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Animal Model Studies Yield Translational Solutions for Cochlear Drug Delivery

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Abstract

The field of hearing and deafness research is about to enter an era where new cochlear drug delivery methodologies will become more innovative and plentiful. The present report provides a representative review of previous studies where efficacious results have been obtained with animal models, primarily rodents, for protection against acute hearing loss such as acoustic trauma due to noise overexposure, antibiotic use and cancer chemotherapies. These approaches were initiated using systemic injections or oral administrations of otoprotectants. Now, exciting new options for local drug delivery, which opens up the possibilities for utilization of novel otoprotective drugs or compounds that might not be suitable for systemic use, or might interfere with the efficacious actions of chemotherapeutic agents or antibiotics, are being developed. These include interesting use of nanoparticles (with or without magnetic field supplementation), hydrogels, cochlear micropumps, and new transtympanic injectable compounds, sometimes in combination with cochlear implants.

Introduction

The field of hearing loss and deafness research is on the cusp of development and clinical introduction of many new drugs and compounds to prevent or treat hearing loss and

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deafness. Much evidence exists that otoprotective drugs have beneficial or detrimental effects on cochlear auditory processing in animal models, and some of this exciting progress will be reviewed in the present article. Through the investigational new drug evaluation and FDA approval process, some of these chemical, stem cell or gene therapy interventions have been shown or will be found to be efficacious, but will not be translatable clinically due to systemic side effects. Therefore, a great need exists for clinically appropriate techniques for local, inner ear compound delivery. Current and recent animal model experimentation has yielded much hope and translational promise for the development of clinically-suitable cochlear drug or gene therapy delivery paradigms. The present article will summarize these relevant efforts, and provide representative literature references that capture this progress and excitement. More specifically, we will summarize studies that have shown successful inner ear interventional effects with systemic administration, and then compare results with the newer, exciting local drug delivery strategies for the cochleae of lab animals. Our review complements and extends two recent reviews in this area (Ayoob & Borenstein 2015; Lilun et al. 2017).

Systemic Effects: Cochlear Drug Delivery Yields Oto-Protective and Other Actions

Systemic applications of certain preventative agents given orally or in some cases via intraperitoneal (IP) injections have shown that certain types of acquired sensorineural hearing loss (SNHL) can be reduced or prevented. Most of this work has been accomplished utilizing rodents, such as guinea pigs, rats and mice.

Antibiotic-Induced Inner Ear Deficits

Aminoglycosides are cost-effective bactericidal aminoglycosidic aminocyclitols, so they are widely used, but unfortunately ototoxicity is a significant dose-limiting side effect (Guthrie 2008). This collateral damage to the inner ear manifests itself as a bilateral, high-frequency hearing loss and in many cases it is accompanied by vestibular deficits. This permanent hearing impairment is accompanied by loss of hair cells and cochlear spiral ganglion neurons. A likely, primary mechanism is that the antibiotic iron-aminoglycoside complexes potentiate reactive oxygen species, producing ROS-induced cellular degeneration in inner ear sensory cells. Specifically, there are several studies that have shown a relation between endoplasmic reticulum (ER) stress and hearing loss (e.g., Zong et al. 2017). This is a situation when excessive improperly folded proteins accumulate in the ER lumen. Basically, during the ER stress, the unfolded protein response is activated to restore ER proteostasis, but severe ER stress can lead to apoptosis- programmed cell death.

Sha & Schacht (2000) have done some interesting work showing the otoprotective of aspirin and D-methionine (D-Met), an amino acid with chelating and antioxidant properties, on preventing antibiotic-related hearing loss, e.g., for gentamicin, kanamycin. For example, utilizing D-Met, administration both *in vitro* and *in vivo* in guinea pigs, these investigators demonstrated reduction in gentamicin-induced free radical generation. In addition, auditory brainstem response (ABR) threshold shifts due to gentamicin ototoxicity were attenuated with twice-daily injections of D-Met, with once-daily injections being less effective,

pointing to the importance of optimizing dosage timing and pharmacokinetics for antioxidant protection *in vivo*.

