Review Article

The Role of Neurotrophins in Hearing Loss and their Implications in Developing Innovative Therapies

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Abstract

Neurotrophins (NTs) are pleiotropic molecules that can exert a variety of function in both the central and peripheral nervous systems, modulating survival, development and function of neurons. Due to their crucial involvement in the development and innervation of the inner ear, NTs have been considered as potential therapeutic approaches for the treatment of hearing loss. Positive results obtained in various preclinical models of hearing loss opened the way for the clinical use of NTs to counteract synaptopathy, improve cochlear implant performance or prevent long-term neural loss after noise exposure. However, although promising results have been obtained also in clinical trials, NT treatments for hearing loss have not yet achieved the clinical practice. Here, we will review the repair and regeneration potential of inner ear cells and discuss how NTs can contribute to these processes and can thus be used for the treatment of hearing loss. In this context, we will examine the limitations of current NT treatments and the status of development of novel NT-based potential therapeutic approaches for hearing diseases.

1. Introduction

Today, hearing loss affects over 460 million people worldwide, and numbers are expected to increase, reaching over 900 million people by 2050 [1]. The causes that can lead to hearing loss are several, and different are the phenotypes of the disease, as well as the severity grades of the pathology, depending on the specific component or structure of the auditory system that has been damaged. Despite the significant efforts and investments that have been recently made to uncover the principal mediators and mechanisms involved in this disease, still no drug-based therapy has been approved by the Food and Drug Administration, and treatment options for hearing loss are limited to devices and cochlear implants [2]. Among the factors that have been investigated as potential treatments aimed at ameliorating or even restoring the underlying pathology, neurotrophins (NTs) have emerged as promising approaches, progressively and increasingly attracting the interest of researchers and clinicians. In this review, we will provide an overview of the auditory system and the possible causes of hearing loss, thus discussing the repair and regeneration potential of inner ear cells, focusing on the potential use of NTs for the treatment of hearing loss and the current status of development of NT-based therapeutic approaches.

2. Auditory system: anatomy and function

The auditory system is one of the mechanically most sensitive organs of the human body [3], and it is composed by the outer, middle, and inner ear (Figure 1), the auditory nerve and the central auditory pathways. The outer ear has a complex structure and shape and consists of the auricle (or pinna) and the ear canal. It collects sound waves and guides them to the tympanic membrane in the middle ear [4], which is composed also by 3 ossicles (malleus, incus and stapes) with the associated muscles, tendons, and ligaments, as well as the Eustachian tube. From the tympanic membrane, sound vibrations are conveyed to the cochlea in the inner ear via the auditory ossicles [5]. The cochlea is a spiral fluid-filled tube that is part of the peripheral nervous system; it contains the organ of Corti, a specialized sensory epithelium, in which Hair Cells (HCs) allow for the transduction of sound vibrations into neural signals. Cochlea's functions crucially depend on the integrity of the HCs, their postsynaptic partners and the Spiral Ganglion Neurons (SGNs), which convey the information to the auditory brainstem of the central nervous system [6].
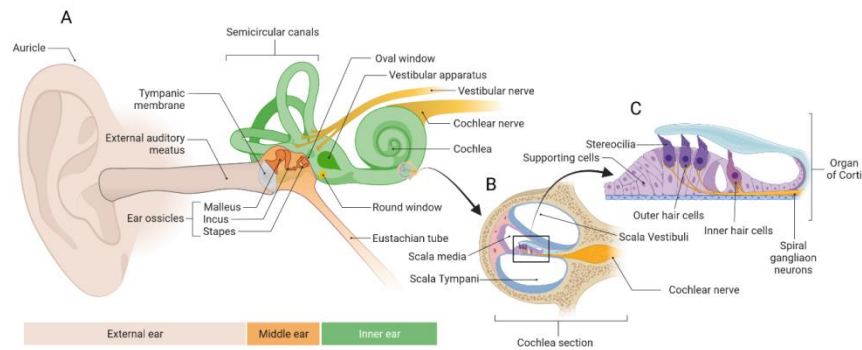
Figure 1: Auditory system anatomy.

