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Adipose-derived stromal cells enhance auditory neuron survival in an animal model of sensory hearing loss

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Abstract

Background. A cochlear implant (CI) is an electronic prosthesis that can partially restore speech perception capabilities. Optimum information transfer from the cochlea to the central auditory system requires a proper functioning auditory nerve (AN) that is electrically stimulated by the device. In deafness, the lack of neurotrophic support, normally provided by the sensory cells of the inner ear, however, leads to gradual degeneration of auditory neurons with undesirable consequences for CI performance. *Methods.* We evaluated the potential of adipose-derived stromal cells (ASCs) that are known to produce neurotrophic factors to prevent neural degeneration in sensory hearing loss. For this, co-cultures of ASCs with auditory neurons have been studied, and autologous ASC transplantation has been performed in a guinea pig model of gentamicin-induced sensory hearing loss. *Results. In vitro* ASCs were neuroprotective and considerably increased the neuritogenesis of auditory neurons. *In vivo* transplantation of ASCs into the scala tympani resulted in an enhanced survival of auditory neurons. Specifically, peripheral AN processes that are assumed to be the optimal activation site for CI stimulation and that are particularly vulnerable to hair cell loss showed a significantly higher survival rate in ASC-treated ears. *Discussion/Conclusion.* ASC transplantation into the inner ear may restore neurotrophic support in sensory hearing loss and may help to improve CI performance by enhanced AN survival.

Key Words: *adipose-derived stromal cells, clinical translation, inner ear, neuritogenesis, transplantation*

Introduction

According to the World Health Organization, 360 million people worldwide have a disabling hearing loss [1]. Loss of the inner ear sensory cells, called "hair cells", is the most important reason for profound hearing impairment or deafness. In these cases, cochlear implants (CI) are the treatment of choice [2]. For this, a multielectrode array is inserted into the cochlea that allows direct electric stimulation of the auditory nerve (AN). Clinical studies show that the majority of CI subjects demonstrate clear improvements in speech discrimination, at least under quiet conditions. However, speech understanding in background noise and perception of music are still limited [3,4]. It is assumed that these limitations are largely based on poor channel selectivity that is caused by the distance between electrode contacts and stimulated neuronal structures and the large spread of electric excitation, i.e., the activation of overlapping populations of auditory neurons (spiral ganglion neurons [SGNs]) elicited by each electrode contact [5].

It has been suggested that this non-selective pattern of electric neuronal activation might be overcome by a more specific bioelectric interface between electrode contacts and SGNs [6,7]. In the healthy inner ear, hair cells secrete neurotrophic factors that are important for maintaining SGNs [8]. Loss of neurotrophic support leads to deterioration of the synaptic contact, degradation of peripheral processes and, subsequently, the entire auditory neuron [9–11]. The application of neurotrophic factors, primarily brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), is known to provide enhanced survival of SGNs, increased neuritogenesis and directed outgrowth of neurites [12–14]. In animal models, drug delivery systems, e.g., osmotic pumps either alone or in combination with CIs, have been successfully tested [15–17] for the release

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of neurotrophic factors that can be provided by this means over a certain period of time. However, after the delivery was discontinued, the positive effects in the cochlea decreased [18]. Moreover, the continuing use of a pump system might be associated with the risk of infection. Cell therapy, in contrast, might enable a continuous supply of neurotrophic factors without the risk of infections [19,20]. In particular, virally transfected cells have been investigated. These, however, bear the risk of spreading viral particles intracranially [21]. Thus, both approaches are not suitable for application in patients.

Adipose-derived stromal cells (ASCs) are multipotent stem cells that can be harvested from adult individuals and transplanted autologously [22,23]. ASCs are known to secrete neurotrophic factors like BDNF, glial cell–derived neurotrophic factor (GDNF) and NT-3 [24,25] and, thus, may serve as a suitable source of neurotrophins. It has been shown that ASCs enhance the survival and neuritogenesis of auditory neurons *in vitro* [26]. In animal models with spinal cord injury, ASCs are capable of neurite guidance and enhanced neurite outgrowth [27–29]. The successful clinical application of ASCs has already been proven in different clinical fields [23,30].