Campbell and colleagues have also recently presented evidence for antibiotic neuroprotection utilizing D-Met (Campbell et al. 2016). Guinea pig were administered an ototoxic dose of kanamycin, with D-met and ABR thresholds were measured at 4, 8, 14, and 20 kHz at baseline and two, four, and six weeks after kanamycin and D-met administration injections. Specifically, the guinea pigs were administered a subcutaneous kanamycin sulfate (250 mg/kg/day) injection once per day for 23 days to induce cochlear toxicity. D-met was injected intraperitoneally twice daily at 0 (saline), 120, 180, 240, 300, 360, 420, or 480 mg/kg/day, 15 minutes prior to and seven hours after kanamycin injection for the same 23 days. Six-weeks after ABR testing, outer hair cell (OHC) quantification was conducted. Their study sample included: eight groups of 10 male pigmented guinea pigs that were administered a subcutaneous kanamycin (250 mg/kg/dose) injection once per day, and an IP D-met injection (0 (saline), 120, 180, 240, 300, 360, 420, or 480 mg/kg/day) twice per day, for 23 days. They observed significant ABR threshold shift *reductions* and *increased* number of surviving outer hair cells in the D-met treated group. Specifically, a 300 mg/kg/day optimal otoprotective D-met dose provided 34–41 dB ABR threshold shift reductions.

Additional studies by University of Michigan auditory investigators, such as Miller and LePrell, have shown that an antioxidant cocktail given orally can be effective in rodents for reducing both antibiotic and noise-induced (SNHL). Much of their success was achieved utilizing a combination of vitamins A (converted by the body from beta-carotene), C (ascorbic acid) and E (trolox), together with magnesium (Mg, a vasodilator). This cocktail primarily works by scavenging and reducing free radicals, including oxygen and peroxy, and singlet oxygen (Miller 2012). The Mg, as well as nitric oxides, counteract the reduced cochlear blood flow that often occurs during cochlear stress induced by antibiotics or noise overexposure; improving cochlear energy-producing and oxygenation respiratory processes (Vass et al. 1996).

Using an alternative strategy to attenuate aminoglycoside-induced hearing loss, Fetoni and her team (2003) gave α -tocopherol, which interferes with gentamicin-induced free radical formation, suggesting that this drug may be useful in preventing aminoglycoside ototoxicity. Specifically, they treated groups of adult guinea pigs, and treated them for 2 weeks as follows: Group A, controls; Group B, gentamicin plus corn oil; Group C, gentamicin only; and Group D, gentamicin plus α -tocopherol. To assess the effect of the otoprotectant on hearing, they measured the compound action potential (CAP- a measure of auditory nerve activity) recorded near the round window membrane (RWM); and to record vestibular function, they monitored the vestibulo-ocular reflexes (VORs). They discovered that α -tocopherol co-therapy slowed the progression of SNHL and significantly reduced permanent threshold shifts (PTS). In addition vestibular function was improved versus controls, as manifested by an increase in VOR gain. Structurally, in control animals, a massive loss of outer hair cells was observed in the cochlear basal turn, as well as the degeneration of cristae ampullaris stereocilia in gentamicin-treated guinea pigs; and this damage did not occur in the inner ear of animals treated with α -tocopherol. Recently, Le Prell et al. (2014) showed that this type of diet can produce statistically reliable increases in

plasma levels of vitamins C and E and magnesium, while significantly decreasing the gentamicin-induced hearing loss at frequencies including 12 kHz and below in guinea pigs.

Noise-Induced Hearing Loss

A major story here is the impressive work of the research groups of Colleen LePrell, Joseph Miller and Kathleen Campbell, where they administered antioxidants, such as Vitamins A, C, E, and Mg, or D-Met, to reduce or prevent noise-induced hearing loss in rodent animal models via systemic drug delivery to the cochlea. A primary mechanism in play here is that noise overexposure induces oxidative stress in cochlear cells, as a result of mitochondrial bioenergetics mis-regulation during and after the noise stimulation. Another major mechanism is that noise trauma induces a down-regulation in cochlear glutathione (GSH) and therefore, strategies to enhance cochlear GSH levels may reduce noise-induced cochlear injury (Kopke et al. 2002). D-met is an agent that replenishes GSH levels.

Specifically, Campbell and co-workers (2007) exposed chinchillas to 105 dB SPL, octave-band noise centered at 4 kHz for 6 hrs. The experimental animals then received a treatment of 200 mg/kg D-met IP injections starting 1 hr after cessation of the noise exposure, with an additional four injections given at 12 hr intervals. D-met treatment resulted in up to a 15 dB improvement in the ABR threshold shifts between 2 and 8 kHz, following acoustic trauma; and rescue of inner and outer hair cells (Kopke et al. 2002). This team continued their elegant studies by investigating treatment timing effects, namely they tested whether D-met protected against noise trauma if treatment was given for intervals at 3, 5 and 7 hr post-noise exposure (Campbell et al. 2011). They measured OHC loss in cochleae obtained at sacrifice 21 days post-exposure. Administration of D-met starting at any of the delay times of 3-7 hr post-noise exposure significantly improved day 21 ABR threshold shifts at 2 and 4 kHz and also showed less OHC loss at all hair cell regions measured (places on the cochlear tonotopic map corresponding to 2, 4, 6 and 8 kHz).

