mediated drug delivery of rolipram to tyrosine kinase B positive cells in the inner ear with targeting peptides and agonistic antibodies.Front Aging Neurosci. 2015 May 19;7:71.
35.LIU, H., WANG, Y., WANG, Q., LI, Z., ZHOU, Y., ZHANG, Y., LI, S., Protein-bearing cubosomes prepared by liquid precursor dilution: inner ear delivery and pharmacokinetic study following intratympanic administration. J Biomed Nanotechnol. 2013 Oct;9(10):1784-93.
36..McCALL, A.A., SWAN, E.E., BORENSTEIN, J.T., SEWELL, W.F., KUJAWA, S.G., MCKENNA, M.J., Drug delivery for treatment of inner ear disease: current state of knowledge. Ear Hear. 2010 Apr;31(2):156-65.
37.GOYCOOLEA, M.V., CARPENTER, A.M., MUCHOW, D., Ultrastructural studies of the round-window membrane. Arch Otolaryngol Head Neck Surg. 1987;113:617–624.
38.NORDANG, L., LINDER, B., ANNIKO, M., Morphologic changes in the round window memebrane after topical hydrocortisone and dexamethasone treatment. Otology and Neurotology. 2003;24:339–43.
39. TANAKA, K., MOTOMURA, S.,Permeability of the labyrinthine window in guinea pigs. Arch Otorhinolaryngol. 1981;233:67–75.
40.SALT, A.N., MA, Y., Quantification of solute entry into cochlear perilymph through the round window membrane. Hear Res. 2001 Apr;154(1-2):88-97.
41.GOYCOOLEA, M.V., Clinical aspects of round window membrane permeability under normal and pathologic conditions. Acta Otolaryngol. 2001;121:437–47.
41. GOYCOOLEA, M.V., Clinical aspects of round window membrane permeability under normal and pathologic conditions. Acta Otolaryngol. 2001;121:437–47.
42.HOFT, J., The permeability of the round window membrane and its changes by pantocaine (tetracaine). Arch Klin Exp Ohren Nasen Kehlkopfheilkd. 1969;193(2):128-37.
43. CHANDRASEKHAR, S.S., RUBINSTEIN, R.Y., KWARTLER, J.A., GATZ, M., CONNELLY, P.E., HUANG, E., BAREDES, S., Dexamethasone pharmacokinetics in the inner ear: comparison of route of administration and use of facilitating agents. Otolaryngol Head Neck Surg. 2000 Apr;122(4):521-8.
44.MIKULEC, A.A., HARTSOCK, J.J., SALT, A.N., Permeability of the round window membrane is influenced by the composition of applied drug solutions and by common surgical procedures. Otol Neurotol. 2008 Oct;29(7):1020-6.
45.ZOU, J., HANNULA, M., MISRA, S., FENG, H., LABRADOR, R.H., AULA, A.S., HYTTINEN, J., PYYKKÖ, I., Micro CT visualization of silver nanoparticles in the middle and inner ear of rat and transportation pathway after transtympanic injection. J Nanobiotechnology. 2015 Jan 27;13:5.
46.HAHN, H., SALT, A.N., BIEGNER, T., KAMMERER, B., DELABAR, U., HARTSOCK, J.J., PLONTKE, S.K., Dexamethasone levels and base-to-apex concentration gradients in the scala tympani perilymph after intracochlear delivery in the guinea pig. Otol Neurotol. 2012 Jun;33(4):660-5.
47.HAHN, H., SALT, A.N., SCHUMACHER, U., PLONTKE, S.K., Gentamicin concentration gradients in scala tympani perilymph following systemic applications. Audiol Neurootol. 2013;18(6):383-91.
48.SHEPHERD, R.K., COLREAVY, M.P., Surface microstructure of the perilymphatic space: implications for cochlear implants and cell- or drug-based therapies. Arch Otolaryngol Head Neck Surg. 2004 May;130(5):518-23.
49.GE, X., JACKSON, R.L., LIU, J., HARPER, E.A., HOFFER, M.E., WASSEL, R.A., DORMER, K.J., KOPKE, R.D., BALOUGH, B.J., Distribution of PLGA nanoparticles in chinchilla cochleae. Otolaryngol Head Neck Surg. 2007 Oct;137(4):619-23.

50.TAMURA, T., KITA, T., NAKAGAWA, T., ENDO, T., KIM, T.S., ISHIHARA, T., MIZUSHIMA, Y., HIGAKI, M., ITO, J., Drug delivery to the cochlea using PLGA nanoparticles. Laryngoscope. 2005 Nov;115(11):2000-5.
51.DU, X., CHEN, K., KURIYAVAR, S., KOPKE, R.D., GRADY, B.P., BOURNE, D.H., LI, W., DORMER, K.J., Magnetic targeted delivery of dexamethasone acetate across the round window membrane in guinea pigs. Otol Neurotol. 2013 Jan;34(1):41-7.
52.NIBUYA, M., NESTLER, E. J., DUMAN, R. S. (1996). Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J. Neurosci. 16, 2365–2372.
53.SCHUBERTOVA, V., MARTINEZ-VERACOECHEA,F..J, VÁCHA, R.,Influence of ligand distribution on uptake efficiency.Soft Matter. 2015 Apr 14;11(14):2726-30.
54.LIU, W., KINNEFORS, A., BOSTROM, M., RASK-ANDERSEN, H., (2011). Expression of TrkB and BDNF in human cochlea-an immunohistochemical study. Cell Tissue Res. 345, 213–221. doi: 10.1007/s00441-011-1209-3.
