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Advances in Inner Ear Drug Delivery in Mice

Yanru Li*

QU-Drake United College, Qingdao University, 308 Ningxia Road, Qingdao, Shandong Province, P.R.China

**Corresponding author: Yanru Li.*

Abstract

Drug delivery to the inner ear is a key technology for treating sensorineural hearing loss. Due to the presence of physiological barriers such as the blood-labyrinth barrier and the round window membrane, it is difficult for drugs to effectively reach target tissues in the inner ear. Mouse models, with their well-defined genetic backgrounds and short breeding cycles, have become the primary tools for research on inner ear drug delivery. This article reviews the main obstacles and delivery strategies of drug delivery in the inner ear of mice, focusing on the research progress of intratympanic injection, direct cochlear injection, nanocarriers, hydrogels and gene delivery vehicles. The advantages and disadvantages of each method and the corresponding precise delivery strategies for different deafness types are analyzed, and future directions in this field are discussed.

Keywords

inner ear drug delivery, mouse model, blood-labyrinth barrier, nanocarriers, sensorineural hearing loss

1. Introduction

Hearing loss is one of the most common sensory impairments worldwide. According to the World Health Organization, more than 1.5 billion people globally suffer from varying degrees of hearing loss [1]. Among these, sensorineural hearing loss is the most common, with its pathological basis primarily involving damage to or degeneration of the cochlear hair cells and spiral ganglion cells [2]. Currently, clinical interventions primarily rely on hearing aids and cochlear implants. However, these methods are essentially functional compensations and have limited ability to fundamentally repair or regenerate diseased inner ear tissue [3]. Therefore, developing drug delivery strategies capable of effectively delivering therapeutic agents to target cells in the inner ear has become a key direction in hearing loss treatment research.

The core challenge in inner ear drug delivery lies in the unique anatomical location of the inner ear. Deeply embedded within the petrous portion of the temporal bone, it is one of the most inaccessible organs in the human body. More importantly, a physiological barrier known as the “blood-labyrinth barrier” exists between the inner ear and the bloodstream. This barrier, composed of the stria vascularis and tight junctions between capillary endothelial cells, selectively restricts the passage of substances from the blood into the inner ear fluids. When administered systemically, the vast majority of drugs have difficulty crossing this barrier, resulting in insufficient local drug concentrations in the inner ear while potentially causing toxic side effects in other organs [3, 4].

As a classic experimental animal model, mice offer unique advantages in the field of inner ear research. Mice have a short reproductive cycle and a well-defined genetic background, and various models of hearing

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loss have been established, such as models of noise-induced hearing loss and cisplatin-induced ototoxicity. However, the temporal bone of mice has a delicate anatomical structure, the volume of the inner ear is only one-third that of a guinea pig, and the stapedial artery continuously covers the surface of the cochlea, which places high demands on surgical techniques.

2. Major Strategies for Drug Delivery to the Mouse Inner Ear

2.1 Intratympanic Injection and Round Window Membrane Route

Intratympanic injection is currently the most commonly used method of local drug delivery to the mouse inner ear. In this method, the drug is injected into the middle ear cavity through a puncture of the eardrum, and it subsequently diffuses through the round window membrane into the perilymph of the cochlea [5]. In mouse experiments, researchers typically use a retroauricular approach: after anesthesia, the hair behind the ear is shaved and the area disinfected. The skin and subcutaneous tissue are incised; the muscles are dissected to expose the tympanic bulla; the bulla is punctured with a 25-gauge needle and the opening enlarged; and the round window niche is exposed under a microscope. Next, a borosilicate glass capillary pipette is used to gently puncture the center of the round window membrane; surface fluid is blotted dry with sterile filter paper. After waiting 5–10 minutes for fluid outflow to stabilize, a drawn-glass capillary pipette with a tip diameter of approximately 15 micrometers is used to draw up 1–2 microliters of the drug solution, which is then microinjected into the scala tympani through the same opening.

The following points should be paid special attention to during the operation: First, the facial nerve of mice is close to the round window, so the course of the facial nerve must be carefully identified and protected during the operation to avoid facial paralysis caused by injury. Second, the volume of injection should be strictly controlled, about 1.5 microliters per cochlea in newborn mice and 2.0 microliters per cochlea in adult mice. The injection time should be controlled within 1 minute to avoid pressure damage to the inner ear. Third, the pipet should be carefully held and advanced to minimize the perforation of the round window membrane and reduce the risk of postoperative middle ear effusion.