Specific, exemplary results by LePrell et al. (2007) include measurement of ABR thresholds prior to and 10 days post-noise exposure (octave-band noise centered at 4 kHz, 120-dB SPL, 5 hours). ABR threshold shifts were improved at 4, 8, and 16 kHz by the A, C, E and magnesium (ACEMg) combination. Interestingly, treatment with the antioxidants (ACE) or magnesium (Mg) alone did not result in significant otoprotection. Similar effects were obtained regarding inner and outer hair cell loss: maximal protection with the combination cocktail, with very little benefit from the ACE or Mg alone.

Another pharmacological, cochlear drug delivery approach employed by Eric Bielefeld's team for achieving protection from the deleterious effects of loud noise is to utilize Src protein tyrosine kinase (PTK) inhibitors as protective or therapeutic treatments (Bielefeld 2015). They employed subcutaneous injection of PTK inhibitors in chinchillas before noise exposure and showed similar protective effects to N-acetyl cysteine (NAC) as well as significant otoprotective effects when this compound was injected in combination with NAC. In addition, these authors discussed challenges of the blood-labyrinth barrier pertaining to systemic injection cochlear drug delivery methodologies. They highlighted the extra procedures needed for dose-response pharmacokinetics to quantify effective dosages when using systemic cochlear drug delivery strategies. Constraints on the schedule of

delivery were also discussed, all pointing to need for local, controllable inner ear drug delivery systems.

Unfortunately, the positive rodent findings on reduction of noise-induced hearing loss have not yet translated well for human clinical trials, illustrating that rodent studies do not always bode well for clinical trials (e.g., LePrell et al. 2016); including those using noise exposure during routine training by military service members on military bases in the US and Europe. These may be due to species differences, or variations in dosage magnitudes, durations or timing relative to differential responses to the noise exposures. Also, most humans are exposed to noise, more or less, throughout their lives; whereas most rodent studies of prevention of noise-induced hearing loss have employed acute, versus chronic, exposures to loud acoustic stimulation.

Chemotherapy-Related Hearing and Balance Problems, Tinnitus

As oncologists become more effective in saving the lives of cancer patients, both pediatric and adult, minimizing side effects becomes a more intensive area of enquiry and clinical research. This is particularly important in children whose lives are saved, as they have to live with the platinum-induced sequelae and other side effects for many years. A major side effect of platinum-based and certain other types of cancer chemotherapeutic agents is ototoxicity (Travis et al. 2014; Frisina et al. 2016; Wheeler et al. 2016). So, hearing researchers are now turning their attention to drug development, to identify agents, first in animal model studies, that can attenuate the hearing loss and balance problems induced by cancer chemotherapy, without interfering with the anti-neoplastic efficacy of the chemotherapy.

Campbell and colleagues (2007), similar to their work with reducing noise-induced hearing loss, report significant ABR threshold shift reductions with both oral and IP D-Met for cisplatin-induced ototoxicity in guinea pigs. Similarly, Parham (2011) showed that intratympanic injections of D-Met can attenuate cisplatin-induced hearing loss at low frequencies in young adult CBA/CaJ mice and at high frequencies in aging mice. Another study by Sun and co-workers (2015 (2016) in guinea pigs explored protective effects of systemic (IP) administration of dexamethasone (DEX) and DEX encapsulated in polyethylene glycol-coated polylactic acid (PEG_PLA) nanoparticles (NP). Treatment one hour before cisplatin administration showed protection of cochlear structures and function (ABR) for DEX-NP, equivalent to a multidose (3 days) for DEX alone. So, the nanoparticles enhanced protective efficacy of DEX for an acute, systemic application for cisplatin-induced hearing loss. But in this case, the effect was limited to apical regions (low frequency), and the protective effects, while enhanced, were not sufficient to completely prevent hearing loss. Additionally, Ramaswamy's investigative team (2017) delivered prednisolone-loaded nanoparticles magnetically to the cochlea of cisplatin-treated mice. Hearing protection at high frequencies was observed, with some outer hair cell protection, even over intratympanic injections of methylprednisolone. As a final exemplary current study of protection for chemotherapy-induced ototoxicity, Kaur and colleagues (2016) delivered the drug A1AR via transtympanic injection in rat inhibited cisplatin induced ototoxicity (ABR). The presumed mechanism for this hearing protection is suppression of the NOX3 inflammation

pathway in the cochlea that is involved in cases of acute hearing loss stress, such as chemotherapy-related hearing loss.

Can Cochlear Drug Delivery Slow Down the Progression of Age-Related Hearing Loss?

