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Research Paper

Encapsulated cell device approach for combined electrical stimulation and neurotrophic treatment of the deaf cochlea

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ABSTRACT

Profound hearing impairment can be overcome by electrical stimulation (ES) of spiral ganglion neurons (SGNs) via a cochlear implant (CI). Thus, SGN survival is critical for CI efficacy. Application of glial cell line-derived neurotrophic factor (GDNF) has been shown to reduce SGN degeneration following deafness. We tested a novel method for local, continuous GDNF-delivery in combination with ES via a CI. The encapsulated cell (EC) device contained a human ARPE-19 cell-line, genetically engineered for secretion of GDNF. *In vitro*, GDNF delivery was stable during ES delivered via a CI. In the chronic *in vivo* part, cats were systemically deafened and unilaterally implanted into the scala tympani with a CI and an EC device, which they wore for six months. The implantation of control devices (same cell-line not producing GDNF) had no negative effect on SGN survival. GDNF application without ES led to an unexpected reduction in SGN survival, however, the combination of GDNF with initial, short-term ES resulted in a significant protection of SGNs. A tight fibrous tissue formation in the scala tympani of the GDNF-only group is thought to be responsible for the increased SGN degeneration, due to mechanisms related to an aggravated foreign body response. Furthermore, the fibrotic encapsulation of the EC device led to cell death or cessation of GDNF release within the EC device during the six months *in vivo*. In both *in vitro* and *in vivo*, fibrosis was reduced by CI stimulation, enabling the neuroprotective effect of the combined treatment. Thus, fibrous tissue growth limits treatment possibilities with an EC device. For a stable and successful long-term neurotrophic treatment of the SGN via EC devices in human CI users, it would be necessary to make changes in the treatment approach (provision of anti-inflammatories), the EC device surface (reduced cell adhesion) and the ES (initiation prior to fibrosis formation).

1. Introduction

The treatment of choice for most profound hearing impairments is direct electrical stimulation (ES) of the auditory nerve via a cochlear implant (CI); (Clark et al., 1984; Kral and O'Donoghue, 2010). A substantial degeneration of spiral ganglion neurons (SGNs) after hearing loss has been observed both in animal models (Zilberstein et al., 2012) and in humans (Nadol, 1997). The extent of degeneration strongly depends on the cause of hearing loss and which cochlear structures are affected. SGN survival is critical for CI efficacy (e.g. Scheper et al., 2009; Seyyedi et al., 2014) and may reduce variability in CI users' speech perception. Thus, preventing or at least reducing SGN degeneration has been a major target of recent CI research. ES from a CI may reduce degeneration of SGNs (e.g. Leake et al., 2013) via depolarization induced neurotrophic signaling pathways (Hansen et al., 2001; Roehm and Hansen, 2005).

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Abbreviations

aABR	acoustically evoked auditory brainstem response
CI	cochlear implant
eABR	electrically evoked auditory brainstem response
EC device	encapsulated cell device
ES	electrical stimulation
GDNF	glial cell line-derived neurotrophic factor
NTF	neurotrophic factor
RMS	root mean square
RMSMAX	upper asymptote of the RMS input-output function
SGN	spiral ganglion neuron

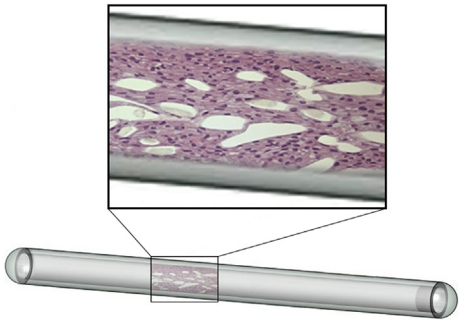

Fig. 1. Schematic representation of the encapsulated cell (EC) biodelivery device. Detail view with scaffold material (white) and ARPE-19 cells (red) filling the lumen of the hollow-fiber membrane capsule. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The extent to which ES alone is capable of reducing SGN degeneration *in vivo* is still under debate (Li et al., 1999; Agterberg et al., 2010) but may depend on several factors, e.g. the onset and duration of ES or the stimulation parameters (Araki et al., 1998; Leake et al., 1999). Additionally, exogenous application of neurotrophic factors (NTFs) has been shown to be effective in reducing SGN degeneration both *in vitro* (e.g. Hegarty et al., 1997; Schwieger et al., 2015) and *in vivo* (e.g. Shepherd et al., 2005; Scheper et al., 2009; Leake et al., 2011). The neuroprotective effect has been found to persist for several weeks after cessation of the neurotrophic treatment (Maruyama et al., 2008; Agterberg et al., 2009; Fransson et al., 2010). However, it can be expected that in humans, with years of deafness, a long-term application of NTFs is required for a stable neuroprotective effect (Gillespie and Shepherd, 2005). A safe and continuous drug delivery method to the human inner ear has not yet been established, mainly due to difficulties translating current methods from animal research into long-term stable clinical solutions, applicable to humans (El Kechai et al., 2015).