The advantages of this approach include relatively minimal trauma, the ability to repeat the procedure, and the fact that drug administration via the round window membrane is non-invasive in newborn mice. However, the disadvantages are equally evident: the round window membrane exhibits significant selectivity regarding drug permeability. Although small-molecule lipophilic drugs can partially cross the membrane, drug concentrations in the inner ear fluids are often low and vary considerably among individuals [3]. For large-molecule biologics such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), passive diffusion is virtually negligible. Furthermore, mild hearing loss has been observed in adult mice following injection through the round window membrane, which may be related to postoperative fluid accumulation in the middle ear. Recent studies have shown that nanoparticle crossing of the round window membrane involves the synergistic action of paracellular and transcellular pathways, including transient regulation of tight junctions and the involvement of various endocytic pathways, providing a theoretical basis for the design of more efficient delivery carriers [6].

Notably, high-resolution MRI studies suggest that the oval window pathway may be a key route for drug entry into the vestibule following intratympanic administration, indicating that the previously held view of the round window membrane as the “sole pathway” may have been overestimated. The stapes annular ligament possesses a mesh-like tissue structure that may allow drugs to enter the vestibular cistern via the oval window, with a transport efficiency even higher than that of entry into the scala tympani via the round window membrane [7]. A study in guinea pigs demonstrated that approximately 90% of the drug enters the inner ear via the oval window rather than the round window membrane. This finding provides new ideas for vestibular-targeted drug delivery and also suggests that the choice of solvent carrier can significantly affect the main pathway of drug entry into the inner ear.

2.2 Direct Cochlear Injection

Direct cochlear injection is another option for studies that require rapid and precise delivery to specific cochlear regions. This method can achieve high local drug concentrations by injecting drugs directly into the perilymph through a round window or a microhole in the bone wall of the cochlea [3, 8]. In mice, new methods

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for rapid drug delivery to the vestibular endorgan through the oval window and the scala vestibuli have been reported.

The advantages of direct cochlear injection are that it can achieve high local drug concentration, high delivery precision, and is suitable for experimental studies that require rapid onset of action. However, its disadvantages are equally prominent: direct injection has a higher risk of damage to the cochlear structure and may lead to a decline in residual hearing [3, 8]. Studies have shown that direct injection into the scala media causes structural destruction of the cochlea and mixing of endolymph and perilymph, often leading to severe hearing loss. In addition, direct cochlear injection is technically demanding. The mouse cochlea is small and has a thin bony wall. The procedure requires advanced surgical skills and carries a high risk of postoperative complications. Therefore, this method is mostly used in acute experimental studies rather than long-term treatment evaluation.

Compared with the round window membrane route, the direct cochlear injection causes greater trauma but higher delivery efficiency, which is suitable for acute experiments requiring rapid action of high concentrations of drugs. The round window membrane route is less invasive but its delivery efficiency is limited by the permeability of the round window membrane, which is more suitable for chronic studies requiring repeated drug administration. Investigators should choose the appropriate delivery route according to the experimental purpose and the characteristics of the drug.

3. Research Progress of New Drug Delivery Vectors

In order to overcome the low permeability of the round window membrane and the rapid clearance of drugs in the cochlea, a variety of new drug delivery vehicles have been developed [1, 9].

3.1 Nanoparticle Carrier

Nanocarriers have become a hot spot in the research of drug delivery in the inner ear due to their tunable particle size, surface properties and drug release behavior [4, 9]. After intraintratympanic injection, chitosan nanocarriers can remain on the round window membrane for a long time and enter perilymph via paracellular and transcellular pathways [6]. Poly(lactic-co-glycolic acid) (PLGA) nanocarriers possess good biodegradability and biocompatibility. They can encapsulate a variety of therapeutic drugs to achieve sustained, targeted delivery to the cochlea and vestibular structures [2].

The advantages of nanoparticle carriers are primarily reflected in the following: they can encapsulate poorly soluble drugs to improve their solubility and bioavailability. They can protect drugs from degradation by the in vivo environment, thereby prolonging their half-life. They can enable sustained release, reducing the frequency of administration [2]. And surface functionalization (such as PEGylation or conjugation with cell-penetrating peptides) can enhance the drug's ability to cross the round window membrane [4].

However, nanoparticle carriers also have significant drawbacks and limitations. First, the issue of inner ear toxicity cannot be overlooked: new materials may possess inherent ototoxicity, carry the risk of inducing inflammatory responses, and may accumulate within the cochlea. Their long-term safety requires further in-depth study [2, 4]. Secondly, there is a lack of effective evaluation models that can visually or quantitatively assess the distribution and movement of nanocarriers in the inner ear. In addition, the large-scale production and quality control of nanocarriers are also challenges for clinical translation [2, 4].

