Hearing Preservation and Nanotechnology-based Targeted Drug Delivery Future in Cochlear Implantation?

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Cochlear Implantation (CI) remains as one of the greatest medical achievements in modern medicine. New and innovative strategies continue to be developed to optimize and improve the functional results of CI surgery. Preservation of residual hearing through a-traumatic surgical techniques and electrode arrays may alter indications. Conditions with profound SNHL with preserved low tone hearing may have several causes and pathology may vary accordingly. In patients with progressive adult-onset SNHL neurons may be conserved even after long duration of deafness. IHCs and OHCs, supporting cells, ganglion cells and dendrites may be preserved in the apical region while in the lower turn despite atrophic organ of Corti and loss of lamina fibers ganglion cells can be present even after 28 years duration of deafness. These spiral ganglion cells may be excellent targets for electric stimulation using EAS technique that combines electric and acoustic stimulation in the same ear and utilizes both low frequency acoustic hearing and electric stimulation of preserved neurons. At the moment we are trying to elucidate the mechanism responsible for this preservation in humans and to use this knowledge for future therapy. Nano-technology may offer new possibilities for focused release of drugs and possibly genes to the inner ear. European project “NanoEar” is a concerted action to develop 3rd generation of nanoparticles (NP) for treatment of inner ear deafness. One goal is to target drugs and genes to specific inner ear cells through so-called multifunctional NP which are degradable, non-toxic, traceable and can be released in a controlled and biocompatible way. The small size of the NP may give new properties for technical advancement but risks must also be thoroughly evaluated. Uppsala is a Swedish partner to evaluate NP uptake in vitro in both human and animal spiral ganglion neurons. In my presentation I will show the system of culture spiral ganglion cells and demonstrate their locomotive behaviour and NP intracellular uptake using time lapse video recording and combined immunofluorescence and confocal microscopy.

Key words: Cochlear implantation, residual hearing, spiral ganglion, nano-technology, NanoEar

INTRODUCTION

New treatment strategies emerge in our field of “implantology” and Clinical Otology at present. We see new advancements in CI treatment with smaller and softer electrode arrays aiming to preserve residual hearing being constructed. These delicate devices can limit intra-cochlear damage at surgery. Such strategies must be adopted in children where we can envision several revision surgeries or technical “updatings” during a child’s lifetime. In children it will be important not only to preserve inner ear structures for later possible cell replacement but also to enable extraction of devices without causing major trauma. In addition, we may envision new tools to pharmacologically treat or protect
the inner ear possibly including gene therapy. Hybrid devices may combine electric stimulation and nanotechnology-based targeted drug delivery in the near future and increase pharmacological bioavailability and selectivity to treat specialized inner ear cells.

Our on-going research in nanoscience related to the ear is a part of the European community 6th Framework Programme on Research, Technological Development and Demonstration (Nanotechnology-based targeted drug delivery with acronym NANOEAR).

**EAS and Hearing Preservation Surgery**

Preservation of residual hearing is now a goal in CI surgery that should always aim to limit intra-cochlear damage. Combined electro-acoustic stimulation in the same ear, so-called EAS strategy, uses both acoustic and electric stimulation of preserved residual auditory nerve structures (von Ilberg et al., 1999; Kiefer et al., 2002, 2005; Gantz and Turner, 2003; Skarzynski et al 2003, Gstoettner et al., 2004, 2006; James et al., 2006; Turner et al., 2008). As the success rate of hearing preservation increase, patients with more residual hearing may become candidates for EAS surgery (Skarzynski, et al., 2003). The technique was proposed already in 1994 by William House.

When performing CI surgery in a patient intended for combined acoustic and electric hearing it is necessary to consider the great anatomic variations that exist in man. In Uppsala we created a collection of micro-dissected human temporal bones produced initially for studies of the inner ear aqueducts during the seventies. Moreover, our collection includes 325 plastic molds (methacrylate) of normal human inner ears. These specimens can be utilized today for surgeons studying cochlear anatomy (Figure 1).