A. Overview of the ear (external middle and inner ear). **B.** Section of a turn of the cochlea. **C.** Organ of Corti which contains the hair cells.

3. Causes of hearing loss

Degeneration of cells in the cochlea and loss of their connection with the auditory brainstem of the central nervous system lead to hearing loss. The damage and permanent loss of HCs in the cochlea are typical features of sensorineural hearing loss, which is the most common form of deafness [7] and most frequently occurs because of aging, pathogen infections, ototoxic drug or noise exposure [7,8].

3.1 Aging

Age-Related Hearing Loss (ARHL), also known as presbycusis, is the most common cause of hearing loss and one of the most prevalent sensory deficits affecting elderly worldwide [9]. Sixty millions of Americans between the ages of 50 and 65 are expected to be affected by ARHL by 2025, with an increase of prevalence from 9.3% in 2007 to 19% in 2025 [10]. Although often underestimated, the impact of ARHL on patients' quality of life is enormous with repercussions on physical, psychological and social levels; in some cases, it might be also a predictor of dementia [11-13].

ARHL is a polygenic/multifactorial disorder, the onset of which is mainly caused by age-related cumulative effects of extrinsic damages, such as noise exposure or ototoxic medications, intrinsic systemic disorders (e.g. diabetes, hypertension) and genetic predisposition [10, 14]. Although the cochlea is severely affected by the aging process, histopathologic findings have shown that ARHL can be caused by the alteration of different auditory structures, each of which can result in different phenotypes: the degeneration of cochlear inner and outer HCs (sensory) leads to high-frequency hearing loss; strial vascularis atrophy (strial or metabolic) causes diminished pure tone thresholds in all frequencies; and the degeneration of the auditory nerve (neural) reduces word discrimination scores and relatively stable pure tone thresholds [15-17]. In most cases, a mixture of these and other indeterminate pathological changes have been reported [16,18], indicating that the mechanisms underlying ARHL are multiple and still not completely understood. In this context, also alterations in genes involved in cochlear function can influence susceptibility to ARHL; however, to date, ARHL

genome wide association studies have identified only few genes that are linked to hearing loss [19, 20]. Hopefully, whole-genome sequencing approaches would identify genetic variants involved in ARHL, and thus lead to the development of novel pharmaceutical intervention and to a better defined patient stratification.

3.2 Infectious pathogens

Hearing loss can be caused also by infections of pathogens such as bacteria, viruses, protozoons, or mycetes [21, 22]. Infections by Treponemas pallidum and Borrelia burgdorferi, which are the causative pathogens of syphilis and Lyme disease, respectively, can cause severe labyrinthitis of the inner ear. Bacteria that cause meningitis, including meningococcus, pneumococcus, and Haemophilus influenzae, can directly infect labyrinth and cochlea, leading to hearing loss. The bacterium Toxoplasma gondii, which causes toxoplasmosis, is known to damage the auditory system via calcifications in cochlea and spiral ligaments [23]. Viruses, on the other hand, might increase the susceptibility to bacterial infection and induce auditory system damage at various levels, altering patient immune-response or directly damaging inner ear structures. About 20–65% and 50% of individuals with congenital Cytomegalovirus and Rubella viral infections, respectively, experience hearing loss, labyrinthitis and death of sensorial cells in the organ of Corti [24], while Morbillivirus and viral meningitis may also lead to severe sensorineural hearing loss. The prevalence of hearing loss in patients with HIV is 14% to 49%, which can be exacerbated by ototoxic drug for HIV treatment [25, 26]. Recent studies revealed that Sensori Neural Hearing Loss (SNHL) might be a consequence of also COVID-19 infection; however, this finding need to be further elucidated [27].