A more cutting-edge strategy is to combine nanocarriers with barrier regulation functions. A multi-stage biomimetic nanocomposite (tFNA-RA@G-Ang2), which integrated tetrahedral framework nucleic acids, gelatin nanocarriers, and Ang2 peptide modification, was developed to achieve dynamic particle size regulation through MMP2-responsive degradation and cross the blood-labyrinth barrier through LRP1-mediated transcytosis. In a mouse model of noise-induced hearing loss, hearing and synaptic integrity can be restored with a single dose, demonstrating the great potential of nanocarriers for barrier crossing and targeted delivery [10]. However, whether the long-term retention of the system in the inner ear of mice will cause chronic inflammation or cumulative toxicity still needs to be verified by longer-term animal experiments.

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3.2 Hydrogel Sustained Release System

Hydrogel is a kind of polymer material with three-dimensional network structure, which can form a drug reservoir on the surface of round window membrane for sustained release [11]. Chen et al. designed a glucan-PEG hydrogel delivery system (DEX-SILK) loaded with dexamethasone and delivered through a round window membrane, which showed significant protection against cisplatin ototoxicity in a mouse model. This system sustained the release of dexamethasone in the inner ear for up to 21 days and provided significant protection against hearing loss at 4, 8, and 16 kHz frequencies compared with free dexamethasone administration [12].

The advantages of hydrogel systems include precise control of drug release kinetics by hydrolysis or diffusion. Hydrogel systems can be precisely positioned on the surface of the round window membrane. It can effectively solve the problem of drug loss through the Eustachian tube [11]. Injectable hydrogel microspheres are another important advance in recent years. gelatin methacrylamide microspheres prepared by microfluidic technology are modified with polydopamine to enhance their adhesion to round window membrane, and show significant hearing protection effect in noise-induced hearing loss mouse model.

However, hydrogel systems still face several technical barriers and difficulties. First, the filling of the middle ear cavity with hydrogels may cause conductive hearing loss, which needs to be accepted clinically [11]. Second, the long-term safety of hydrogels has not been fully validated, especially the potential effects of their degradation products on the structure of the inner ear. Thirdly, optimizing drug loading capacity and controlling the degradation rate to match the disease progression remain challenges. Fourth, evidence for clinical translation is insufficient, and most of the current research remains at the animal-experiment stage [11].

In the mouse operation, it should be noted that the viscosity of the hydrogel is high, the injection requires the use of a larger-bore needle or special injection device, and the injection volume needs to be precisely controlled to avoid excessive pressure in the middle ear. In addition, the volume of the middle ear cavity in mice is small, and the amount of hydrogel needs to be adjusted accordingly. Overfilling may affect the function of the Eustachian tube.

3.3 Gene Delivery Vectors

Adeno-associated virus (AAV) vectors have attracted extensive attention in gene therapy of the inner ear due to their low immunogenicity and long-term stable expression. AAV vectors have become the preferred platform for gene delivery in the inner ear, which has the advantages of good safety, non-dividing cell transduction, and long-term gene expression [8]. In recent years, a variety of AAV serotypes with different tissue tropism have been obtained by engineering. For example, AAV-Anc80L65 and AAV9-PHP.B can efficiently target inner ear hair cells and successfully restore hearing function in multiple mouse models of hereditary deafness [8]. However, the newly discovered ShH10 serotype is highly specific to supporting cells and can efficiently target all subtypes of supporting cells along the entire length of the cochlear duct without spreading to the central nervous system or the contralateral ear, providing a new tool for more precise targeted therapy.

AAV vectors are mainly delivered to the inner ear by round-window membrane injection or cochleostomy. Round window membrane injection has become the preferred method because of its minimally invasive nature, but the injection parameters need to be precisely controlled to ensure transduction efficiency [3, 8].

The advantages of AAV vectors in inner ear gene therapy are obvious: low immunogenicity, long-term stable expression, and targeting specific cell types. However, several key challenges remain. First, the limited carrying capacity of AAV restricts the size of therapeutic genes that can be delivered [8]. Second, pre-existing immunity to AAV serotypes, which is widespread in the population, may affect treatment efficacy [8]. Third, potential inflammatory responses remain a concern. Although local delivery to the inner ear can partially circumvent systemic immune responses, AAV delivery can still elicit a mild host immune response, and immunogenicity varies among different serotypes and promoters. Fourth, the heterogeneity of hereditary hearing loss necessitates the development of personalized treatment strategies [8].

It is worth noting that gene therapy interventions for inner ear disorders are subject to a limited time window [8]. Current research indicates that hereditary hearing loss occurring after birth is an ideal target for gene therapy, whereas severe congenital hearing loss occurring in utero remains difficult to treat with gene therapy

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at present. This finding suggests that early diagnosis and timely intervention are crucial for the success of gene therapy.

In recent years, gene therapy for hearing loss associated with OTOF gene mutations has achieved breakthrough progress. Because the OTOF gene's coding sequence is too long to fit into a single AAV vector, researchers innovatively adopted a dual-vector split delivery strategy, in which the gene is split into parts and simultaneously injected into the inner ear, where it is reassembled into a complete sequence within cochlear cells and expressed as a functional protein [3, 8]. In 2022, the team took the lead in launching the world's first clinical trial of gene therapy for congenital deafness, which confirmed the safety and effectiveness of this strategy through a systematic follow-up of up to 2.5 years. The success of these clinical trials lays an important foundation for the clinical translation of gene therapy for the inner ear.

