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Clinical application of neurotrophic factors: the potential for primary auditory neuron protection

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Abstract

Sensorineural hearing loss, as a result of damage to or destruction of the sensory epithelia within the cochlea, is a common cause of deafness. The subsequent degeneration of the neural elements within the inner ear may impinge upon the efficacy of the cochlear implant. Experimental studies have demonstrated that neurotrophic factors can prevent this degeneration in animal models of deafness, and can even provide functional benefits. Neurotrophic factor therapy may, therefore, provide similar protective effects in humans, resulting in improved speech perception outcomes among cochlear implant patients. There are, however, numerous issues pertaining to delivery techniques and treatment regimes which need to be addressed prior to any clinical application. This review considers these issues in view of the potential therapeutic application of neurotrophic factors within the auditory system.

Keywords

Neurotrophins; Neural protection; Cochlear implant

Introduction

Deafness is one of the most common health conditions in developed countries, with estimates indicating that approximately 70 million people worldwide are deaf (Hoffmann and Strasnick, 2004). Sensorineural hearing loss (SNHL) is a permanent hearing loss typically associated with a loss of the sensory hair cells of the inner ear, and is the most common type of hearing loss in adults, accounting for 80% of cases. In patients with a severe-profound SNHL, the only therapeutic intervention is via a cochlear implant that electrically stimulates residual primary auditory neurons (spiral ganglion neurons, SGNs) to provide the patient with auditory cues necessary for speech perception.

The SGNs are, therefore, the target cells of the cochlear implant. Significantly, following the loss of the sensory epithelium, the SGNs undergo secondary degeneration. Loss of SGNs is an ongoing process and is also dependent on etiology, with greater loss evident in cases of cochlear infection, such as bacterial or viral labyrinthitis, as compared with ototoxic drugs or a sudden hearing loss (Kerr and Schuknecht, 1968; Otte et al., 1978; Nadol et al., 1989). Evidence from animal studies indicates that ongoing SGN degeneration has the potential to compromise the efficacy of the cochlear implant (Shepherd and Javel, 1997; Hardie and Shepherd, 1999;

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Shepherd et al., 2004), and therefore, from a clinical perspective, there are likely to be benefits in performance following the rescue of SGNs from these degenerative processes.

There is now substantial evidence to indicate that SGN degeneration occurs, at least in part, as a result of the loss of neurotrophic support normally provided by the hair cells. These neurotrophic factors therefore offer promise as therapeutic agents for the treatment of hearing loss, with neurotrophins expected to inhibit or delay degenerative processes in SGNs. In the short-term, neurotrophic factor treatment may be useful to halt or slow the degenerative process during the period when patients are undergoing assessment for suitability for a cochlear implant. Additionally, brief neurotrophin treatment may prove beneficial for prophylaxis, to prevent against possible damage from surgical insertion procedures. In the long-term, neurotrophic factors may play a role in regeneration, to stimulate neurite growth towards a cochlear implant electrode array, or even a regenerated hair cell target. Neurotrophic factors may even be used concurrently with stem cell therapies to enhance survival and differentiation for the replacement of damaged or destroyed SGNs and/or hair cells.

This paper reviews experimental findings of neurotrophic factors within the cochlea, and considers the future of neurotrophin-based therapies for hearing loss based upon experimental findings in the auditory system and clinical trials in other neural classes.

The role of neurotrophic factors in the development of the auditory system

Neurotrophic factors regulate survival and differentiation of neurons throughout the nervous system during embryonic and postnatal development, and are also important for maintenance of synaptic connectivity and plasticity in the adult nervous system (Maness et al., 1994; Terenghi, 1999). The *neurotrophins* are the best characterised family of neurotrophic factors, and comprise nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5).

Numerous studies have provided evidence that BDNF and NT-3, but not NGF or NT-4/5, are important for the development and maintenance of the auditory system. Studies using *in situ* hybridisation have revealed higher expression of both BDNF and NT-3 messenger RNA (mRNA) on hair cells during the period when SGNs send their processes to this epithelia (Pirvola et al., 1992; Ylikoski et al., 1993; Pirvola et al., 1994; Schecterson and Bothwell, 1994; Wheeler et al., 1994), and at the same time SGNs express mRNA transcripts for the high-affinity receptors for BDNF and NT-3 – trkB and trkC, respectively (Ylikoski et al., 1993; Pirvola et al., 1994; Zheng et al., 1995). In contrast, mRNA encoding NGF and NT-4/5 are not present in the otic epithelium of the developing rat inner ear (Pirvola et al., 1992; Pirvola et al., 1994; Schecterson and Bothwell, 1994), and the trkA receptor has been shown to be only transiently expressed within the rodent cochleovestibular ganglion (CVG) (Pirvola et al., 1994; Schecterson and Bothwell, 1994). The presence of the low affinity p75 neurotrophin receptor has also been described in cochlear regions of the VIIIth cranial nerve via immunohistochemistry (von Bartheld et al., 1991), and in the CVG using *in situ* hybridisation (Pirvola et al., 1994). The precise role of p75 in the development of the auditory system is still unclear, however, it may be involved in modulation of the binding, specificity or signalling capacities of the high-affinity receptors.

Further developmental studies report that mice lacking the NT-3 gene or its receptor trkC have a massive reduction in the number of SGNs (Farinas et al., 1994), while studies of double mutant mice have reported that the absence of both BDNF and NT-3 or trkB and trkC leads to a nearly complete loss of inner ear neurons (Ernfors et al., 1995) and a complete loss of all afferent innervation of the ear (Fritzsch et al., 1997). Together, these studies provide strong evidence that the neurotrophins BDNF and NT-3 influence SGN development and survival, and do so via their high-affinity trk receptors.