3.3 Ototoxicity

The damage to the inner ear caused by several drugs is defined as ototoxicity [28-31]. Depending on the part of the inner ear that is affected, ototoxicity can involve non-sensory cochlear or vestibular cells, which are crucial for sensitive hair cell function [32]. Among the ototoxic drugs, antibiotics have been associated to permanent damage to sensory cells and neurons, and thus to irreversible hearing loss [33]. Among the aminoglycoside antibiotics, amikacin, neomycin, anamycin, eptomycin, and kanamycin induce cochlear toxicity, while streptomycin and gentamicin primarily cause vestibular deficits [33]. On the other hand, non-aminoglycoside antibiotics as erythromycin [34], vancomycin [35], azithromycin, and clarithromycin [36, 37], can lead to sensorineural hearing loss, dizziness, and tinnitus, particularly in neonates [31]. Besides antibiotics, irreversible and progressive death of cochlear outer hair cells can be also induced by platinum-based drugs, such as cisplatin [38-41]. Cisplatin is an anticancer drug used to treat several tumors, and its ototoxic effects are dose cumulative [42] and related to the number of cycles and route of administration [43]. In pediatric patients, Cisplatin-Induced Hearing Loss (CIHL) develops early during therapy, with clinical consequences that include impairment of speech and language acquisition, as well as cognitive and psychosocial development, leading to isolation and depression [44-47]. Although clinical practice guidelines are available for ototoxicity surveillance, none focuses on interventions to reduce ototoxicity [48]. Treatments able to reduce cisplatin-induced ototoxicity without decreasing survival rate are an urgent and unmet need for cancer patients [48].

3.4 Noise exposure

Noise exposure is responsible for about 10% of hearing loss in adults and it is frequently observed in military service members, which are often overexposed to high intensity noises that frequently and irreversibly damage cellular structures at multiple locations in the auditory system [49, 50]. Although noise exposure is primarily associated with damage and death of cochlear hair cells, prolonged noise exposure has been reported to cause also myelin alterations along the cochlear nerve in both preclinical models and humans [51,52], as well as cochlear synaptopathy, which is an irreversible damage to the synapses between the inner hair cells and auditory nerve fibers within the cochlea [53,54]. In this context, remyelinating compounds [55] could be used to avoid histological cochlear and auditory nerve alterations and to prevent hearing loss induced by acoustic trauma, along with anti-inflammatory drugs [56] or antioxidants, such as ascorbic acid, resveratrol, and glutathione, which may reduce noise-induced oxidative stress, which has been shown to lead to cochlear damage and hearing loss after acoustic trauma [57]. Thus, for all factors described above involved in onset and progression of acquired hearing loss, prevention, early diagnosis, greater insight of pathophysiology and development of proper treatments are fundamental steps to be implemented for the appropriate management and monitoring of patients with hearing loss.

4. Regeneration of the Inner Ear: underlying mechanisms and cell therapies

In recent years, biomedical research in inner ear regeneration has been focused on studying the protection, regeneration, and functional recovery of mammalian auditory HCs and neurons. Unlike birds