Another relatively effective approach to systemic drug delivery involves utilization of subcutaneous, time release drug pellets in animal models. Walton, Frisina and colleagues have built upon the discovery that the naturally occurring hormone aldosterone can modulate a key ion channel in the cochlear stria vascularis. The stria vascularis degenerates with age in mammals, and is the specialized organ on the cochlear lateral wall responsible for maintaining the “cochlear battery”/endocochlear potential (EP) needed for proper acoustic transduction by organ of Corti hair cells. Ding and co-workers (2014) demonstrated that small concentrations of aldosterone, a hormone which also declines with age in mammals, can up-regulate the ion channel expression and physiological viability of the key cochlear ion channel sodium-potassium-chloride-cotransporter-1, NKCC1. Building upon this elegant method for increasing NKCC1 actions, Frisina et al. (2016) and Halonen et al. (2016), utilized subcutaneous, time-release aldosterone pellets to treat the progression of age-related hearing loss, in middle age, aging CBA/CaJ mice. Their multidisciplinary findings included systemic treatment effects over a 4-month therapeutic period of the aldosterone pellets (relative to saline controls): Significant reduction of ABR threshold shifts across the mouse hearing range; increased hearing sensitivity measured behaviorally; rescue of spiral ganglion cell counts in the modiolus; and up-regulation of cochlear NKCC1 and mineralocorticoid ion channel expression levels.

Innovative Techniques for Local Drug Delivery: Obviation of Side Effects, Improved Effective Dosage

Cochlear Implant Drug Delivery

One active area of cochlear local drug delivery in animal models is the approach of placing therapeutic agents onto the electrode arrays of cochlear implants. Since this topic is covered in another article of this Special Issue (Wise et al. 2018), only some recent, exciting advances in this area will be summarized here (e.g., Liu et al. 2013). For example, Wise et al. (2011) and Mallick et al. (2013) delivered neurotrophin agents (NT3-neurotrophin 3, and BDNF- brain derived neurotrophic factor) in several mammalian species, including guinea pig and cats, and found that spiral ganglion cell (SGN) survival increased over time relative to control animals who were deafened but did not receive the neurotrophins. Scheper et al. (2017) in a guinea pig model, delivered DEX, and looked at possible protective effects with and without electrical stimulation with the cochlear implant. DEX delivery alone did not affect SGN density, but DEX when combined with chronic electrical stimulation did promote increased spiral ganglion cell health. Salt and colleagues (2017) infused FITC-labeled dextran markers with a cannula incorporated into a cochlear implant in guinea pigs. They looked at concentration gradients for up to 7 days. Based upon the concentration gradients measured over time, they hypothesized that there may be permeabilization of the blood-labyrinth barrier as part of the immune response to the implant. Additional studies using DEX, and other trophic agents such as growth factors, have demonstrated, primarily in

guinea pigs, that these drugs can be delivered with cochlear implants made of different biomaterials, including silicone, a star-shaped polyethylene glycol prepolymer (sPEG) or PDMS, and those coated with the newer hydrogels (Farhadi et al. 2013; Kikkawa et al. 2014; Stathopoulos et al. 2014; Wrzeszcz et al. 2014; Liu et al. 2016). And lastly, an interesting variation, which is to have living cells generate the drug of interest, with confirmation of efficacy tested *in vitro* so far, brings in an alternate approach to sustained, cochlear implant drug delivery. In this application, Hütten et al. (2013) used an ultra-high viscous alginate matrix to house NIH3T3 fibroblasts producing endogenous BDNF. They examined this drug delivery strategy in culture for 30 days to demonstrate viability, and then used the supernatant in a primary spiral ganglion cell culture to demonstrate neuroprotective effects.

Approaches Using Injections and Nanoparticles

Innovative new approaches are emerging with the use of nanoparticles, hydrogels, magnetic particles and other new carriers for targeted, cochlear drug delivery in animal models.

Chandrasekhar and colleagues (2000) made pioneering progress regarding intra-tympanic, middle ear cochlear drug delivery in guinea pigs. In their study they compared perilymph dexamethasone (DEX) concentrations after systemic (IV) and intratympanic administration, and assessed the role of 3 potential transport facilitators of intratympanic-DEX into perilymph, measured by radioimmunoassay. Intratympanic-DEX resulted in higher perilymph steroid levels than intravenous dexamethasone. Histamine co-administration resulted in significantly higher perilymph steroid levels than intratympanic-DEX alone. In contrast to histamine, neither hyaluronic acid nor dimethyl sulfoxide was a potent facilitator.