Neurotrophic factors other than members of the neurotrophin family have also been reported to play a role in auditory development and maintenance, such as glial cell-line derived neurotrophic factor (GDNF). *In situ* hybridisation studies have shown that the inner hair cells of both the neonatal and mature rat cochlea synthesize GDNF, while the GDNF-receptor-α is expressed by the SGNs (Ylikoski et al., 1998).

Members of the fibroblast growth factor (FGF) family are also known to be critical for some stages of inner ear development (Pickles and Chir, 2002). For example, FGF-3 gene knockout in the mouse leads to developmental abnormalities of the endolymphatic duct, cochlear spaces and the spiral ganglion (Mansour, 1994), while FGF receptor-3 (FGFR-3) knockout mice fail to differentiate pillar cells, leading to profound deafness (Colvin et al., 1996; Mueller et al., 2002). In addition, signalling through the FGFR-2(IIIb) receptor isoform has been described as critical for the morphological development of the inner ear, with FGF-3 and FGF-10 likely to play a role in the activation of this receptor for inner ear development (Pirvola et al., 2000; Pickles, 2001). Furthermore, FGF-1 (acidic FGF) mRNA has been shown to be present in SGNs through all turns of the rat cochlea from late embryonic stages to approximately postnatal day 14 (P14), and is also observed in the hair cells during the first postnatal week, suggesting involvement in the establishment of cochlear innervation (Luo et al., 1993). FGF-1 and FGF-2 (basic FGF) are also reported to act as trophic factors and play important roles in the migration and initial differentiation of SGNs and auditory nerve fibres in the mouse (Hossain and Morest, 2000).

Insulin-like growth factor-1 (IGF-1) has also been reported to play a role in auditory development and maintenance. Mice with a targeted disruption of the IGF-1 gene show a significant reduction in the size of the cochlea and cochlear ganglion, a decrease in the number and size of SGNs, and a general delayed maturation in the innervation of the organ of Corti (Camarero et al., 2001; Camarero et al., 2002).

Various neurotrophic factors therefore play many important roles during the development of the auditory system, from morphological development of the endolymphatic duct and cochlear spaces to the size of the SGN population and the establishment of neural circuitry. These latter points, in particular, led to early hypotheses that these same neurotrophic factors may also be able to rescue SGNs following trauma. Indeed, numerous neurotrophic factors have been shown to prevent SGN degeneration in animal models of deafness.

Neurotrophic factors can have protective effects on SGN survival in deafness

In addition to their role in inner ear development and innervation, the neurotrophins have also been reported to play a role in survival and maintenance of SGNs in experimental animal models of deafness.

In vitro studies of neurotrophic factors on SGN survival

BDNF, NT-3 and NT-4/5 have all been reported to promote the survival of early postnatal rat SGNs in culture. BDNF can enhance SGN survival significantly over neurotrophin-free control cultures (Malgrange et al., 1996a; Marzella et al., 1999); (Zheng et al., 1995). NT-3 is also reported to elicit survival effects on early postnatal rat SGNs *in vitro*, although the effects were not as potent as BDNF (Zheng et al., 1995; Malgrange et al., 1996a; Marzella et al., 1999). Both BDNF and NT-3 have also been demonstrated to dramatically increase neuronal survival rates in cultures of adult rat SGNs (Lefebvre et al., 1994), supporting suggestions that neurotrophins are required for ongoing trophic support in the auditory system throughout adulthood.

NT-4/5 has also been shown to promote survival of early postnatal rat SGNs, with the survival-promoting effects reported to be equivalent to that of BDNF and stronger than that of NT-3 (Zheng et al., 1995). In addition, the neurotrophins can act together to further enhance SGN survival. For example, BDNF and NT-3 can act in a synergistic fashion to enhance SGN survival rates (Marzella et al., 1999). Interestingly, NGF has been shown to have no detectable survival effects on postnatal SGNs *in vitro* (Lefebvre et al., 1994; Zheng et al., 1995; Malgrange et al., 1996a).

Further to enhancing survival, *in vitro* studies have shown that each of BDNF, NT-3 and NT-4/5 can protect SGNs from the ototoxic effects of aminoglycosides, such as gentamicin; therapeutic drugs, such as salicylates, including sodium salicylate (aspirin); and chemotherapeutic agents, such as cisplatin (Zheng et al., 1995; Zheng and Gao, 1996; Duan et al., 2002). These findings indicate a role for neurotrophic factors in the prevention of the damaging effects of therapeutic ototoxic agents, which are one of the major causes of damage in the peripheral auditory system leading to SNHL.

In addition to the neurotrophins, GDNF has been shown to support survival in dissociated cultures of early postnatal rat SGNs, eliciting survival effects almost as potent as NT-3 (Ylikoski et al., 1998; Qun et al., 1999). Another family of growth factors with demonstrated survival-promoting activity within the cochlea is the transforming growth factor-β (TGF-β) family. Both TGF-β3 and TGF-β5 can promote the survival of SGNs *in vitro* in a concentration dependent manner, and can synergistically potentiate neurotrophin-induced SGN survival (Marzella et al., 1998; Marzella et al., 1999).