[58] and fish [59] in fact, where the spontaneous ability of HCs to regenerate after damage has been widely demonstrated, in mammals the regeneration process of HCs is still controversial. The first piece of evidence of HC regeneration in humans has been reported about 40 years ago, when a 37-year-old man developed bilateral deafness after gentamicin administration, but then, progressively showed an improvement in hearing at 3 weeks and 8 months from treatment discontinuation [60]. In a larger case study, Fee and colleagues reported a series of 138 patients, of which approximately 50% recovered hearing and vestibular function between 1 week and 9 months after ototoxic treatment with tobramycin and gentamicin [61]. In the last decade, numerous studies have investigating the mechanisms and cellular components that could be involved in mammalian HC regeneration. Among them, a recent one has demonstrated that c-myc and Notch pathways co-activation can induce renewed proliferation of inner ear cells and the regeneration of HCs in adult mice [62]. Another study in newborn mice, on the other hand, showed that the suppression of Notch signaling in the cochlea triggered the trans-differentiation of supporting cells into HCs [63,64]; in line with this, a combination of four transcription factors (Six1, Atoh1, Pou4f3, and Gfi1) has been identified as able to convert mouse embryonic fibroblasts and postnatal supporting cells into HCs [65]. Moreover, a population of supporting cells was found capable to proliferate and differentiate into new HCs in neonatal mammalian cochlea [66-68]. Although supporting cells, under specific stimuli, may thus regenerate lost HCs, the whole process still remains difficult to observe in mature mammalian cochlea. Therefore, further understanding of the molecular mechanisms underlying HCs regeneration might represent an opportunity to implement new strategies to

restore the lost connections between HCs and neurons in hearing loss.

Another approach that has been investigated for the regeneration of the remaining cells of the inner ear is the replacement of lost and injured cells with new transplanted progenitor cells. Studies have shown that stem cells can engraft in the inner ear [69] and may differentiate either into inner ear cells or into hair cells, thus contributing to the regeneration of the sensory epithelium [70]. Probably, the most suitable and renewable source for the generation of sensory hair cells are pluripotent stem cells, such as Embryonic Stem Cells (ESCs) and Induced Pluripotent Stem Cells (iPSCs). These cells can in fact functionally and morphologically differentiate into hair cells and promote the organization of the cells into hair-like cells with stereociliary bundles [71]. Alternatively, the administration of Mesenchymal Stem Cells (MSCs) has been found safe in a human study [72] and efficacious in repairing spiral ganglion in cochlear cultures from neonatal rats injured by gentamicin [73] and in rats with cochleae damaged by noise or ototoxic drugs [74]. Interestingly, in this latter study, the migration of MSCs into the cochlea was associated with the expression of the brain-derived neurotrophic factor (BDNF) [74], suggesting that neurotrophins can be involved and play an important role in mediating inner ear regeneration.

5. Neurotrophins in the ear: expression and functions

Neurotrophins (NTs) are a family of growth factors that are crucially involved in the regulation of neural survival, development, differentiation, and plasticity [75]. Produced by the cells that are the targets of innervation, NTs are synthetized as precursors (proneurotrophins) and subsequently cleaved intracellularly and extracellularly to obtain the mature form [73]. In mammals, four NTs have been characterized: the Nerve Growth Factor (NGF) [76], the Brain-Derived Neurotrophic Factor (BDNF), the Neurotrophin-3 (NT-3), and the neurotrophin-4/5 (NT-4/5). They exert different functions via two classes of receptor: the Tropomyosin Receptor Kinase (Trk), which belongs to the family of receptor tyrosine kinases and includes TrkA, TrkB and TrkC subtypes, and the p75 neurotrophin receptor (p75NTR) [77]. Upon ligand binding on their extracellular domain, Trk receptors dimerize and activate, leading to receptor autophosphorylation and subsequent activation of several signaling cascades [78]. The high structural homology, both among the 3 subtypes of Trk and among the NTs, allows for a cross-activation between factors and receptors, but usually, the binding between mature NTs and Trk subtypes occurs with different specificities. NGF, for example, preferentially binds TrkA, BDNF and NT4/5 have higher specificity for TrkB, while NT3 primarily binds TrkC [78]. The p75NTR, on the other hand, binds all mature NTs with similar low affinity, while it binds with high affinity the proneurotrophins [78]. Depending on the class of receptor activated, NTs can trigger two different cellular pathways: the interaction of mature NTs with Trk receptors leads to survival signals, whereas the binding to p75NTR leads to apoptosis [79]. BDNF and NT-3, with their high-affinity receptors TrkB and TrkC, respectively, have been identified as key factors for the innervation, development and maintenance of the ear [80]. BDNF is expressed by HCs of all sensory epithelia in late embryonic life, while NT-3 is mainly expressed by the supporting cells of the cochlea, saccule and utricle [80].