In figure 2 we see plastic corrosion casts of several human cochleae with different morphology. Surprisingly, the human cochlea is individually shaped and varies greatly in length, width and coiling pattern. It may be beneficial to acknowledge this when performing inner ear hearing preservation surgery since these minor variations can be difficult to see on X-rays. Since surgeons now perform surgery on the round window (RW) these extensive anatomic variations get more relevance. The shape and three-dimensional position vary as well as its relationship to the facial nerve. Preoperative high resolution CT is of value prior to EAS surgery.

![Figure 1. Uppsala collection of human inner ear molds.](image)

There are now many different electrode arrays present in the market. These can be custom-made and used in diverse situations depending on cochlear morphology and residual hearing. Hearing preservation surgery is also performed differently among surgeon. The electrode is inserted either through an otic capsulostomy, drilled at the anterior/inferior rim of the RW, or through the RW. The latter technique is preferred in Uppsala with drilling away of the bony overhang. This bone varies in thickness and it is important to remove the posterio/medial bony ridge so that the electrode projection is not directed against the modiolus (Roland et al. 2006). Such removal can sometimes be difficult.

Moreover, the position of the RW relative to the 3rd portion (vertical) of the facial nerve diverges commonly and its exposure can be difficult through the facial recess. We found that facing the exposure of the RW may depend on the direction of the external bony canal and the antero/posterior distance between the facial nerve and the RW. When this distance is short the RW may be “hidden” behind the facial nerve and
drilling of the overhang is more difficult. With longer distance exposure and incision of the membrane is more easy. In these instances longer electrodes can also be inserted.

Despite attempts to preserve hearing after cochlear implantation, a small group of patients may lose their residual hearing that become dependent on their implant. A shallow insertion reduces the risk of damage to apical cochlear structures, whereas a deep insertion of the array may improve CI performance in case residual hearing is lost. According to Gstoettner

Figure 2. Corrosion casts of human cochlea showing anatomical variations. Due to different coiling characteristics the distance between the oval window and the upper basal turn can differ (†). Reprinted with permission (Erixon E, Högstorp H, Wadin K, and Rask-Andersen H: Variational Anatomy of the Human Cochlea: Implications for Cochlear Implantation. Otology & Neurotol 2009; 30:14:22.

Figure 3. Micro-dissection of a left human cochlea show the round window opening (arrow) and its relation to the cochlear aqueduct.

Figure 4. Patient operated with a 24 mm long CI electrode (EAS) through the round window. Due to the short distance between the facial nerve and the round window (a) the exposure of the RW was difficult through the facial recess. Postoperative electrode position is shown in figure 7.
et al. 2006 and Adunka et al. 2006, the anatomic variations of cochlear diameters led to significant variations of insertion degrees at constant surgical depths. They developed a radiological method to preoperatively predict the required insertion depth to achieve a 360-degree insertion. This protocol was based on a high-resolution computer tomography and a 3-D placement of referral points including the basal and first parts of the middle cochlear turn. A 360-degree insertion of the array entering the 1,000-Hz region, which defines the end of electric stimulation and beginning of acoustic stimulation corresponds to 18 to 24 mm (Erixon et al. 2009, table). This depth was found to provide good cochlear implant performance for both combined acoustic and electric hearing and electrical stimulation alone in case of loss of residual hearing without the need for re-implantation. Although not all factors leading to hearing loss after electrode insertion are known, to date, the extensive anatomic variations most likely play one important role.
Ganglion Cells and Dendrite Populations in EAS Ears

A key issue for a positive outcome with EAS technique may be the neuron potential in the “deaf” part of the cochlea (usually the basal part where the electrode lies close to the high- and mid-frequency coding neurons). How important these neurons really are for the results of CI in general is still under debate (Linthicum and Galey 1983, Linthicum et al. 1991, Nadol et al. 2001, Khan et al. 2005, Fayad and Linthicum et al. 2006, Nadol and Eddington 2006). Fewer neurons than earlier thought seem needed and if this also holds true for the EAS principle is not known. We need to know more about the stimulation pattern of ganglion cells or axons within the modiolus and acoustic nerve. Neurons are placed near the perilymph in scala tympani and currents can reach the central of the cochlea. It seems hard to conceive how selective stimulation within such small distances using conventional monopolar stimulation are reached.

