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Cell-based neurotrophin treatment supports long-term auditory neuron survival in the deaf guinea pig

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ABSTRACT

The cochlear implant provides auditory cues to profoundly deaf patients by electrically stimulating the primary auditory neurons (ANs) of the cochlea. However, ANs degenerate in deafness; the preservation of a robust AN target population, in combination with advances in cochlear implant technology, may provide improved hearing outcomes for cochlear implant patients. The exogenous delivery of neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 is well known to support AN survival in deafness, and cell-based therapies provide a potential clinically viable option for delivering neurotrophins into the deaf cochlea. This study utilized cells that were genetically modified to express BDNF and encapsulated in alginate microspheres, and investigated AN survival in the deaf guinea pig following (a) cell-based neurotrophin treatment in conjunction with chronic electrical stimulation from a cochlear implant, and (b) long-term cell-based neurotrophin delivery. In comparison to deafened controls, there was significantly greater AN survival following the cell-based neurotrophin treatment, and there were ongoing survival effects for at least six months. In addition, functional benefits were observed following cell-based neurotrophin treatment and chronic electrical stimulation, with a statistically significant decrease in electrically evoked auditory brainstem response thresholds observed during the experimental period. This study demonstrates that cell-based therapies, in conjunction with a cochlear implant, shows potential as a clinically transferable means of providing neurotrophin treatment to support AN survival in deafness. This technology also has the potential to deliver other therapeutic agents, and to be used in conjunction with other biomedical devices for the treatment of a variety of neurodegenerative conditions.

1. Introduction

The World Health Organisation has estimated that over 5% of the world's population has a disabling hearing loss [1]. Hearing loss can range from mild to profound, and can impact upon quality of life by affecting the development of language in children, and by having adverse educational and social ramifications. People with a severe hearing loss also typically have a much higher unemployment rate and a higher rate of mental health concerns such as depression [2].

Sensorineural hearing loss (SNHL) is the most common form of deafness and affects the sensory and neural elements of the inner ear, or cochlea (Fig. 1). Specifically, SNHL is characterised firstly by a loss of auditory hair cells, which are located in the cochlea and are the first link in the chain for sound transduction, and subsequently by degeneration of the auditory neurons (ANs) which transmit the sound signals to the central auditory centres in the brain.

Damage to or loss of hair cells can occur through a number of causes, including noise trauma, aging and ototoxic drugs. Patients with a partial SNHL can benefit from hearing aids, which amplify sound signals for acoustic stimulation of residual surviving hair cells. In comparison, the only therapeutic intervention for patients with a severe-to-profound SNHL is the cochlear implant, which bypasses the missing hair cells to directly electrically stimulate the ANs to provide auditory cues. However, the progressive degeneration of ANs in SNHL ultimately results in significant neuronal loss after long periods of deafness [3,4], and experimental studies indicate that this ongoing AN degeneration compromises the efficacy of cochlear implants [3,5]. Significantly, results from a recent study indicate that, for individual patients, a larger number of surviving ANs results in better performance after cochlear implantation [6], highlighting the importance of the preservation of a maximal viable population of ANs in order to optimise the clinical benefits to cochlear implant patients. In addition, future advances in cochlear implant technology, such as new stimulation strategies and electrode designs that increase the spatial resolution of stimulation, may benefit from enhanced and more viable populations of ANs [7] to provide even greater outcomes.

Neurotrophic factors, which are naturally occurring proteins important for the development and maintenance of the auditory system, have been shown both in vitro and in vivo to have potential as clinically useful compounds to address SNHL. Indeed, the delivery of neurotrophins

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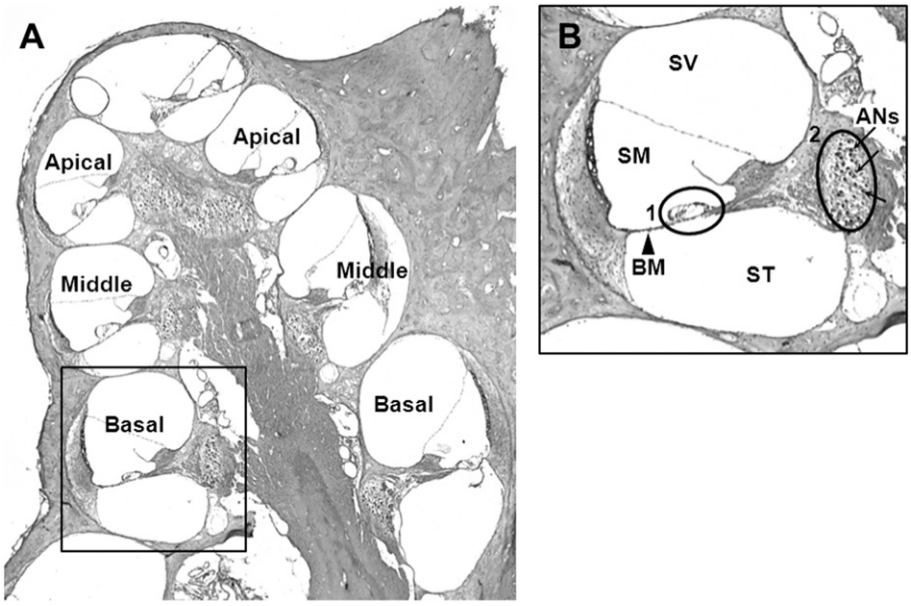