Pursuing a nanoparticle approach, Buckiova and colleagues (2012) utilized liposome and polymersome nanoparticles (NPs), both formed from amphiphilic molecules (lipids in liposomes and block copolymers in polymersomes), and tested them as tools for cochlear drug delivery via placement onto the RWM in adult mice. After one day, they observed the presence of both types of NPs labeled with fluorescent markers in the spiral ganglion in all cochlear turns without producing any distinct morphological or functional damage to cochlear cells. To discover if these NPs could carry a bound substance into the cochlea via the round window, they attached disulfiram, a neurotoxic agent, as a model payload. Following this toxic delivery, they found a significant decrease in the number of spiral ganglion neurons, but no changes in the structure or functionality of the hair cells; indicating that the NPs could carry a neuro-active agent into the cochlea.

Tan and colleagues (2012) carried this approach forward innovatively by placing BDNF-loaded poly- γ -glutamic acid (PGA) particles in guinea pigs. Specifically, they delivered these particles (3.4×10^8 particles per animal) into the cochlea of guinea pigs that were systemically deafened with high doses of aminoglycoside antibiotics. Their delivery method involved soaking small pieces of surgical grade Gelfoam® (Pfizer, Germany) made from gelatin sponge in the nanoparticle solution, and inserting them into the cochlea through the RWM. They achieved some beneficial effects, including increased spiral ganglion cell survival relative to deafened animals without the encapsulated PGA particle therapy.

Shi et al. (2013) utilized a very innovative approach of utilization of microbubbles with ultrasound induced cavitation in the middle ear cavity to enhance transport across the RWM by 3.5 to 38 times that of diffusion transport alone in a guinea pig model. They visualized increased permeability of the RWM. Interestingly, there was no damage to RWM integrity, nor were hearing thresholds elevated as assessed by click ABRs. This is quite important, since RWM delivery is a very practical route for all drug and gene therapies, and permeability remains a major challenge. This type of approach would be broadly applicable to both animal studies and human translation.

Bielefeld (2013) continued his interesting studies of noise over-exposure in the chinchilla animal model employing local delivery of an NADPH oxidase inhibitor (AEBSF) (4-[2-aminoethyl] benzene sulfonyl fluoride). They exposed the chinchillas to one of two loud noises: a 4 kHz octave band noise at 106 dB SPL for 6 hr or an impulse noise that consisted of 75 pairs of 155 dB pSPL impulses. When injecting this therapeutic compound near the round window, they found that this intervention reduced permanent ABR threshold shifts for their impulse noise, but not for the continuous noise, and did not reduce temporary threshold shifts (TTS). Outer hair cell losses were no different between treated animals and controls. Bielefeld explained that the lack of protection for the longer-duration continuous noise might be attributed to a number of possibilities related to dose level and delivery schedule; i.e. the round window drug application was before the noise exposure, so it is possible that if it were applied immediately after the continuous noise exposure some TTS or PTS protection might have been achieved. The latter dosing strategy may be required since it is known that the physiological processes responsible for cochlear damage from noise overexposure, such as free radical damage, continue to occur even after the termination of the noise stimulus, sometimes for hours or even days.

Pettingill and colleagues (2011) examined SGN survival in guinea pig, following an antibiotic-based ototoxic insult (furosemide/kanamycin deafness), and found that SGN survival was enhanced with the co-administration of an innovative application of Schwann cell secreting BDNF capsules injected into the cochlea. Specifically, 5 days after being deafened, alginate capsules (10–12) containing BDNF-Schwann cells (eBDNF-SC cohorts) or empty capsules (control cohorts) were slowly injected into the cochlea via a cannula placed in a cochleostomy opening. This innovative utilization of a neurotrophin-based capsule system holds much promise to enhance spiral ganglion neuron survival when used in conjunction with cochlear implants for the deaf.

Along the same vein, liposome drug nanocarriers have been tested via intratympanic injection delivery in rat, tested for biocompatibility, including inflammatory response, apoptosis, and auditory function, and determined to be safe, paving the way for conjugation with therapeutic agents such as trophic compounds or growth factors (Zou et al. 2017). Zou and colleagues point out that advantages of Liposome nanocarriers (LPNs) for effective inner ear therapy are related to their high drug loading capacity and efficient uptake in the cochlea after a minimally invasive intratympanic administration. In sum, they hold much promise for being an effective carrier for a variety of potentially therapeutic compounds and drugs to be applied to the inner ear.

Other possible nanoparticle drug carriers being tested in rodent animal models for biocompatibility and cochlear uptake actions include modified Poly(lactic-co-glycolic acid)-PLGA nanoparticles which are particularly effective for outer hair cell uptake (Wen et al. 2016); and nanoengineered silica supraparticles loaded with BDNF that can enhance spiral ganglion survival, and have versatility for delivering a variety of otoprotective agents (Wise et al. 2016).