55.BOURZAC, K., Nanotechnology: Carrying drugs. Nature. 2012 Nov 22;491(7425):S58-
56. SAGAR V, PILAKKA-KANTHIKEEL S, POTTATHIL R, SAXENA SK, NAIR M. Towards nanomedicines for neuroAIDS. Rev Med Virol. 2014 Mar;24(2):103-24.
57.DI TORO, R., BETTI, V., SPAMPINATO, S., Biocompatibility and integrin-mediated adhesion of human osteoblasts to poly(DL-lactide-co-glycolide) copolymers. Eur J Pharm Sci. 2004 Feb;21(2-3):161-9. PubMed PMID: 14757487.
58.GE, X., JACKSON, R.L., LIU, J., HARPER, E.A., HOFFER, M.E., WASSEL, R.A., DORMER, K.J., KOPKE, R.D., BALOUGH, B.J., Distribution of PLGA nanoparticles in chinchilla cochleae. Otolaryngol Head Neck Surg. 2007 Oct;137(4):619-23.
59.WANG, Y., GAO, X., KURIYAVAR, S., BOURNE, D., GRADY, B., CHEN, K., DORMER, K., KOPKE, R., Incorporation, release and effectiveness of Dexamethasone in poly(lactic-co-glycolic acid) nanoparticles for inner ear drug delivery. J Nanotechnol Eng Med. 2011.
60.SUN, C., WANG, X., ZHENG, Z., CHEN, D., WANG, X., SHI, F., YU, D., WU, H.,A single dose of dexamethasone encapsulated in polyethylene glycol-coated polylactic acid nanoparticles attenuates cisplatin-induced hearing loss following round window membrane administration. Int J Nanomedicine. 2015 May 14;10:3567-79.
61.PRAETORIUS, M., BRUNNER, C., LEHNERT, B., KLINGMANN, C., SCHMIDT, H., STAECKER, H., SCHICK, B., Transsynaptic delivery of nanoparticles to the central auditory nervous system. Acta Otolaryngol 2007 May;127(5):486-90.
62.FENSKE, D.B., CULLIS, P.R., Acyl chain orientational order in large unilamellar vesicles: comparison with multilamellar liposomes: a 2H and 31P nuclear magnetic resonance study.Biophys J. 1993 May;64(5):1482-91.
63.FENSKE, DB., MACLACHLAN, I., CULLIS, P.R., Stabilized plasmid-lipid particles: a systemic gene therapy vector. Methods Enzymol. 2002;346:36-71.
64.LOTEM, M., HUBERT, A., LYASS, O., GOLDENHERSH, M.A.,INGBER, A., PERETZ, T., GABIZON, A., Skin toxic effects ofpolyethylene glycol-coated liposomal doxorubicin.Arch Dermatol 2000;136:1475-1480.
65.CHAN, A., SHIH, V., THAM, CHEE, KIAN., Liposomal doxorubicin-associated acute hypersensitivity despite appropriate preventive measures.J Oncol Pharm Pract. 2007 Jun;13(2):105-7.
66.STEELE, J.P., O'DOHERTY, C.A., SHAMASH, J., EVANS, M.T., GOWER, N.H., TISCHKOWITZ MD, RUDD RM. Phase II trial of liposomal daunorubicin in malignant pleural mesothelioma.Ann Oncol. 2001 Apr;12(4):497-9.
67.SZEBENI, J., BARANYI, L., SÁVAY, S., BODÓ, M., MILOSEVITS, J., ALVING, C.R., BÜNGER, R., Complement activation-related cardiac anaphylaxis in pigs: role of C5a anaphylatoxin and adenosine in liposome-induced abnormalities in ECG and heart function. Am J Physiol Heart Circ Physiol. 2006 Mar;290(3):H1050-8. Epub 2005 Oct 7.
68.ZOU,J., HANNULA,M., SUPERB MISRA, HAO FENG, LABRADOR,R.H., ANTTI S AULA, JARI HYTTINEN., ILMARI PYYKKÖ., Micro CT visualization of silver nanoparticles in the middle and inner ear of rat and transportation pathway after transtympanic injection Journal of Nanobiotechnology2015 13:5.
69.OKADA, M., KAWAGUCHI, A.T., HAKUBA, N., HYODO, J., HATO, N., GYO, K., Liposome-encapsulated hemoglobin alleviates hearing loss after transient cochlear ischemia: an experimental study in the gerbil. Neurosci Lett. 2013 Oct 11;553:176-80. doi: 10.1016/j.neulet.2013.08.043. Epub 2013 Aug 27.
70.EL KECHAI, N., MAMELLE, E., NGUYEN, Y., HUANG, N., NICOLAS, V., CHAMINADE, P., YEN-NICOLAY, S., GUEUTIN, C., GRANGER, B., FERRARY, E., AGNELY, F., BOCHOT, A., Hyaluronic acid liposomal gel sustains delivery of a corticoid to the inner ear.J Control Release. 2016 Mar 28;226:248-57.
71.WAREING, M., MHATRE, A.N., PETTIS, R., HAN, J.J., HAUT, T., PFISTER, M.H., HONG, K., ZHENG, W.W., LALWANI, A.K., Cationic liposome mediated transgene expression in the guinea pig cochlea. Hear Res. 1999 Feb;128(1-2):61-9.
72.TOMALIA, D.A., BAKER, H., DEWALD, J., HALL, M., KALLOS, G., MARTIN, S., ROECK, J., RYDER, J., SMITH, P., A New Class of Polymers: Starburst-Dendritic Macromolecules. Polymer Journal 1985;17:117-132.

Manuscript received: 15.01.2016