Table 1  The total length of the outer wall excluding the basal half of the round window (RW). SD; standard deviation. n; number of specimens. (from Erixon et al. 2009)

<table>
<thead>
<tr>
<th>Description</th>
<th>Mean</th>
<th>Range</th>
<th>SD</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer wall length (mm)</td>
<td>42.0</td>
<td>38.6-45.6</td>
<td>1.96</td>
<td>58</td>
</tr>
<tr>
<td>Half diameter of the RW</td>
<td>1.1</td>
<td>0.3-1.6</td>
<td>0.21</td>
<td>65</td>
</tr>
<tr>
<td>First half of first turn</td>
<td>13.5</td>
<td>12.1-15.0</td>
<td>0.73</td>
<td>67</td>
</tr>
<tr>
<td>First turn (quadrant 1-4)</td>
<td>22.6</td>
<td>20.3-24.3</td>
<td>0.83</td>
<td>65</td>
</tr>
<tr>
<td>Second turn (quadrant 5-8)</td>
<td>12.4</td>
<td>10.7-13.3</td>
<td>0.63</td>
<td>63</td>
</tr>
<tr>
<td>Third turn (quadrant 9 to 11(12)</td>
<td>6.1</td>
<td>1.5-8.2</td>
<td>1.40</td>
<td>58</td>
</tr>
</tbody>
</table>

Figure 9. Flex soft electrode (31 mm long) inserted through the RW in the patient shown in figure 3. The first electrode reaches up one and a half turn (1). The last electrode (12) is positioned just outside the round window.

loss with some residual low tone hearing. Some of these patients had low tone hearing making them candidates for EAS surgery (Rask-Andersen et al 2009). Remarkable is the high degree of preservation of neuron perikarya in the basal part of the spiral ganglion. These neurons lack peripheral processes or dendrites due to retrograde degeneration caused by the loss of structural integrity of the organ of Corti (Fig. 10). These neurons may be targeted in EAS surgery. The mechanisms behind the preservation are discussed in more detail in the next section.

Nanotechnology-based Targeted Drug Delivery

On-going progressive cell degeneration may be caused by genetic or secondary environmental factors. These changes could theoretically be influenced by delivery of therapeutic drugs or genes in a controlled way. Inner ear drug delivery could lead to better cellular concentrations and selectivity to certain cell components such as treatments for in Meniere’s disease, tinnitus, hearing loss and NF2. The concept of multi-functional nanoparticles, which are targetable, biodegradable and traceable, has lead to new approaches for such controlled drug release. Tissue specific delivery can be achieved by functionally “addressed” nanostructures loaded with a therapeutic molecule. Such drug delivery could be accomplished either through application in the middle ear or through specially designed implant systems.
Targeting of neurons and other cells in the inner ear requires that we obtain more knowledge about the expression of surface markers and receptors in order to construct suitable ligands and surface structure on the NPs. We therefore studied the human spiral ganglion cells for expression of various surface receptors. Important markers are neurotrophin and nerve growth factor receptors such as Trk B, C-ret, and other GDNF family ligand (GFLs) receptors (Fig. 11). These receptors may play an important role for the survival of neurons and their interaction with glia/satellite cells. These factors may explain the unique survival of neurons in the human spiral ganglion making CI treatment possible.

**NP incorporation in spiral ganglion cells**

The feasibility to use nanoparticles (NP) for intracellular delivery was investigated. We studied the incorporation, distribution and toxicology of amphiphilic block copolymer NPs (Southampton, England), lipid nanocapsules (Anger, France) and lipoplexes (Helsinki, Finland) in spiral ganglion (SG) cell cultures. Human surgical specimens were isolated during petroclival meningioma surgery and adult guinea pigs (aged 3-6 months) and used in this study. The study was approved by the local ethics committee (no. 99398, 22/9 1999, no. C254/4, no C45/7 2007), and patient consent was obtained (Rask-Andersen et al. 2005, 2006, Boström et al. 2007, Anderson et al. 2006, 2009). Dissections of the SGs from human and guinea pig
were performed. Briefly, the SG was dissected out from the cochlea together with the distal part of the cochlear nerve. Adult human and guinea pig SG neurons and glia/Schwann dissociated cell cultures were expanded, grown for several weeks and studied live using time lapse video microscopy and high-resolution light microscopy. The cells were further characterised using immunocytochemistry for the neural marker Tuj1 and the glia cell markers S-100 and GFAP and their morphology was studied in more detail using scanning electron microscopy (SEM). The cell cultures were exposed to fluorescent (DiI), FITC or TRITC loaded NPs for different time periods and at different concentrations and the uptake was studied using fluorescence microscopy. The study demonstrates that DiI-loaded NPs can be internalised into SG neurons as well as SG glia/Schwann cells without indication of toxicity or reduced viability (Fig. 12). Through time lapse video recordings of live cells the NPs were detected in small vesicles surrounding the nucleus and in the periphery of the cytoplasm. This information could lead to the development of more specialised NPs, only targeting SG neurons or Schwann cells.