Other approaches have been found to be better suitable for sustained release of therapeutic compounds. For example, biodegradable silk-PEG hydrogel with DEX has been demonstrated to provide sustained delivery of this corticosteroid for a period of 10 days, with normal hearing verified following the administration to confirm the safety of this approach (Yu et al. 2016); PHEA oligoarginine-conjugated nanoparticles have worked for RWM applications in mice (Yoon et al. 2015). This team actually investigated 3 carrier molecule formulations: PHEA-g-C18, PHEA-g-Arg8, and PHEA-g-C18-Arg8. A series of *ex vivo* initial experiments and *in vivo* procedures in C57 mice revealed that the arginine conjugate, PHEAg-C18-Arg8 nanoparticles were successfully transported into the inner ear through an intratympanic route, transfected hair cells, and showed to be a promising delivery carrier for future inner ear disease treatments.

Another approach involves utilization of albumin particles (10-100nm) loaded with Rhodamine B, in an attempt to provide higher surface area for drug binding, due to the unique, flower shape of these particles (Yu et al. 2014). A limitation of Yu's investigation was that although they reported fluorescence of albumin particles in the middle ear of guinea pigs, they did not look at concentration in the cochlea, so it is not clear if the Rhodamine was being released into the cochlea or not. Another strategy involved self-assembled cubic liquid crystalline nanoparticles (cubosomes) 100-200nm size (Liu et al. 2013). This approach needs further investigation since good transport across the RWM following intratympanic injection was achieved, i.e., they saw a 2.6× increase in intracochlear concentration of the drug using the cubosomes as compared to the free drug delivered intratympanically; however some cell toxicity also appeared. For a review of other nanoparticle approaches, including liposomes, polymersomes, lipidic nanocapsules, see Pritz et al. (2013).

Wang et al. (2014) developed mesoporous silica nanoparticles (diameter ~400nm) with a closely packed structure under capillary force action. The result was a macroporous structure with a pore size of ~100-200nm that can then be loaded with drug. The goal of this approach was to greatly increase drug loading capacity relative to nanoparticles generally used for delivery. They tested sustained delivery of BDNF in pre-deafened guinea pigs with cochleostomy at the basal turn, and implantation of 7 of these BDNF loaded mesoporous supraparticles. After 28 days of implantation, they did histology and observed improved survival of auditory nerve fibers.

Another innovative approach to nanoparticle delivery to the cochlea is utilization of magnetic particles placed on the RWM and then applying an electric field near the cochlea to facilitate penetration of the particles into the cochlea. For example, Du et al. (2013) used PLGA-magnetite-DEX-Ac nanoparticles (482 nm± 158 nm with a zeta potential of -19.9

mv) applied to the RWM in guinea pigs. A permanent magnet (0.26 mT) was placed opposite the RWM for one hour to pull the particles across the RWM. They saw a significant increase in intracochlear concentration with the magnetic assisted delivery over diffusion alone. They also observed RWM loading with nanoparticles, and expect this would provide sustained delivery via passive diffusion.

A unique methodology has appeared relatively recently: Intratympanic aerosol delivery. Li et al. (2013) used a silicon-based ultrasonic nozzle with a syringe pump to provide constant flow. The ultrasonic nozzle aerosolized the liquid with an average drop diameter of 7µm. They looked at protection from acoustic trauma in albino guinea pigs by delivering methylprednisolone with this innovative system. Very little protection was seen for auditory brainstem response thresholds or inner hair cells, but some protection from outer hair cell loss was reported. So, although there results for attenuating noise-induced hearing damage were not impressive, this strategy may prove useful with further development for introducing therapeutic agents into the intact cochlea.

Very recently, employing the CRISPR gene editing technique, Gao and colleagues (2018) have succeeded in improving auditory function, measured physiologically and behaviorally in mice with a single nucleotide mutation that causes deafness. Specifically, they microinjected, via a cochleostomy, cationic lipid-mediated Cas9–guide RNA complexes that preferentially disrupt the dominant deafness-associated allele in the Tmc1 (transmembrane channel-like gene family 1) Beethoven (Bth) mouse model of congenital SNHL. Mice with this mutation in the Tmc1 gene are profoundly deaf. Aside from functional improvements in the mice receiving this cochlear gene therapy, they also observed improved hair cell survival relative to mice with the same deafness allele, but who received gene therapy for a different gene.

Hydrogel Methodologies for Inner Ear Drug Applications

For hydrogel applications in the middle ear for localized cochlear drug delivery, hyaluronic acid liposomal gel delivery of dexamethasone phosphate, which converts to the active form DEX, has been injected into the middle ear for successful delivery in the guinea pig for sustained periods of 30 days (El Kechai et al. 2016). This hyaluronic acid gel has a unique advantage of selective thinning properties, making injections more advantageous, as well as the long period of sustained release. Similar approaches have been used by Havenith et al. (2015) involving delivery of brain-derived neurotrophic factor (BDNF) via Gelfoam placement to a perforated or intact RWM in a guinea pig model. They observed enhanced neuronal survival in the basal turn with both BDNF treated groups, but with ABR responses similar to normal-hearing controls, only in the perforation group, suggesting that RWM perforation may be a clinically relevant approach for local drug delivery.