The osmotic pump is the most common application method for neurotrophic supply in animal research is (Brown et al., 1993). Thereby, a cannula is led from a reservoir containing an NTF solution to the inner ear (Scheper et al., 2009). This approach has two major limitations if intended to be used for continuous application: 1) the reservoir has to be refilled periodically and 2) a pump offers a permanent, possible entrance for infectious agents to the inner ear. An alternative is inoculation of the inner ear by viral vectors (Geschwind et al., 1996) to transduce cochlea cells to over-express the desired NTF (Kanzaki et al., 2002). This approach allows a long-term stable application without a permanent opening of the cochlea. However, viral inoculation comes with several safety concerns (Sacheli et al., 2014), such as control of NTF dosage, choice of transfection site/volume and no means of stopping NTF expression after transduction. Another approach for NTF supply are encapsulated cells (Zanin et al., 2012), which are genetically engineered to overexpress a desired NTF (e.g. Emerich et al., 2014). So far, alginate microspheres with encapsulated cells have been successfully implanted in the cochleae of both guinea pigs (Pettingill et al., 2011; Gillespie et al., 2015) and cats (Wise et al., 2011). Microspheres have the disadvantage that they cannot be easily explanted and replaced, which is likely to be necessary in human CI users, considering implantation duration of many years. This constraint can be overcome if cells are encapsulated in a hollow-fiber membrane capsule, i.e. via an encapsulated cell biodelivery device (EC device); (Fig. 1). These EC devices (NsGene Inc., Providence, USA; Wahlberg et al., 2012b) have already been successfully used in the brain of patients with Alzheimer's (Wahlberg et al., 2012a; Eyjolfsdottir et al., 2016) or Parkinson's disease (Emerich et al., 2014). The membrane of the capsule both allows for the diffusion of nutrients and NTFs and physically and immunologically shields the cells from the host tissue. The encapsulated cells in the present study came from an allogeneic human retinal cell line (ARPE-19). The genetic modification was performed using the sleeping beauty transposon expression technology (Fjord-Larsen et al., 2012). The EC device, including the modified ARPE-19 cells, is approved for human application (Emerich et al., 2014) and the medical grade components allow function for more than one year *in vivo* (Fjord-Larsen et al., 2012).

Several NTFs have already been successfully applied to reduce progressive SGN degeneration in both developing and adult cochleae (Roehm and Hansen, 2005). In the present study, we applied glial cell line-derived neurotrophic factor (GDNF), which is a distant member of the transforming growth factor-β (TGF-β) superfamily that activates intracellular signaling cascades via the RET receptor tyrosine kinase by first binding the glycosylphosphatidylinositol-anchored GDNF family receptor GFRα1 (Saarma, 2000; Sariola and Saarma, 2003). GDNF and its receptor GFRα1 have been found in the developing (Ylikoski et al., 1998) and adult cochlea (Stöver et al., 2000), including the SGN (Stöver et al., 2001). Exogenous application of GDNF has been shown to be protective for hair cells (Kawamoto et al., 2003), to provoke neuritogenesis *in vitro* (Euteneuer et al., 2013) and to reduce SGN degeneration *in vivo* (Scheper et al., 2009). The simultaneous administration of GDNF and ES has additive SGN-preserving effects (Kanzaki et al., 2002; Scheper et al., 2009). The protective effect of GDNF on SGNs has so far only been analyzed in deafened guinea pigs (Ylikoski et al., 1998; Yagi et al., 2000; Kanzaki et al., 2002; Maruyama et al., 2008; Scheper et al., 2009; Fransson et al., 2010), who show fast SGN degeneration (within weeks) and were treated for periods less than two months (38 days after viral inoculation; Kanzaki et al., 2002). To reveal GDNF effects over a relatively long time-span, we used cats deafened with aminoglycoside as an animal model for sensorineural hearing loss. In this animal model, SGNs significantly degenerate over several months (e.g. Shepherd et al., 2008; Leake et al., 2013).

The aim of the present study was to evaluate the safety, effectivity and stability of the EC device (Emerich et al., 2014; Eyjolfsdottir et al., 2016) to assess its feasibility as an approach for NTF treatment of the CI-implanted cochlea. First, we assessed *in vitro* whether the GDNF-release from the EC device remained stable during ES applied via a CI. We furthermore assessed whether ES as delivered during CI usage was sufficient to reduce fibrous tissue growths over an active electrode contact. After confirmed stability of the device *in vitro*, we tested if chronic implantation of GDNF-releasing EC devices, with or without additional ES, had a neuroprotective effect on SGNs *in vivo*. The safety of the EC device