The neuronal cytokines ciliary-derived neurotrophic factor (CNTF) and leukaemia inhibitory factor (LIF) have also been reported to increase the survival of SGNs in early postnatal rat cultures (Staecker et al., 1995; Hartnick et al., 1996; Marzella et al., 1997). LIF can also act in an additive fashion with both TGF-β3 and TGF-β5 (Marzella et al., 1999), and both LIF and CNTF can enhance the survival-promoting capacities of BDNF and NT-3 (Staecker et al., 1995; Hartnick et al., 1996; Marzella *et al.*, 1997; Marzella et al., 1999).

SGN rescue following in vivo application of neurotrophic factors

In addition to *in vitro* survival studies, and perhaps more significantly, *in vivo* studies have demonstrated that the delivery of exogenous neurotrophic factors to the mammalian inner ear can prevent the SGN degeneration that is normally seen following loss of hair cells (Summarised in Table 1). For example, intracochlear infusion of NT-3 yields greater than 90% SGN survival, as compared to only 14-24% survival in contralateral untreated ears in ototoxically deafened guinea pigs (Ernfors et al., 1996;Staecker et al., 1996).

BDNF can also prevent SGN degeneration in ototoxically deafened guinea pigs, providing statistically significant enhanced SGN survival over untreated ears following either two- (Miller et al., 1997), four-weeks (Gillespie et al., 2003) or eight weeks of treatment (Staecker et al., 1996). Furthermore, intracochlear BDNF infusion has been used simultaneously with patterned electrical stimulation in deaf guinea pigs, resulting in significantly enhanced SGN survival compared to BDNF treatment alone (Shepherd et al., 2005). A similar study also reported an additive protective effect from electrical stimulation and a combination of neurotrophic factors, including BDNF, GDNF and FGF-1 on SGN survival *in vivo* (Miller and Altschuler, 2004). Such anatomical changes have also been shown to correspond with functional changes. For example, intracochlear infusion of BDNF in combination with CNTF can enhance the functional responsiveness of the deaf auditory system, as measured using electrically-evoked auditory brainstem responses (EABRs) (Shinohara et al., 2002). In addition, a significant reduction in EABR thresholds is seen following BDNF infusion (Shepherd et al., 2005).

Despite being shown to have limited survival effects *in vitro*, NGF has been shown to have protective effects in the guinea pig cochlea *in vivo*, with early studies reporting that intracochlear infusion of NGF via a mini-osmotic pump could prevent the degeneration of auditory nerve fibres after unilateral neomycin exposure (Schindler et al., 1995; Shah et al., 1995). However, SGN survival due to NGF was greatest in the basal turn of the cochlea, closest to the neurotrophin source (Shah et al., 1995). The differences observed between the *in vitro* and *in vivo* studies suggest that NGF plays different roles in the auditory system in immature stages as compared to the adult.

Delayed neurotrophin treatment, when the degenerative processes are more established, has also been shown to have protective effects on SGNs. For example, after a two week period of deafness, each of BDNF, NT-3, NT-4/5 and NGF can prevent further SGN degeneration (Gillespie et al., 2004), while a combination of NT-3 plus BDNF had similar protective capacities after a four-week period of deafness (Richardson et al., 2005; Wise et al., 2005). The presence of each of the high-affinity neurotrophin receptors (trkA, trkB and trkC) has also been shown in the cochleae of normal, deafened, and deafened plus neurotrophin-treated adult guinea pigs (Gillespie et al., 2004). This supports the theory that the neurotrophins are important for SGN integrity in the mature cochlea, and indicates that these neurons may respond to any or all of these trophic factors for survival.

Delayed treatment using a combination of BDNF and CNTF can also have protective effects on the inner ear when treatment commences at either two or six weeks post-deafening (Yamagata et al., 2004). Furthermore, EABR thresholds in the deaf control subjects increased following deafening, however these thresholds decreased significantly following the commencement of the neurotrophic factor treatment (Yamagata et al., 2004). It is again important that the anatomical effects of enhanced SGN survival are supported by functional data. However, while protective effects were still elicited in the six-week delayed treatment group, the extent of the effect was diminished in comparison to the survival effects in the two-week delayed group. This suggests that longer periods of deafness may lead to decreased efficacy in terms of SGN rescue, and therefore there may be an optimum treatment period following the onset of deafness for neurotrophic factor administration to be effective and/or worthwhile.

GDNF is another neurotrophic factor that has been demonstrated to rescue SGNs from degeneration following deafening , via both mini-osmotic pump and gene therapy techniques (Ylikoski et al., 1998; Yagi et al., 2000). In addition, GDNF gene therapy used in combination with electrical stimulation, via a scala tympani electrode, has been shown to provide significantly greater SGN preservation than either treatment alone (Kanzaki et al., 2002).

Combined, results from these *in vivo* studies provides further evidence that neurotrophic factors that play a role in auditory development and maintenance are also prime candidates for therapeutic agents to protect SGNs from degeneration following SNHL. Furthermore, it is also apparent that the administration of neurotrophic factors in conjunction with cochlear implantation may provide enhanced physiological and functional benefits.

Neurotrophic factors can protect SGNs in numerous animal species

Studies using neurotrophic factors for the prevention of SGN degeneration in guinea pigs are now being extended to other species. For example, BDNF has been shown to have protective effects on SGNs in ototoxically deafened rats (McGuinness and Shepherd, 2005), and BDNF gene therapy has been shown to have protective effects on SGNs in deaf mice (Staecker et al., 1998).

This finding, that neurotrophin survival effects on SGNs are observed in the rat and the mouse as well as the guinea pig, gives promise for therapeutic application of neurotrophic factors in further species, including the human.