In vivo studies on mutant BDNF or NT-3, or their specific receptors, have demonstrated that the spatio-temporal expression pattern of these two NTs is fundamental to provide trophic support for inner ear sensory neuron afferents, and thus to guarantee the correct development of the inner ear [81-83]. Depletion of both NT-3 and BDNF or of their receptors during development determines the complete loss of SGNs [84], indicating that the remaining NTs cannot suffice to support SGN survival in the cochlea [85]. Interestingly, the presence of either NT-3 or BDNF is sufficient to support neuronal survival in ear development, suggesting a comparable role of these two NTs in supporting neuronal survival in ear development [80]. While also NT-4/5 has been shown to exert pro-survival effects on SGNs during development [86], available data suggest that NGF is not crucially involved in the development of the auditory system [87].

5.1. Neurotrophins: Potential therapeutic approaches for hearing loss

Due to their involvement in several neuronal processes, including neuron development and function [88], NTs have been considered as potential therapeutic approaches for the treatment of hearing loss. To test the potential therapeutic effects of NTs, several studies have investigated the exogenous NT delivery in different animal models (Table 1), showing that NTs can be effective in preserving the function and morphology of the auditory nerve from both noise exposure-induced hearing loss and drug-induced ototoxicity and can increase cochlear neuron survival [89-91]. In guinea pigs deafened by loud noises, a single dose application of NT-3 or BDNF to the round window restored hearing function to the damaged cochlea and reduced internal HC synaptopathy [92]. Confirming these data, local

delivery of NT-3 to the inner ear in noise-exposed mice induced neurite growth from available auditory neurons and regenerated HC synapses, partially reversing cochlear synapthopathy [93]. BDNF strongly reduced auditory thresholds and repaired the damaged connections between inner HCs and auditory nerve fibers also when administered to guinea pigs deafened by kanamycin [94,95], and similarly, in a guinea pig model of cisplatin-induced ototoxicity, BDNF treatment led to an improvement in hearing [96]. Exogenous administration of BDNF has led to an enhancement of SGC survival in different species, such as cats [97,98], rats [99] and guinea pigs [100]; in particular in deafened guinea pigs, short-term treatment with BDNF has been shown to prevent long term auditory nerve degeneration and to recover SGC function, ensuring a successful cochlear implant [90]. Notably, also in rat models, the administration of both BDNF and NT-3 enhanced spiral ganglion neurites length and number toward cochlear implants [101], suggesting a new potential therapeutic approach for cochlear implantation. The higher success rate of this approach in patients has been in fact associated with the number of surviving SGNs or HCs, suggesting that neuronal survival might represent a limiting factor for implant success [102] and thus, that the combination of cochlear implant with NTs may represent an advantage for the success of cochlear implant by stimulating innervation toward the implant. Despite its role in the development of the auditory system is still undefined, exogenous NGF administration exerted protective and beneficial effects both *in vitro* and *in vivo*. *In vitro*, NGF treatment induced dose dependent growth of Statoacoustic Ganglion (SAG)-derived Neural Progenitor (NP) and stimulated their neuronal/glutamatergic differentiation, while *in vivo*, it also dramatically enhanced SAG-NP survival rate after

implantation into adult mammalian inner ear. Exogenous administration of NGF enhanced Dorsal Root Ganglia (DRG) survival and stimulated their extensive neurite projections following transplantation into adult rat cochlea [103,104]. Moreover, NGF treatment preserved SGNs and outer hair cells in the cochlea of mice with early onset of hearing loss [105], while it exerts otoprotective effects in neomycin-induced auditory neural degeneration in guinea pigs [106]. Overall, these results provide a solid proof-of-concept for the use of NTs in patients to counteract synaptopathy, improve cochlear implant performance or prevent long-term neural loss after noise exposure [90, 91, 94, 107, 108]. Clinical and audiometric improvement has been found in