Lajud et al. (2015) obtained similar promising results utilizing a Chitosan-hydrogel-based nanoparticle delivery to the RWM in the mouse. Specifically, they employed nanoparticles of 160 nm in diameter, which were functionalized NPs with a phospholipid bilayer shell and aqueous core are able to cross cellular membranes and persist in physiologic conditions for at least two weeks without significant degradation. Indeed they crossed the RWM and were

identified in the perilymph and cochlear cells. So, effective use of a unique nanoparticle delivery vehicle coupled with a controlled release hydrogel.

Hütten et al. (2014) utilized a unique approach to extending hydrogel delivery. They had a DEX PEG-based hydrogel, but rather than allowing the hydrogel to directly interface with the physiological environment, they put it in a silicone reservoir (a tube). This slowed down release, and allowed sustained release over several weeks. They tested their innovative technique in a guinea pig cochlear trauma model and showed some hearing protection and reduced fibrosis. An advantage highlighted was that you could replenish the therapeutic agent in the tube. Lastly, Sircoglou and colleagues (2015) took a very innovative approach and developed silicone-based thin film implants for application to the oval window (stapes footplate), achieving noteworthy sustained release of DEX within 2 min, with a peak at 1 week, and a sustained presence a month later.

Micropumps Can Deliver Efficacious Actions through the Middle Ear or via Cochleostomy

Utilization of micropumps allows for very carefully controlled drug delivery to the middle ear for compounds that can cross the RWM, and also for direct infusions into the cochlea or vestibular system via tiny holes that can be made in the bony labyrinth, such as cochleostomy into the scala tympani or scala media. For direct injections or fluid sampling of scala media, another surgical approach is used in the mouse. The mouse is the most challenging of our animal models for cochlear drug delivery because of its extremely small size; i.e., the entire volume of the mouse cochlea is less than 1 μL , and the dimensions are on the order of 1-2 mm, requiring typical infusion rates in the low nanoliter per minute range.

Borkholder and Frisina's team have utilized salicylate delivery via cochleostomy or middle ear infusions to induce temporary threshold shifts (TTS) in rodents to trace and quantitatively modeled solute and fluid flow through the mouse cochlea using nanoliter flow rates. Salicylate (aspirin) has the pharmacological property of temporarily inhibiting outer hair cell function, which reduces the responses of otoacoustic emissions (lowers OAE amplitudes, and raises OAE thresholds) in a reversible way. This team specifically assessed cochlear function using a closed-system distortion product otoacoustic emissions (DPOAE) measurement setup from 8 to 49 kHz. When salicylate was infused directly into the basal region of mouse scala tympani via cochleostomy, using a table top syringe pump which simulated the flow rates of the micropump. Reversible changes in outer hair cells responses were observed, with maximal effects in the cochlear base (high frequencies) near the infusion site, and smaller, delayed changes in the apex (low frequencies) (Borkholder et al. 2010). This strong basal-to-apical concentration gradient was found to be consistent with a diffusion-driven distribution. This gradient limits the efficacy of therapeutic approaches for apical structures, and puts constraints on practical therapeutic dose ranges. To address this, they found that making an opening, or fluid vent in the vestibular system (superior semicircular canalostomy) can decrease the base-to-apex concentration gradients and therefore, increase the perfusion of the apical, low frequency cochlear regions, presumably via increased fluid flow out of scala vestibuli (via the helicotrema) and into the vestibular labyrinth. Computer simulations modeling diffusion, volume flow, interscala transport, and physiological clearance mechanisms gave estimates of drug concentration as a function of

time and cochlear position. Variations in infusion flow rate on frequency-dependent DPOAE threshold shifts were also evaluated. Both the canalostomy technique and a flow rate increase were found to *enhance* apical responses for lower frequencies.

This group continued its cochlear micropump studies utilizing a RWM approach for delivering salicylate (Borkholder et al. 2014). Part of the motivation was related to the fact that cochleostomies in some rodents such as mice, although not inducing a temporary threshold shift, generally cause a permanent hearing loss, as measured with ABRs a week or more after the cochleostomy. So, salicylate studies were repeated by this investigative team, except for in this recent study, a hole in the bulla was made and the microtubing from the lab syringe micropump was surgically placed in the middle ear, with the tubing tip near the RWM. Results were similar to their previous cochleostomy study, in that hearing measures were most affected in the base, and the base-to-apex concentration gradient was reduced by utilizing the posterior semicircular canal vent hole. Also, for investigating clinical translational implications, it was found that the middle ear/round window approach *did not cause a permanent hearing loss*, as determined by measuring ABRs and DPOAEs a week after these middle ear infusions.