Neurotrophic factors can enhance regrowth of auditory axonal processes

A long-term goal of auditory research is to develop a means for the biological replacement of the missing sensory and neural cells of the inner ear, followed by reestablishment of a correct tonotopic map. In the short-term, an improved electro-neural interface, by growing axons of SGNs towards the electrode array of the cochlear implant, may enhance the efficacy and benefits of the cochlear implant. As such, regeneration, or “re-sprouting”, of SGNs could provide significant functional benefits for users of cochlear implants, as well as play an important role in regeneration of a fully functional auditory system.

Neurotrophic factors stimulate neurite outgrowth in vitro

In addition to supporting SGN survival, numerous neurotrophic factors can stimulate auditory nerve fibre growth. For example, BDNF treatment can stimulate neurite outgrowth from early postnatal SGNs in dissociated cultures (Hartnick et al., 1996; Malgrange et al., 1996b; Gillespie et al., 2001). However, neither BDNF nor NT-3 can stimulate neurite outgrowth from adult SGNs *in vitro* (Lefebvre et al., 1994), suggesting that the tropic effects of the neurotrophins alters in the mature system. Both CNTF and LIF have also been identified as a neuritogenic factors for SGNs in dissociated cultures (Hartnick et al., 1996; Gillespie et al., 2001), with LIF reported to be more potent than BDNF for promoting neurite outgrowth from SGNs (Gillespie, 2001). Furthermore, CNTF can act in an additive fashion with BDNF (Hartnick et al., 1996), while LIF and BDNF act in a strongly synergistic fashion to dramatically enhance neuritic outgrowth (Gillespie, 2001).

Axonal outgrowth from SGNs in vivo

Numerous studies have reported that damaged SGNs can re-sprout axons following various forms of cochlear insult *in vivo*. For example, spontaneous regeneration of auditory nerve fibres in the mammalian cochlea has been described in both chinchillas and guinea pigs following noise-induced damage (Wright, 1976; Bohne and Harding, 1992; Strominger et al., 1995; Lawner et al., 1997); in ototoxin-deafened guinea pigs (Terayama et al., 1977; Terayama et al., 1979; Webster and Webster, 1982; Wise et al., 2005); and also after VIIIth nerve transection in the cat (Spoendlin and Suter, 1976). Regenerated nerve fibres were found to be present within regions of the organ of Corti where the sensory epithelium had been destroyed, suggesting regeneration from within the osseous spiral lamina (OSL). These fibres were observed to follow abnormal, disorganised and tangled courses within the OSL and along the basilar membrane. More recently, a study investigating changes in the morphology of the peripheral processes of SGNs in deaf guinea pigs used whole mount preparations to demonstrate that peripheral processes re-sprout following ototoxic deafening (Wise et al., 2005). Moreover, this study showed that concurrent intracochlear infusion of BDNF and NT-3 enhanced this effect. Re-sprouting axons were typically observed to project through the habenula perforata, and then coursed back between the cells of the inner sulcus in a honeycomb-like fashion, towards the spiral limbus (Wise et al., 2005).

Aberrant sprouting of peripheral nerve fibres has also been observed within the organ of Corti and the spiral limbus of ototoxically deafened, NT-3-treated guinea pigs (Ernfors et al., 1996), while Staecker et al. (1996) reported growth of axonal processes of SGNs into the scala tympani following intracochlear infusion of BDNF and/or NT-3 in deaf guinea pigs. A cocktail of neurotrophins – including BDNF, NT-3, CNTF and GDNF – was also reported to lead to regrowth of auditory fibres curling under the OSL into the scala tympani, as identified by immunostaining for the growth cone marker GAP-43 (Miller et al., 1997).

Findings that neurotrophic factors can enhance axonal growth following denervation in the cochlea are of great clinical interest. A closer interface between the cochlear implant electrode array and the neural elements of the cochlea, specifically, the peripheral SGN processes, may enhance the efficacy of the cochlear implant by, for example, decreasing excitation thresholds, decreasing power consumption, and increasing the range of stimulation. In addition, in the long-term, stimulation of axonal growth towards, and correct tonotopic connectivity with, a regenerated hair cells target could lead to a fully functional regenerated auditory system.

Clinical Application of Neurotrophic Factors

In view of the important physiological functions that neurotrophic factors play within the auditory system, as well as the protective effects they have demonstrated in the experimental scenario, they are considered prime candidates for SGN rescue in humans.

Interestingly, preliminary studies have reported that the neurotrophin receptors trkB and trkC are present on human SGNs grown in culture, and that the neurotrophic factors BDNF, NT-3 and GDNF can have survival benefits and increase neuritic outgrowth in human SGN cultures (Miller et al., 2002). Such findings may provide a basis for clinical trials in humans to promote the survival and regrowth of the auditory nerve following hearing impairment.

While clinical trials using neurotrophic factors in the human cochlea have not as yet commenced, some growth factors have been trialled in a number of other neurodegenerative diseases. For example, NGF has been tested in clinical trials to arrest the degeneration of cholinergic neurons in Alzheimer's Disease (Ebendal, 1989; Olson, 1993; Eriksdotter Jonhagen et al., 1998). Both BDNF (Mitsumoto et al., 1994; Bradley, 1995; Group, 1999a; Ochs et al., 2000) and CNTF (Group, 1996a; Miller et al., 1996) have been trialled for the treatment of the degenerative effects of Amyotrophic Lateral Sclerosis (ALS), while GDNF has been investigated as a therapeutic agent for Parkinson's Disease (Gash et al., 1998; Nutt et al., 2003). The precise details of each of these studies obviously vary considerably in terms of dose rate, treatment course and number of patients, however, the overall outcome was generally the same, with no clinical benefit reported in any case.