patients affected by SNHL and tinnitus after autologous stimulation of NGF in nasal fluid [109]. Moreover, a recruiting, randomized, double-blind, placebo-controlled Phase 1/2 clinical trial reported that a single intratympanic injection of BDNF was well tolerated in subjects with speech-in-noise hearing loss, and the proportion of subjects with a clinically meaningful improvement was higher in the BDNF-treated group than in the placebo (NCT04129775). Despite these promising results however, significant safety and clinical data are still needed to grant the pharmacological use of NTs in clinical practice to treat hearing loss.

Neurotrophin	Species	Method of hearing loss	Treatment	Outcome	References
NGF (200 μg/ml)	Guinea pig	30% of neomycin solution by osmotic pump	2 weeks, infusion by osmotic pump	Protection of the auditory nerve	Schindler RA et al., 1995 [127]
NGF (200 μg/ml)	Guinea pig	30% of neomycin solution by osmotic pump	2 weeks, infusion by osmotic pump	Spiral ganglion survival	Shah SB et al., 1995 [106]
NT-3 and BDNF (1 mg/mL^{-1})	Guinea pig	Kanamycyn (400 mg Kg^{-1}) followed 2 h later by ethacrynic acid (40 mg Kg^{-1})	8 weeks, infusion by osmotic pump	Prevention of the degeneration of auditory hair cells	Staecker H et al., 1996 [128]
NT-3	Guinea pig	Oxytetracycl	2 weeks,	Protecting spiral	Ernfors P al., 1996 [129]

(62 µg/ml)		in (10 mg/kg)	infusion by osmotic pump	ganglion neurons	
BDNF (62.5 µg/mL)	Guinea pig	Ketamine (40 mg/kg) and xylazil (4 mg/kg)	28 days, infusion by osmotic pump	Prevention of the degeneration of auditory neurons	Gillespie LN et al., 2003 [130]
BDNF, NT-3, NT-4/5, NGF (10 mg of neurotrophin delivered into the scala tympani)	Guinea pig	Ketamine (40 mg/kg) and xylazil (4 mg/kg)	28 days, infusion by osmotic pump	Auditory neuron survival	Gillespie LN et al., 2004 [131]
NT-3 (50 µg/ml)	Guinea pig	Kanamycin (400 mg/kg) and frusemide (100 mg/kg)	28 days, infusion by osmotic pump	Trophic effects on SGN cell bodies	Richardson RT et al.,2005 [132]
BDNF (62.5 µg/mL)	Guinea pig	Kanamycin (400 mg/kg) and frusemide (100 mg/kg),	28 days, infusion by osmotic pump with chronic electrical stimulation	Trophic or survival advantage in the base of the cochlea	Shepherd RK et al., 2005 [133]
BDNF and NT-3 (50 µg/ml)	Guinea pig	Kanamycin (400 mg/kg) and frusemide (100 mg/kg),	28 days, infusion by osmotic pump	Re-sprouting of peripheral processes	Wise AK et al., 2005 [134]
BDNF (5.4µg/ml)	Wistar rat	Furosemide (175mg/kg) followed by gentamicin (350mg/kg)	28 days, infusion by osmotic pump	SGN rescue	McGuinness SL, Shepherd RK, 2005 [99]