Borkholder and Frisina's team also investigated cochlear transport, and reported some of the first murine inner ear micro-computed tomography (μ CT) images and precise quantitative modeling of continuous flow of contrast agents (Haghpanahi et al. 2013). This approach was an advance over previous investigations where the mathematical models describing the pharmacokinetics of drug delivery to the cochlea were based on larger rodent studies with invasive measurements of concentration at only a few cochlear locations. For those prior reports, estimates of clearance and diffusion parameters were based on fitting measured data with limited spatio-temporal resolution. In the μ CT study, measurement accuracy was improved by performing a subject-mouse atlas image registration. In sum, this in vivo μ CT approach can quantify concentrations at any point along the fluid-filled scalae of the inner ear, permitting calculation of spatially dependent diffusion and clearance parameters for enhanced modelling.

In addition to lab syringe micropumps, neurotrophins, such as brain-derived neurotrophic factor (BDNF) treatments have been applied to the cochlea with a mini-osmotic pump (Gillespie et al. 2003). Neurotrophic factors are critical for the normal development and healthy survival of the auditory system, acting as survival factors in animal models of hearing loss and deafness. Gillespie's team infused the cochleae of deafened guinea pigs with BDNF for 28 days via a mini-osmotic pump, and neuronal survival was analyzed at various stages after the completion of treatment. They discovered that this sustained BDNF therapy reduced the degeneration of auditory neurons that normally is seen after a loss of hair cells in deafened guinea pigs (furosemide/kanamycin). They also discovered that cessation of the BDNF therapy led to an accelerated decline in auditory neuron survival, as compared to that in deafened, untreated guinea pig cochlea.

Lastly, the Borenstein group at Draper Labs and MIT has pioneered the development of microfabricated mini-pumps for cochlear drug delivery, which is covered in detail in their article elsewhere in this Special Issue. One of their innovative techniques is the utilization of

the reciprocating pumping approach that is unique to this group (see Tandon et al. 2016; for one of their most recent reports). This unique infuse-withdraw and on-demand technique has a major microfluidic advantage that agents can be introduced into the cochlea at low flow rates and deliver drug without increasing the volume of fluid in the cochlea; an important consideration on the clinical translational pathway of cochlear drug delivery. Testing has been successfully done both on the bench and in guinea pig animal models with pumps mounted in a backpack on the guinea pig.

Borkholder and Frisina's team has further miniaturized micropump technologies to enable implantation in the mouse with flow rate control consistent with the small murine cochlear volumes. Microelectromechanical System (MEMS) approaches have been coupled with additive manufacturing direct write 3D printing to create planar peristaltic micropumps optimized for flows between 18-104 nl/min (Johnson et al. 2011; Johnson and Borkholder, 2016). The design was fully biocompatible, with use of Parylene-C coating of the microfluidic flow channels and on the exterior of the micropump. In-plane microfluidic interconnects provide a form factor consistent with subcutaneous implantation for long-term delivery of agents to the cochlea via either RWM or cochleostomy.

Summary and Future Directions

The present article has made clear that we have learned much about successful protection of acquired ototoxic insults due to drugs (antibiotics, chemotherapeutics), noise overexposure and aging, primarily from rodent studies using systemic cochlear drug delivery, including oral and IP systemic administration. More recently, innovative new technological approaches to local inner ear drug delivery have demonstrated some promising clinical, translational trajectories based upon provocative evidence from animal models. Most of the work presented in the present report has looked at polymers (fixed and biodegradable) for sustained release, or nanoparticle carriers, or direct delivery pumps. Advances in the carrier molecule constructs and delivery techniques, like the bicompartamental microparticles recently described by Rahmani and colleagues (2016), are capable of encapsulating and releasing two distinct therapeutics to achieve independent release profiles; and when these are coupled with advances in inner ear micropumps described above and in other articles in this Special Issue, such as the report by Borenstein and colleagues, new opportunities will be created for complex, and controlled delivery profiles that take us well beyond the current single dose/profile systems; to achieve higher levels of protection for acquired hearing loss and treatment of permanent SNHL and deafness.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

Efficacious cochlear drug delivery results have been obtained with animal models

Animal models have been used to demonstrate protection against acute hearing loss

Acute hearing loss includes loud noise exposure, antibiotics and cancer chemotherapy

Exciting new options for local drug delivery are being developed and tested

These include interesting use of nanoparticles, hydrogels and cochlear micropumps