In addition, each of these clinical studies reported a range of negative side effects, including injection site reactions, fever, cough, nausea, weight loss and anorexia. However, these adverse reactions were mostly considered mild to moderate in severity, and the application of neurotrophic factors has generally been considered to be well tolerated (Group, 1999b; Ochs et al., 2000).

It is important to note that each of these clinical trials utilised either systemic administration or administration via a route that introduced neurotrophic factors to the central nervous system (CNS). A means of delivering neurotrophic factors directly and exclusively to the area of interest – in our case, the inner ear – without diffusion to other regions of the nervous system, may shift the balance of outcomes in favour of the positive effects.

These outcomes therefore highlight the need for further experimental studies before these potentially therapeutic proteins can be used clinically, regardless of the neurodegenerative condition. In particular, special consideration needs to be given to the route of administration, the delivery mechanisms employed, the concentrations of the trophic factors required to elicit an effect, treatment regimes, and potential adverse effects.

The future of neurotrophic factors as therapeutic agents for SGN rescue

Effective delivery of neurotrophic factors requires that the trophic agent(s) reach the targeted neurons at doses that are sufficient to stimulate neuron function and prevent cell death. The concentration required to elicit an effect will undoubtedly differ amongst the different trophic

factors, as well as the neuronal class in question. As such, it is important to realise that results obtained with one neuronal class or one neurotrophic factor can not – and indeed, should not – be extrapolated to other trophic agents or types of neurons. Preclinical studies will therefore be required to determine optimal dosing regimens to maximise efficacy and minimise toxicity for the administration of neurotrophic factors for SGN rescue in deaf patients.

The time course of treatment is also an aspect of neurotrophic factor therapy that requires consideration. Animal studies to date have investigated various neurotrophin treatment regimes, including infusion periods of two- (Ernfors et al., 1996; Miller et al., 1997), four- (Shinohara et al., 2002; Gillespie et al., 2003; Gillespie et al., 2004; Shepherd et al., 2005; Wise et al., 2005), and eight-weeks (Staecker et al., 1996). In each case, protection of SGNs was evident in comparison to untreated controls. However, it has been reported that these survival effects are lost immediately following the cessation of the treatment. While intracochlear BDNF infusion was shown to protect SGNs from degeneration in deafened guinea pigs, survival rates as early as two weeks following the completion of treatment were not statistically different from contralateral untreated cochleae (Gillespie et al., 2003).

Studies in other neuronal classes have also observed a loss of neurotrophin-induced survival effects following cessation of treatment. For example, intravitreal BDNF injections led to increased numbers of surviving retinal ganglion cells following optic nerve transection in the rat; however, most of the rescued cells died soon after the neurotrophin was discontinued (Mansour-Robaey et al., 1994). In addition, NGF administration was not sufficient to permanently rescue cholinergic neurons following rescue of the septohippocampal pathway, with animals that survived for four weeks beyond the treatment period showing a loss of neurons (Montero and Hefti, 1988).

Neurotrophic therapies may therefore require ongoing administration for maintained survival effects. As such, another issue which must be overcome is the development of a method for delivering neurotrophic factors to the ear over extended periods of time, in order to afford long-term protection of SGNs from deafness-induced degeneration. A further point for consideration here, however, is the ongoing bioactivity of neurotrophic factors at body temperature. The vast majority of neurotrophic studies have used osmotic pumps as the delivery technique, and have clearly demonstrated that neurotrophic factors can remain bioactive in this environment for up to four weeks. It remains to be determined, however, how long this bioactivity can last. This is obviously an important factor when considering long-term clinical application of neurotrophic factors.

Adequate delivery methods will also need to be developed, since the techniques used for the delivery of neurotrophic factors to experimental animals are not clinically viable. For example, current commercially available osmotic pumps have a limited life-span of only up to four weeks. Hence, using this technique, long-term administration would require repeated replacement of the pumps, thereby increasing the risk of infection associated with such surgical procedures. Alternative implantable pump systems are also available, which can be refilled on a regular basis via a transcutaneous port, and have been shown to function adequately for up to eight months in rats (Praetorius et al., 2001). These pumps still, however, involve the insertion of a device into the cochlea and, therefore, the regular refilling of such devices would mean that the potential for introduction of infection directly into the inner ear remains.

Minimisation of infection is of particular importance within the inner ear. In the first instance, infection would lead to a reduction in the number of surviving SGNs, further compounding the degenerative effects of hair cell loss. More importantly, however, the cochlea is continuous with the CSF via the cochlear aqueduct, a bony channel that connects the perilymphatic space of the basal turn of the cochlea with the subarachnoid space of the posterior cranial cavity. This

means that infection within the cochlea has the potential to spread throughout the brain and CNS, and may therefore lead to such conditions as meningitis.

One potential alternative method of neurotrophin administration may involve less invasive techniques, such as localised drug delivery via the round window. When NT-3-loaded alginate beads are placed on the round window of deaf guinea pigs, significant survival effects on SGNs are observed over a period of four weeks in comparison to unloaded beads or untreated cochleae (Noushi et al., 2005). These results indicate that neurotrophins can be released from such matrices and can pass through the round window membrane. The precise degradation of matrices such as alginate, and the subsequent release profile of neurotrophins over a long time scale remains to be elucidated; however, such techniques may provide a potential clinical means of neurotrophin delivery to the deaf ear.

Alternatively, gene and cell-based therapies are emerging as potential therapeutic options for delivery of neurotrophic factors for neurodegenerative disease, including loss of SGNs.