In this study we examined the application of ASCs to the inner ear as a potential way to overcome the detrimental effect of lacking neurotrophic support in the absence of hair cells in a guinea pig (gp) model. Initially, we confirmed the efficacy of a gpASC co-culture on rat SGN survival and neuritogenesis *in vitro*. Moreover, we show that ASC transplantation to the inner ear significantly improves survival of auditory neurons and peripheral processes in the osseous spiral lamina *in vivo* and that this effect is strongest at the region of transplantation.

Methods

Animals and treatment

For this study, a total of 15 female adult albino Hartley gp with a body weight ranging from 200–300 g and a litter of postnatal day 5/6 rats were used (Charles River). All experimental procedures were performed according to the regulations of the German Protection of Animals Act and were approved by and reported to the authorities responsible (Regierung von Unterfranken, Germany). All experimental procedures except euthanization of the rats were performed under general anesthesia with ketamine (Pfizer) and xylazine (CP-Pharma) under sterile conditions.

Isolation and expansion of gpASCs

gpASCs were isolated from adipose tissue of the neck of each individual animal. A horizontal incision was made in the skin of the dorsal neck. Subcutaneous fat was harvested and stored in sterile phosphate-buffered saline (PBS; Roche Diagnostics GmbH) with 2% penicillin/streptomycin (P/S; Biochrom AG) until further processing, and the wound was closed with absorbable sutures. The isolation of ASCs was performed according to the protocol described by Zuk *et al.* [31] and has been modified for the use in gp [32]. Briefly, the adipose tissue was digested in collagenase P solution (Roche Diagnostics GmbH; 10 mg/100 mL in PBS) for 3 h at 37°C. The adipose cell fraction was separated by centrifugation and removed. Erythrocytes were lysed by erythrocyte lysis buffer (4.1 g ammonium chloride [Merck] and 0.5 g potassium hydrogen carbonate [Merck] dissolved in 100 mL distilled water with 0.2 mmol/L ethylenediaminetetraacetic acid [EDTA; Sigma-Aldrich] at pH 8.0). Subsequently, cells were washed in PBS and after a further centrifugation step were resuspended in ASC medium consisting of Dulbecco's modified Eagle's medium (DMEM; Invitrogen), 10% fetal calf serum (FCS; Linaris) and 1% P/S. The cell suspension was filtered with a 100-µm cell strainer (BD Bioscience) before plating the cells in culture flasks. After 24 h, ASCs were rinsed with PBS to remove non-adherent cells and debris. The ASC medium was changed every other day. At 70–80% confluence, ASCs were detached with 0.25% trypsin solution containing 1 mmol/L EDTA (Gibco) and transferred to the next passage. ASCs from passages 2–4 were used for this study. Cells were counted using an automated cell counter (Casy Technologies, Innovatis AG).

Co-culture experiments

To evaluate the effects of gpASCs on auditory neurons *in vitro*, isolated gpASCs were co-cultivated with SGNs isolated from Sprague Dawley rats at postnatal day 5 or 6 in a 24-well Transwell system (Falcon, BD Biosciences). The isolation of SGNs was performed as described earlier [26]. Briefly, rats were decapitated, the brains were removed, and the cochleae were extracted. Then the capsule of each cochlea was opened with a fine forceps, the stria vascularis, the tectorial membrane and the organ of Corti were removed and the spiral ganglion was collected from the Rosenthal's canal that is not ossified at this age. The spiral ganglion was enzymatically dissociated in Hank's balanced salt solution (Gibco), supplemented with trypsin (final concentration 0.1%; Worthington), collagenase 1 (final concentration 0.1%; Worthington) and DNAse 1 (final concentration 0.01%; Worthington) at 37°C for 30 min in a shaker. Next, trypsin inhibitor (Sigma-Aldrich) was added at a final concentration of 0.1%. The suspension was triturated and centrifuged. The resulting cell pellet was resuspended in