4. Precision Delivery Strategies for Different Types of Hearing Loss

There are numerous types of inner ear diseases, and the causes and mechanisms of different types of hearing loss vary. Therefore, the choice of delivery strategy must be tailored to the specific condition.

For hereditary deafness, gene therapy is the fundamental treatment strategy. The feasibility of AAV-mediated gene-replacement therapy has been demonstrated in clinical trials of OTOF-related deafness. Round window membrane injection combined with dual AAV vectors is the current mainstream delivery method [3, 8]. For GJB2-related deafness, because the GJB2 gene is expressed in supporting cells, it is necessary to select AAV serotypes such as ShH10 with high selectivity for supporting cells to achieve effective gene delivery.

For noise-induced hearing loss, treatment focuses on protecting hair cells and spiral ganglion cells from oxidative stress damage. Nanoparticle carriers and hydrogel systems have obvious advantages in this field due to their ability to sustainably release antioxidants or anti-apoptotic drugs [2,4]. The previously described tFNA-RA@G-Ang2 system can restore hearing and synaptic integrity with a single dose in a mouse model of noise-induced hearing loss, demonstrating the potential of nanocarriers for protection against acute acoustic injury [10].

For drug-induced deafness (such as that caused by cisplatin or aminoglycoside antibiotics), prophylactic administration is key. The hydrogel sustained-release system can continuously release protective drugs during chemotherapy [2, 11]. DEX-SILK hydrogel can sustainably release dexamethasone for 21 days in the cisplatin ototoxicity model, and has a significant protective effect on hearing loss at multiple frequencies [12].

Glucocorticoids are commonly used in the treatment of sudden sensorineural hearing loss [5]. Intraintratympanic injection of dexamethasone is the standard protocol, but the problem of rapid loss of drug through the Eustachian tube limits its efficacy. The application of hydrogels and nanoparticle carriers is expected to prolong the retention time of drugs in the middle ear and improve the treatment efficiency [4, 11].

In summary, gene delivery vectors are preferred for hereditary deafness, while nanoparticle carriers or sustained-release hydrogel systems are preferred for noise-induced and drug-induced deafness. For sudden deafness, intratympanic injection combined with sustained-release hydrogel delivery may be considered.

5. Summary and Prospect

Drug delivery in the mouse inner ear has made great progress in recent years. In terms of delivery routes, intratympanic injection combined with round window membrane penetration is still the mainstream strategy, and the re-understanding of the oval window pathway opens up a new direction for vestibular targeted drug delivery [5, 7]. In terms of carrier technology, nanocarriers and hydrogel systems have significantly improved the retention time and targeting efficiency of drugs in the inner ear, and some systems have shown clear hearing protection effects in mouse models [2, 4, 11]. In terms of gene delivery vectors, AAV-mediated gene therapy has progressed from basic research to clinical trials, with particularly notable achievements in gene therapy for OTOF-related deafness [3, 8].

The advantages and disadvantages of each delivery strategy can be summarized as follows: intratympanic injection combined with round window membrane approach is minimally invasive and repeatable, but the delivery efficiency is limited by the permeability of round window membrane, and there is a risk of

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postoperative middle ear effusion [3, 5]. Direct cochlear injection offers high delivery efficiency but causes substantial trauma, which is suitable for acute experiments. Nanoparticle carriers can improve drug solubility and targeting, but long-term safety needs to be verified [2, 4]. The hydrogel sustained-release system can achieve sustained release, but it may cause conductive hearing loss and there is insufficient clinical evidence [11]. AAV gene delivery vector has good safety and durable expression, but its carrying capacity is limited and its immunogenicity is still a problem [8].

However, several key challenges remain in the field. The precise regulatory mechanisms of the blood-labyrinth barrier are not fully understood, and the balance between vector performance and biosafety needs to be evaluated more systematically. In addition, there is still a long way to go from mouse model to clinical application, and the anatomical dimensions and pharmacokinetic characteristics of the mouse inner ear are significantly different from those of humans. Different types of deafness correspond to their own pathogenic causes and mechanisms. Delivery carriers and administration strategies need to be selected according to the specific disease.. This idea of precision medicine should be used throughout the research of drug delivery in the inner ear. Future research should focus on the development of intelligent responsive vectors, the optimization of barrier regulation strategies, and the exploration of multimodal combined treatment regimens. As these problems are gradually solved, inner ear drug delivery is expected to bring a truly effective drug treatment for patients with sensorineural hearing loss.

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