The potential of gene and cell-based therapies for the application of neurotrophic factors to the inner ear

Numerous studies have tested both gene and cell-based techniques in a number of neuronal classes, including the SGNs of the inner ear (refer to Table 2). These methods may, therefore, prove useful in addressing some of the problems associated with pump-based neurotrophin administration.

Gene transfer is a method whereby a new gene(s) can be incorporated into cells or organisms, leading to the synthesis of the encoded protein, and provides a potentially powerful method of introducing ‘desired molecules’ into the inner ear. These molecules may be neurotrophic factors, or even genes identified to be defective. Studies investigating cochlear gene therapy have typically used viral vectors as the gene delivery vehicle. For example, viral-mediated gene transfer of BDNF using a HSV-1 vector has been shown to have a significant positive effect on the survival of denervated SGNs in the mouse (Staecker et al., 1998). In addition, introduction of the GDNF transgene into the scala tympani of deaf guinea pigs via adenoviral vectors led to significantly greater SGN survival than in untreated ears (Yagi et al., 2000; Kanzaki et al., 2002). The use of viral vectors does, however, raise issues associated with toxicity and cell targeting, since it is not possible to select or control for the type or number of cells infected.

The patency of the cochlear aqueduct, therefore, also poses concerns in this instance, with gene-based studies demonstrating that unilateral viral transduction of the inner ear also led to transduction within the contralateral cochlea. For instance, following unilateral infusion of adeno-associated viral vectors encoding for the reporter genes green fluorescent protein (Lalwani et al., 1997) or lacZ (Stover et al., 2000) into the guinea pig cochlea, viral particles were found to be present in the contralateral cochlea and in the brain.

The use of viral vectors for gene therapy within the cochlea, therefore, has the potential to deliver viral particles throughout the nervous system. As such, human gene therapy in the future will likely utilise non-viral techniques to maximise safety.

An alternative approach for cochlear gene transfer for the maintenance of SGNs is the use of cell transplantation as an *ex vivo* means of providing neurotrophic support, whereby a population of cells producing neurotrophic factors are transplanted into the cochlea. An advantage of such a cell-based technique includes the fact that a preselected host population is utilised, thereby avoiding the issue of viral vectors where any type and number of cells may potentially be infected.

To date, the majority of studies testing cell transplantation within the cochlea have aimed to replace damaged or degenerated SGNs and/or hair cells using stem cells, bone marrow stromal cells or dorsal root ganglion neurons (Table 2), and have not attempted cell-based gene transfer. These cochlear cell transplant studies have typically demonstrated that transplanted cells have the capacity to migrate, with transplanted cells reported to be localised within all turns of the cochlea (Iguchi et al., 2003;Naito et al., 2004;Coleman et al., 2005), in vestibular regions (Tateya et al., 2003;Sakamoto et al., 2004), and in the modiolus (Olivius et al., 2003;Kojima et al., 2004;Naito et al., 2004). Importantly, these studies report survival of transplanted cells within the mammalian cochlea for periods of up to 3-4 weeks (Ito et al., 2001;Iguchi et al., 2003;Olivius et al., 2003;Tateya et al., 2003;Hu et al., 2004;Naito et al., 2004;Coleman et al., 2005). These issues – migration and survival – will be important factors for consideration when future studies assess the feasibility of cell-based gene transfer for the maintenance of SGNs in the deaf cochlea.

An additional study of note demonstrated that the transplantation of Schwann cells into the scala tympani of ototoxically-deafened guinea pigs could lead to enhanced SGN survival, in comparison to survival rates in cochleae not receiving transplants (Andrew, 2003). Since Schwann cells are known to inherently produce small quantities of a variety of neurotrophic factors, including BDNF, CNTF, GDNF and FGF-2, it is likely that secretion of these proteins from the cell transplants elicited survival effects on the remaining SGNs.

Cell transplants are now considered a clinically viable technique for treatment of numerous neurodegenerative diseases. In an ongoing phase I clinical trial, Alzheimer's patients received autologous grafts of skin fibroblasts transfected with the human NGF gene into the basal forebrain. Both the genes and the vector have been deemed safe, with no adverse side effects of implantation of NGF-expressing cells reported to this date. Promisingly, in comparison to how quickly the patients had deteriorated before entering the trial, the rate of cognitive decline in these patients has been shown to be reduced by almost 50% (Tuszynski, 2004).

Cell therapy techniques may also incorporate the use of encapsulation technology, to prevent migration and dispersal by providing a matrix within which to contain cell growth, while still allowing molecular exchange between the enclosed cells and host tissue. Previous studies investigating cell-based therapies in other neural systems – in particular, the septohippocampal system used to study Alzheimer's disease – have demonstrated that transplanted cells have the capacity to grow beyond the implantation site, and in some instances form tumours (Hoffman et al., 1993; Winn et al., 1994). However, transplantation of encapsulated cells demonstrated that the cells remained confined to the capsule space, and that cells genetically engineered to release NGF remained viable and released measurable quantities of the neurotrophin, such that significant neuroprotective effects were observed (Hoffman et al., 1993; Winn et al., 1994). Furthermore, these cells have been shown to be able to release biologically active neurotrophin for greater than 12 months (Winn et al., 1996).

Therefore, cell transplantation, in particular, encapsulated genetically modified cells may prove useful for future applications involving delivery of neurotrophic factors. Such techniques would likely prove useful in the cochlea by preventing migration and dispersal of transplanted cells.