BDNF (100 µg/ml)	Guinea pig	Kanamycin (400 mg/kg) and frusemide (100 mg/kg)	4 weeks, infusion by osmotic pump	SGC protection and rescue	Agterberg MJH et al., 2008 [95]
BDNF (94µg/ml)	Cat	Neomycin sulfate (60 mg/kg)	10 weeks, infusion by osmotic pump	SG survival neurons	Leake PA et al., 2010 [97]
BDNF (62.5 µg/mL)	Guinea pig	Kanamycin (400 mg/kg) and frusemide (100 mg/kg),	4 weeks, infusion by osmotic pump	SGN survival HC protection	Sly DJ et al., 2011 [94]
BDNF (100 µg/ml)	Guinea pig	Kanamycin (400 mg/kg) and frusemide (100 mg/kg),	4 weeks, infusion by osmotic pump	long-term SGC survival and protection	Ramekers D et al., 2015 [90]
NT-3 (1µg/µl) or BDNF (1µg/µl)	Guinea pig	2 hours to 4 to 8 kHz noise at either 95 or 105 dB SPL	A 4µl test bolus was delivered to the round window	Rescue auditory function and inner HC synaptopathy reduction	Sly DJ et al., 2016 [92]
NT-3 (300 ng/µl /30 ng/µl)	CBA/CaJ mice	Noise (8–16 kHz) for 2 hours at 98 dB sound pressure level (SPL)	Local delivery of NT-3 to the round window niche	Cochlear synaptic regeneration	Suzuki J et al., 2016 [93]
mNGF (3.6×10^{-3} µg/g body mass)	A/J mice	Age-related	Intramuscular injection in the hips once	SGN and OHC protection	Gao L et al., 2017 [105]

		every other day.from postnatal day 7			
BDNF (50 ng/mL)	Guinea pig	Kanamycin (400 mg/kg) two hours later 40 mg/kg ethacrynic	mini-osmotic pump for 14 days delivery	SGN protection	Scheper V et al., 2020 [100]
BDNF (0.05 μg)	Guinea pig	Cisplatin 4 mg/kg per 3 doses on alternate days for a total of 12 mg/kg.	BDNF injected into the round window	Hearing restoration	Blakley BW, Seaman, M. & Alenezi, A, 2020 [96]

Table 1: Neurotrophins treatment in preclinical studies (in chronological order).

6. Novel neurotrophin-based approaches for the treatment of hearing loss

One of the most significant challenges for the treatment of hearing loss is to effectively deliver the drug, as the anatomy of the ear makes it difficult to achieve the uniform delivery of the drug and a residence time that could allow reaching therapeutically meaningful concentrations. As systemic administration has been limited so far by the onset of side effects, in the last years different approaches have been investigated to optimize NT delivery to ultimately protect the residual cochlear function [110-112]. Among the available administration routes, the intratympanic injection has been widely used, but the leakage of liquid formulations through the Eustachian tube represents the major limitation of this approach. On the other hand, the use of specific biomaterials [113] and advanced microneedles [112] has highly improved the rate of drug delivery from middle to inner ear, preventing the drawbacks of liquid formulations. Among the biomaterials, hydrophilic polymers in particular, such as hydrogels, have attracted the interest of drug researchers because of their chemical functionality, biocompatibility, physical properties and drug loading and degradation capabilities [114,115]. Indeed, their high viscosity and viscoelastic properties allow hydrogel delivery systems to locally retain drugs in a solidified status, preventing NTs from rapidly flowing through the Eustachian tube in the middle ear. This increases NT drugs residence time and extend their diffusion across the Round Window Membrane (RWM), thus achieving a more uniform delivery to the inner ear

and allowing to reach therapeutically meaningful concentrations [116]. In line with these data, the application of hydrogels loaded with NTs onto the RWM of deafened guinea pigs resulted in SGC survival [117] and slowed neuron loss [118]. Another possible approach for the targeted delivery of drugs is the nanoparticle (NP). NPs are less-than-100-nm diameter solid particles that are synthesized from compounds such as Polylactide-Co-Glycolide (PLGA) and a diblock copolymer containing Poly-L-Lactide And Polyethylene Glycol (PLLA–mPEG). As alternative nonbiological carriers, NPs carry small hydrophobic drugs, hydrophilic substances and biomolecules, such as peptides and proteins [119], that are delivered to specifically targeted cells or organs [120]. With the aim of increase the half-life of drugs and achieve their sustained or targeted release, NPs have been thus also tested for the delivery of NTs through the RWM. NGF functionalized NPs have been investigated in particular for their ability to target cells of the inner ear in organotypic explant cultures of the mouse inner ear and PC-12 rat pheochromocytoma cells. Notably, these NPs did not show any signs of toxicity, thus implying the potential application of NT-loaded NPs as therapeutic approaches for the stimulation of nerve and hair cell regeneration or repair [121].

