Journal of Controlled Release 270 (2018) 275–281

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

The feasibility of an encapsulated cell approach in an animal deafness model

Anette Fransson[a,*], Jens Tornøe[b], Lars U. Wahlberg[b], Mats Ulfendahl[a]

[a] Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden
[b] NsGene A/S, 2750 Ballerup, Denmark

ARTICLE INFO

Keywords:
EC-device
Cochlear implant
Spiral ganglion neuron
Neurotrophic factor
Encapsulated cells
Drug delivery

ABSTRACT

For patients with profound hearing loss a cochlear implant (CI) is the only treatment today. The function of a CI depends in part of the function and survival of the remaining spiral ganglion neurons (SGN). It is well known from animal models that inner ear infusion of neurotrophic factors prevents SGN degeneration and maintains electrical responsiveness in deafened animals. The purpose with this study was to investigate the effects of a novel encapsulated cell (EC) device releasing neurotrophic factors in the deafened guinea pig.

The results showed that an EC device releasing glial cell line-derived neurotrophic factor (GDNF) or brain-derived neurotrophic factor (BDNF) implanted for four weeks in deafened guinea pigs significantly preserved the SGNs and maintained their electrical responsiveness. There was a significant difference between BDNF and GDNF in favour of GDNF. This study, demonstrating positive structural and functional effects in the deafened inner ear, suggests that an implanted EC device releasing biologically protective substances offers a feasible approach for treating progressive hearing impairment.

1. Introduction

The World Health Organization (WHO) estimates that 360 million people worldwide, or > 5% of the world's population suffers from disabling hearing loss [1]. Loss of auditory function is caused primarily by damage or loss of sensory cells (hair cells) in the inner ear cochlea as a result of acoustic trauma, exposure to ototoxic drugs, cochlear infection, genetic abnormalities, or aging. Loss of sensory cells results in a subsequent degeneration of spiral ganglion neurons (SGNs) [2,3], which are primarily bipolar neurons that relay auditory information from the sensory cells of the organ of Corti to the central auditory system. There are two types of SGNs, Type I and Type II. In mammals, neither the sensory cells nor the SGNs have the ability to regenerate, and there are currently no effective interventions for their repair. Currently, the only available therapeutic intervention for patients with a profound hearing loss is a cochlear implant (CI). With a CI, the malfunctioning sensory cells are bypassed and the subsequent functional level of the auditory system is directly stimulated via an electrode inserted into the fluid-filled cochlea. Type I neurons constitute 90–95% of SGNs and are the targets of CI.

The effectiveness of CI is thought to be related to the number and functional state of SGNs [4,5]. Several animal studies have shown a correlation between electrical responsiveness, measured by obtaining electrically-evoked auditory brainstem responses (eABRs), and the number of remaining SGNs in deafened animals [6–8]. Consequently, extensive experimental research has explored the possibilities of protecting and maintaining SGNs and their electrical responsiveness in order to optimize the beneficial effect of implanted CI. However, due to the natural barriers between fluid compartments accessible via systemic infusions and the compartments related to the inner ear (e.g., the blood-perilymph barrier and the intrastrial fluid-blood barrier), it has proven difficult to pharmacologically target the inner ear. Especially the intrastrial fluid-blood barrier restricts the transport of drugs into the inner ear [9]. Recent studies have therefore focused on developing methods for local inner ear drug delivery mainly using osmotic pumps connected to a cannula positioned in scala tympani [6,7,10,11]. However, approaches using adenoviral transfection [12,13] and encapsulated cells [14] have been explored. It has been shown that neurotrophic factors released by osmotic pumps preserve and maintain SGNs in traumatized inner ear. Moreover, it has been shown that neurotrophic factor treatment results in a significant decrease in eABR thresholds, suggesting that the functionality of SGNs is also preserved [6,7,10,11,15]. However, even though experimental intracochlear treatment using neurotrophic factors has been successful, the administration techniques need further development in order for this therapeutic concept to be tested in a clinical setting.

An interesting approach, which has already been tested clinically for the treatment of neurodegenerative disease [16,17], is using a delivery system based on implantation of an encapsulated cell (EC) device. Briefly, the EC device [18,19] consists of a semi-permeable membrane

* Corresponding author at: Karolinska Institutet, Department of Neuroscience, Retzius väg 8, SE-171 77 Stockholm, Sweden.
E-mail address: anette.fransson@ki.se (A. Fransson).

https://doi.org/10.1016/j.jconrel.2017.12.014
Received 24 August 2017; Received in revised form 15 December 2017; Accepted 17 December 2017
Available online 18 December 2017

enclosing genetically modified human cells that release neurotrophic factors. The semi-permeable thermoplastic membrane protects the encapsulated cells by preventing the entry of damaging elements of the host immune system while allowing the cell-produced neurotrophic factors to diffuse out into the surrounding host tissue. This EC device offers not only sustained local neurotrophic factor delivery, but also the possibility to terminate the treatment by removing the retrievable device [20].

The EC device was designed to fit the guinea pig inner ear and implanted in a CI experimental model in order to monitor the functional effects of neurotrophic factors released from the EC. It is demonstrated that the EC device can preserve the functionality of SGNs in the deafened guinea pig.