Potential side-effects of neurotrophic factor treatment

In addition to the design of delivery methods and treatment regimes, careful consideration also needs to be given to the potential side effects of neurotrophic factor administration. Neurotrophic factors are not specific to SGNs, and will potentially stimulate numerous other cell types within the cochlea. Furthermore, indiscriminate or non-targeted delivery of growth factors may lead to side effects which would render them not useful. For example, appetite

suppression and weight loss appear to be common side effects of systemic and intrathecal neurotrophic factor administration in human patients (Group, 1996b; Eriksdotter et al., 1998; Dechant and Neumann, 2002). As such, any treatment should ideally be localised and specifically targeted to a particular region or neuronal population. This is of particular importance within the inner ear, given the patency of the cochlear aqueduct with the CSF, which would mean that any agent delivered to the cochlea has the potential to enter the brain and CNS, and may therefore elicit effects throughout the nervous system.

Concluding remarks

The application of neurotrophic factors to the cochlea has proven highly successful in animal studies in terms of protecting SGNs from degeneration, and results from studies combining neurotrophins and cochlear prostheses are particularly promising. In terms of clinical application, however, the side effects and risks associated with neurotrophin administration must always be considered, especially in view of the free communication between the cochlea and the CSF. In addition, previous clinical trials highlight the need for thorough laboratory-based research, followed by careful consideration of all aspects of a treatment regime before these treatments reach the clinic.

Clinical application of neurotrophic factors within the inner ear and auditory system will ultimately need to be highly localised and at doses sufficient to stimulate SGN function. Cell-based therapies, perhaps in conjunction with gene transfer, are likely to provide a safe and efficient means of delivering neurotrophic factors to the cochlea at physiologically relevant levels, and over long periods of time.

Neurotrophic factors therefore retain high potential as therapeutic agents for the rescue of SGNs in deafness, for application both in combination with cochlear implants, as well as for innervating regenerated hair cells.

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Abbreviations

ALS, Amyotrophic lateral sclerosis
BDNF, Brain-derived neurotrophic factor
CNS, Central nervous system
CNTF, Ciliary-derived neurotrophic factor
CSF, Cerebrospinal fluid
CVG, Cochleovestibular ganglion
EABR, Electrically-evoked auditory brainstem response
FGF, Fibroblast growth factor
FGFR, Fibroblast growth factor receptor
GDNF, Glial cell line-derived neurotrophic factor
GFAP, Glial fibrillary acidic protein
HSV-1, Herpes simplex virus-1
IGF, Insulin-like growth factor
LIF, Leukaemia inhibitory factor
OSL, Osseous spiral lamina
MAP2, Microtubule associated protein 2
mRNA, Messenger RNA
NCAM, Neural cell adhesion molecule

NGF, Nerve growth factor
NT-3, Neurotrophin-3
NT-4/5, Neurotrophin-4
P, Postnatal day
SGN, Spiral ganglion neuron
SSEA3, Stage-specific embryonic antigen 3
SNHL, Sensorineural hearing loss
TGF-β, Transforming growth factor-β

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Table 1

In vivo studies of neurotrophic factors in the inner ear.

Species	Method of deafening	Period of deafness	Neurotrophin used	Period of treatment	Method of treatment	Electrical stimulation	Major finding[1]	Reference
Guinea pig	Neomycin (in cannula)[2]	1 day	NGF	2 weeks	Osmotic pump	No	↑ nerve fibre survival	(Schindler et al., 1995)
Guinea pig	Neomycin (in cannula)[2]	2 weeks	NGF	2 weeks (after 2 weeks of AP)	Osmotic pump	No	↑ survival in basal turn	(Shah et al., 1995)
Guinea pig	Kanamycin/ Ethacrynic acid	5 days	BDNF and/or NT-3	8 weeks	Osmotic pump	No	↑ survival; resprouting axons	(Staecker et al., 1996)
Guinea pig	Amikacin (in cannula)[2]	24 hours	NT-3	2 weeks	Osmotic pump	No	↑ survival; resprouting axons	(Ernfors et al., 1996)
Guinea pig	Kanamycin/ Ethacrynic acid	7 days 4 days	BDNF BDNF + NT-3 + GDNF + CNTF	2 weeks 28 days	Osmotic pump	No	↑ survival resprouting axons	(Miller et al., 1997)
Guinea pig	Noise	4 days	GDNF	3 weeks	Osmotic pump	No	↑ survival	(Ylikoski et al., 1998)
Mouse	Neomycin	None	BDNF	2 or 4 weeks	Herpes simplex viral vector	No	↑ survival	(Staecker et al., 1998)
Guinea pigs	Aminoglycoside/diuretic	4 or 7 days	GDNF	24 or 21 days	Adenoviral vectors	No	↑ survival	(Yagi et al., 2000)
Guinea pig	Neomycin (in cannula)[2]	2 days	BDNF + CNTF	26 days	Osmotic pump	No; EABRs measured	↑ survival; ↓ thresholds	(Shinohara et al., 2002)
Guinea pig	Kanamycin/ Ethacrynic acid	5 days	GDNF	39 days (36 days ES)	Adenoviral vectors	Yes	↑ survival with GDNF +ES	(Kanzaki et al., 2002)
Guinea pig	Kanamycin/Frusemide	5 days	BDNF	28 days	Osmotic pump	No	↑ survival; ↓ survival after BDNF removed	(Gillespie et al., 2003)
Guinea pig	Neomycin (in cannula)[2] Neomycin (middle ear)	2 weeks 6 weeks	BDNF + CNTF BDNF + CNTF	27 days	Osmotic pump	No; EABRs measured	↑ survival ↑ survival; but less pronounced	(Yamagata et al., 2004)
Guinea pig	Kanamycin/Frusemide	14 days	BDNF, NT-3, NT-4/5 or NGF	28 days	Osmotic pump	No	↑ survival	(Gillespie et al., 2004)
Guinea pig	Kanamycin/Frusemide	28 days	NT-3 NT-3 + BDNF	7 days 28 days	Single pulse infusion Osmotic pump	No	↑ soma size; no survival effect ↑ survival; ↑ soma size	(Richardson et al., 2005)
Guinea pig	Kanamycin/Frusemide	5 days	BDNF	28 days	Osmotic pump	Yes	↑ survival; ↓ thresholds	(Shepherd et al., 2005)
Rat	Gentamycin/Frusemide	2 weeks	BDNF	28 days	Osmotic pump	No	↑ survival; ↑ soma size	(McGuinness and Shepherd, 2005)
Guinea pig	Kanamycin/Frusemide	5 days or 33 days	BDNF + NT-3	28 days	Osmotic pump	No	↑ survival; resprouting axons	(Wise et al., 2005)