Fig. 1. Anatomy of the guinea pig cochlea. (A) The inner ear, or cochlea, is a spiral structure comprised of three turns — the basal, middle and apical regions of the cochlea. (B) Depicts the region outlined in (A), and shows the three chambers, or scalae, that longitudinally divide the cochlea and spiral together along its length — scala tympani (ST), scala media (SM) and scala vestibuli (SV). The auditory hair cells are the sensory cells of the cochlea and are located in the organ of Corti (1) on the basilar membrane (BM; arrowhead). The auditory neurons (ANs; arrows) are located within Rosenthal's canal (2), which spirals around the central core of the cochlea. ANs form synaptic connections with hair cells via their peripheral processes, and neural impulses generated by the hair cells in response to acoustic stimuli are transmitted by the ANs to the central auditory pathway and the auditory cortex where they are decoded, leading to the perception of sound.

such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3) to the deaf cochlea is well known to support AN survival and prevent deafness-induced degenerative changes [8–17]. However, issues relating to safety [18] and the duration of the treatment [19,20] must be addressed before the clinical translation of this therapy can be realised. Specifically, a neurotrophin delivery method designed for use in a clinical setting must be safe and biocompatible; deliver neurotrophins for extended periods in order to support long-term AN survival and function; and avoid the risks of infection associated with implantation into the cochlea [18–20]. Cell-based therapies, which would involve the long-term implantation of cells secreting a desired therapeutic in a consistent and physiologically relevant manner, have the potential to fulfil these criteria. Cell-based neurotrophin delivery has been reported to enhance AN survival in animal models of deafness [21–23]. Cell-based therapies may utilise cells that naturally secrete therapeutic agents, such as Schwann cells and choroid plexus cells, or cells that have been genetically modified to over-express specific therapeutic agents. A number of cell types, including Schwann cells and fibroblasts, have been genetically modified to express neurotrophins (for review see [24]), and long-term expression of over 12 months is possible [25]. This technique would enhance the option for autologous transplantation, whereby a recipient's own tissue could be harvested, manipulated to express the required therapeutic(s) and re-implanted, thus preventing or minimizing the risk of immunorejection.

The use of cell encapsulation technologies greatly increases the safety and biocompatibility of these therapies for use in the cochlea [21,23]. Cell encapsulation is important in order to prevent dispersal of the cells away from the site of implantation [26], and also provides an immunoisolatory barrier, thereby enabling cell implantation without the need for co-treatment with toxic immune-suppressive drugs. Encapsulated cell-based therapies are currently being assessed in clinical trials as long-term treatments for a number of diseases. For example, populations of encapsulated choroid plexus cells (NTCELL®) derived from virus-free porcine colonies are currently undergoing a Phase I/IIa clinical trial for Parkinson's Disease, while encapsulated islet cells from the same source (DIABECELL®) have progressed through Phase IIa dose finding and safety and efficacy studies and are now in late-stage clinical trials for diabetes (Living Cell Technologies, Ltd; http://www.lctglobal.com; http://clinicaltrials.gov/show/NCT01734733; http://clinicaltrials.gov/show/NCT01736228).

Promising experimental results have also been obtained using encapsulated cell-based therapies to support AN survival in the cochlea. We have reported that Schwann cells, genetically modified to express BDNF and encapsulated in a non-biodegradable and biocompatible alginate membrane, can support AN survival in the deaf guinea pig for four weeks [21]. Furthermore, transplantation of NTCELL® into the cochlea can support AN survival in deaf cats for at least eight months [23].

The current study investigated the long-term potential of a cell-based therapy using genetically modified cells to support AN survival in deafness in conjunction with electrical stimulation from a cochlear implant. Specifically, we combined cell- and gene therapies with alginate encapsulation technology to produce encapsulated BDNF-expressing fibroblasts, and assessed the survival-promoting effects on ANs in the deaf guinea pig following implantation for periods of up to six months, and with and without chronic electrical stimulation.

2. Experimental procedures

2.1. Preparation of encapsulated neurotrophin-expressing cells

2.1.1. Isolation and nucleofection of fibroblasts

Fibroblasts that were nucleofected to express BDNF were used for this study based upon findings from in vitro experiments [27], which demonstrated that these parameters were most efficacious in terms of gene transfer, and produced cells which had the greatest duration of neurotrophin expression. Specifically, fibroblasts were isolated from rat sciatic nerve explants using a method similar to that used to isolate Schwann cells [21,28]. Briefly, 8–10-week-old rats were killed using sodium pentobarbitone (150 mg/kg intraperitoneally) and the sciatic nerves removed. The perineurium was removed and 1 mm segments of nerve placed into uncoated 60 mm tissue culture dishes with D10 culture medium, comprised of Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen) containing 10% foetal calf serum (FCS; Invitrogen),