Since the neurotrophic and neuroprotective role of NTs is defined and well established, clinical research has been recently focused on the generation of small molecules and monoclonal antibodies that mimic NTs' function and can target Trk receptors to prevent neural cell death and support neural survival. Among the available NT mimetics, 1Aa and Ris-1Aa and 7,8-Dihydroxyfla-vone (DHF) are a small molecule analogue of NT-3 and a selective agonist of TrkB,

respectively, that have shown to stimulate SGNs and promote regeneration of synapses between SGNs and inner hear cells in *in vitro* studies [122,123]. DHF has been reported to be effective also in protecting and restoring both synapses and neural function in noise-exposed mouse ears [124]. However, these results have not been confirmed using another small molecule TrkB agonist (7,8,30-trihydroxyflavone) that did not affect survival of SGCs in deafened guinea pigs [125]. In a recent report, DHF has been compared to a new class of monoclonal antibody agonists of TrkB or TrkC, known as "M-antibodies" for their effects on rat cochlea *ex vivo* models. Four of these M-antibodies activate TrkB (M3, M4, M5 and M6) and three of them activate TrkC (M1, M2 and M7). Among all, M3 showed the greatest activity on SGN survival, neurite extension and synapse restoration, suggesting that this antibody can be potentially used for further investigations for the treatment of hearing loss [126]. However, additional studies are required for the development of these agonists, including an evaluation of biostability, bioavailability, safety and efficacy in animal models to further investigate their potential to treat hearing loss.

7. Conclusions and future perspectives

Although in the last decade considerable progress has been made in the understanding of the causes and molecular mechanisms underlying hearing loss, the need for new therapeutic approaches for the treatment of this disorder remains high. The crucial and multifaced role of NTs in regulating the development of the mammalian nervous system and the functions of both neuronal and non-neuronal cells also after the developmental stage, have suggested the potential application of NTs as therapeutic approaches for the treatment of hearing loss disorders. Supporting this concept, the treatment with

NTs, and in particular with NGF and BDNF, has shown the ability to induce the regeneration of hair cells, spiral ganglion neurons and stria vascularis, ultimately improving hearing functions in various models of ear diseases. Despite the promising preclinical results and the potential application for the rescue of the SGN degeneration and for patients undergoing cochlear implantation, however, NT treatments have not yet achieved clinical translation. Since this can be attributed mainly to the limitations due to ear delivery and permeation of drugs, the development of new methods of local drug application aimed at reducing possible adverse effects, and the thorough investigation of drug pharmacokinetic properties to ensure the achievement of the target cell, could represent crucial starting points for the development of new therapeutic approaches. Moreover, the exploitation of the knowledge acquired so far on NTs and on their role in ear physiology and pathophysiology, together with the implementation of animal-based experimental research, might improve the knowledge we have already gained in the field and pave the way to new clinical studies that will provide solid support on the safety and efficacy of NT treatment in ear disorders. In conclusion, the development of optimized formulations in combination with the rational choice of the administration route could be the key factor for the selection of new NT-based approaches that can be moved to clinical application for the treatment of hearing loss disorder.

Author Contributions

PC, CG, RN and LB wrote the manuscript. MA and AA substantially revised it. All the authors contributed to the final manuscript. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors PC, CG, RN, AA, MA, LB declare the following competing interest: they are employees of Dompé Farmaceutici s.p.a.

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