[1] As compared to deafened, untreated controls and/or deafened, sham-operated cohorts

[2] In these cases, the animals underwent surgery for aminoglycoside exposure and osmotic pump implantation at the same time. The cannula of the drug delivery system was filled with the aminoglycoside; one end was inserted into the cochlea, while the other was attached to the osmotic pump.

Table 2

Cell transplantation studies in the inner ear.

Recipient species	Hearing status	Donor species & Cell type	Period of treatment	Observations	Reference
Adult mouse	Deaf, 3 days (neomycin)	Mouse; fetal neural stem cells	25 days	- grafted cells observed in perilymphatic and endolymphatic spaces of inner ear, in both cochleae and vestibules - cell survival after 25 days - cells expressed glial (GFAP)[1] and neuronal (MAP2)[1] markers	(Tateya et al., 2003)
Newborn rat	No intervention	Rat; adult neural stem cells	2 or 4 weeks	- cells localised to region of organ of Corti - cell survival for up to four weeks - differentiation into neurons, astrocytes and oligodendrocytes	(Ito et al., 2001)
Adult rat	- Normal hearing - Deaf, 1 week (neomycin)	Mouse; fetal dorsal root ganglia	3, 6 or 10 weeks	- cells survived for up to 10 weeks - localised close to organ of Corti and SGNs	(Hu et al., 2004)
Adult chinchilla	Deaf, 4 weeks (gentamycin/ethacrynic acid)	Chinchilla; bone marrow stromal cells	3 weeks	- cell migration through all cochlear turns - cells in scala vestibuli, scala tympani, cochlear lateral wall and modiolus - cell survival after three weeks - expressed neuronal (neurofilament) or glial (GFAP) markers	(Naito et al., 2004)
Adult mouse	No intervention	Mouse; embryonicneural stem cells	28 days	- migration through all cochlear turns - most surviving cells in perilymphatic space - cell survival after four weeks - most cells expressed glial (GFAP) markers	(Iguchi et al., 2003)
P21 rats	- Normal hearing - Acoustic overstimulation	Rat; fetal otocyst cells	1 month	- cell survival for one month - cells observed in lateral wall, modiolus, scala tympani, scala vestibuli	(Kojima et al., 2004)
Adult mouse	Deaf, 1 day (neomycin)	Mouse; embryonic stem cells	4 weeks	- cells found in vestibular and cochlear regions - most cells expressed markers for undifferentiated stem cells (SSEA3)[1] and for neurons (βIII-tubulin; NCAM)[1]	(Sakamoto et al., 2004)
Embryonic chicken	No intervention	Mouse; embryonic stem cells	48 hours	- cells identified in the otic vesicles, but not as part of the otic vesicle walls	(Sakamoto et al., 2004)
Guinea pig	- Normal hearing - Deaf, 7 days (neomycin)	Guinea pig; fetal dorsal root ganglia	21-22 days	- implant commonly attached close to spiral lamina and organ of Corti - some migration into the modiolus - animals receiving concurrent neurotrophic factor treatment showed increased survival	(Olivius et al., 2003)
Guinea pig	- Normal hearing - Deaf	Mouse; embryonic stem cells	13 days	- good cell survival in scala tympani - labelling of neurofilament and nestin in some of the surviving cells	(Altschuler et al., 2003)
Guinea pig	- Normal hearing - Deaf, 2 days (neomycin)	Mouse; adult neural stem cells	1-4 weeks	- cells located near Rosenthal's canal, organ of Corti and along peripheral processes; also seen in scala tympani and scala vestibuli - poor cell survival - cells expressed neuronal (βIII-tubulin) and glial (GFAP) markers	(Hu et al., 2005)
Guinea pig	- Deaf , 2 or 4 weeks (kanamycin/frusemide)	Rat; sciatic nerve Schwann cells	2 weeks	- cells located within scala tympani of basal turn - enhanced SGN survival	(Andrew, 2003)
Guinea pig	- Deaf , 2 or 4 weeks (kanamycin/frusemide)	Mouse; embryonic stem cells	1,2 or 4 weeks	- cell survival for up to four weeks - cells observed in all turns, in scala tympani and scala vestibuli - some neurofilament labelling	(Coleman et al., 2005)

[1] GFAP = glial fibrillary acidic protein; MAP 2 = microtubule associated protein; SSEA3 = stage specific embryonic antigen-3; NCAM = neural cell adhesion molecule