2. Materials and methods

2.1. Experimental design

Normal hearing guinea pigs were experimentally deafened by locally infusing an ototoxic drug, neomycin, into the middle ear. Three days after the injection, the animals were tested for hearing loss using acoustically-evoked auditory brainstem response (ABR). After three weeks, when ABR recordings demonstrated significant hearing loss, the animals were implanted with an EC device and a stimulus electrode mimicking a CI. The animals were then divided into four groups based on the cellular contents of the implanted EC device: 1) glial cell-derived neurotrophic factor (GDNF)-releasing cells (GDNF/ARPE-19); 2) brain-derived neurotrophic factor (BDNF)-releasing cells (BDNF/ARPE-19); 3) non-modified cells of the parental cell line (ARPE-19); and 4) devices not containing cells (Cell-free). A custom-made stimulus electrode was implanted into the scala tympani of the inner ear in order to elicit evoked potentials (eABRs) that reflect SGN responsiveness. After four weeks, the animals were sacrificed and the EC devices retrieved to measure the final level of neurotrophic factor release.

2.2. EC devices

The EC device contained cells of a genetically modified human cell line enclosed within a semi-permeable fibre membrane having a unique isoreticulated pore structure that allows influx of oxygen and nutrients to nourish the cells, and an outflow of therapeutic factor(s) into the surrounding tissue (Fig. 1A and B). The semi-permeable membrane protects the encapsulated cells from an immune response and rejection, and therefore no immunosuppression is required [16,18]. The release of BDNF and GDNF was confirmed in vitro in a separate study (Dash-Wagh et al., in preparation), in which the encapsulated cell devices were co-cultured with tissue explants from the newborn rat spiral ganglion (postnatal days 3–5). After a three-day period, there was a significant effect on neuronal survival and neurite outgrowth.

The EC device used in the present study was designed to fit the guinea pig cochlea. The diameter was 0.4 mm and the length was 4 mm excluding the tether. The tether was used to steer the device and precisely position it without damaging the membrane. In the present study the devices contained ARPE-19 neurotrophic-factor producing cell line. The parental ARPE-19 cell line is a spontaneously immortalized human retinal pigment epithelial cell line. The native cells were transfected with plasmid DNA encoding GDNF or BDNF using the sleeping beauty technique [21]. Both BDNF and GDNF were cloned from human DNA sequence. Growth factor release levels were measured using enzyme-linked immunosorbent assay (ELISA). A total of 30 EC devices were implanted in four experimental groups: GDNF/ARPE-19 ($n = 9$), BDNF/ARPE-19 ($n = 8$), ARPE-19 (parental cell line, $n = 7$), and Cell-free (empty device, $n = 6$).

The EC devices were maintained in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12; 1:1) and 10% human endothelium serum free medium (Invitrogen). Before implantation, neurotrophic factor release from the majority of the EC devices was measured using ELISA. After the final eABR measurement, the EC device was retrieved from the subjects for a second release measurement.

2.3. Deafening

Normal hearing animals were deafened by transtympanic injections of the ototoxic drug neomycin [6]. Within 48 h, most sensory cells had degenerated. The sensory cell loss resulted in a secondary degeneration of SGNs due to loss of neurotrophic support. In order to mimic the conditions of hearing impaired patients, the animals were deafened three weeks prior to device implantation. In some subjects, neomycin was administered repeatedly (maximum three extra doses) in order to produce significant damage to the auditory sensory cells. For this study, only animals with a threshold shift of 45–65 dB sound pressure level (SPL) were used.

2.4. Implantation surgery

The guinea pigs were deeply anaesthetized using ketamine (40 mg/kg i.m.) and xylazine (10 mg/kg i.m.). An ophthalmic ointment was then applied to the eyes to prevent corneal ulcers due to ketamine-induced suppression of the blink reflex. Marcain, a local anesthetic, was injected subcutaneously on the head, neck, and behind the ear. The middle ear cavity was opened to expose the cochlea and a cochleostomy was made in the basal part of the cochlea in order to get access to the scala tympani. Using the tether connected to the EC device, the device was then inserted into the scala tympani. To prevent perilymph leakage from scala tympani, a small piece of fascia was put around the tether to seal the cochlea hole. The electrode mimicking a CI has previously been described [22]. Briefly, a stimulus electrode was inserted through the round window membrane into scala tympani and the ground electrode was placed against the wall in the middle ear cavity.

After the last eABR measurement, the temporal bone was again opened to retrieve the GDNF- and BDNF-releasing devices (e.g., experimental groups GDNF/ARPE-19 and BDNF/ARPE-19). A final measurement was made to verify the release of neurotrophic factors throughout the experiments (in a few animals, it was not possible to retrieve the EC device as the tether detached or the device was damaged during retrieval).

2.5. Electrically-evoked Auditory Brainstem Response (eABR)

The animals were anaesthetized as previously described and placed in a sound proof box. The eABRs were recorded using a SigGen system 2 signal analyzer (Tucker-Davis Technologies, FL), as previously described [23]. Briefly, responses to monophasic current pulses (50-μs long; presented at 50 pps) with alternative polarity were recorded between a permanent electrode placed at the vertex (active), a subdermal needle electrode placed subcutaneously above the bulla on the deafened ear (reference) and a ground electrode placed in the hind leg. The thresholds were monitored and defined as the lowest stimulus level in 10-μA steps that elicited a reproducible waveform.

2.6. Histology

After the final eABR measurement, the animals were deeply anaesthetized with pentobarbital sodium (25 mg/kg i.p.) and transcardially perfused with saline (37 °C) followed by cold glutaraldehyde (2.5% in 0.1 M phosphate buffer). The temporal bone was removed and opened to expose the cochlea. Small fenestrations were made in the round window membrane and at the apex in order to gently flush glutaraldehyde through the cochlea. After fixation the cochleae were decalcified in 0.1 M ethylenediaminetetraacetic acid (EDTA) in 0.1 M phosphate buffer, and prepared for histology as previously described [6]. In short, the cochleae were rinsed, dehydrated and embedded in