**NP drug delivery**

Several neuroactive agents in the form of peptides, proteins and oligonucleotides may act as therapeutics in neurodegenerative and other disorders. While suitable they are often poorly soluble and protective delivery vehicles must be developed. Most polymer
micelles as drug carriers have been developed for the
delivery of anticancer drugs (Steiniger et al. 2004),
DNA probes (Whittlesey and Shea 2004) and recently
antisense oligonucleotides (Elhamess et al. 2009) who
used systemic delivery targeting the EWS/FLI1 gene
product by a poly-iso-hexyl-cyanoacrylate (PIHCA)-con-
taining polycation (chitosan) to bind and protect the
antisense-oligonucleotide. It was found to dramatically
inhibit tumor growth in a murine model of Ewing’s
sarcoma.

Little is still known about the release, uptake and
subcellular distribution of drug carriers and drugs.
Maysinger et al. (2001) and Luo et al. (2002) focused on
polycaprolactone-polyethylene oxide (PCL21-b-PE044)
micelles containing the fluorescent probe Dil, a
dialkylindocarbocyanine derivative. The NP systems
formed from block copolymers can be considered as
nano-containers from which agents are released in a
sustained manner (Hu et al. 2004). This is of particular
interest for in vivo studies where the success of
micelles for the delivery of neuroactive agents depends
on the stability of the micelle, the stability of the drug
within the micelle core and release kinetics. In addi-
tion, the nature of the corona has a known influence on

NPs, liposomes and other polymeric colloids are pos-
sible carriers for drug delivery into the brain
(Schroeder et al. 1998, LaVan et al. 2003, Whittlesey
and Shea 2004). A major challenge in delivering ther-
apies to neurons in the central nervous system is the
difficulty of penetrating the blood-brain barrier. A
similar barrier, the “blood-labyrinth barrier”, exists in
the ear and may offer resistance for the passage of NP
into the cells. Recently liposomes conjugated to an
antibody raised against the transferrin receptor, were
used to by-pass the brain barrier (Maysinger et al.
2001). In the brain NPs coated with polysorbate 80
and apolipoprotein E displayed improved brain tar-

Tumour cell targeting
The ear can be affected by solitary tumors arising in
the vestibular cranial nerve; so-called vestibular
Schwannomas (often referred to as acoustic neuromas).
These are benign neoplasms that cause hearing loss
due to destruction of the near-by located acoustic
nerve. The tumors can involve both ears; a condition
known as neurofibromatosis type 2 which is an autosom-
dominant inherited condition associated with a
mutation of the NF2 gene coding for merlin. Since
surgical intervention often leads to complete deafness
and mostly aims at removing fatal brain compression
there is a need for medical intervention to arrest or
decrease tumor growth rate. Such therapies may
include the administration of drugs carried by NPs
delivered to the ear through the round window and
not necessarily through systemic delivery. These NPs
may reach the tumor in the internal acoustic meatus
through targeting. Such targeting may exploit axo-
plasmic transport from terminals to neuron cell bodies
located in the acoustic meatus. Such drugs may
include PI3-Kinase-Akt and Ras/Raf/Mek/Erk path-
ways. Their inhibitors have recently been used with
success in disease models for Schwannoma where they
seem to inhibit tumour growth (McCutcheon et al.
2001). Positive results for brain tumors were achieved
with doxorubicin-containing NP which diminished
glioblastomas (Steininger et al 2004). Such cancer ther-
apies were predominantly based on drug-loaded PBCA
[poly(butyl-2-cyanoacrylate)] NPs (Duncan 2003) which
were shown to undergo enzymatic biodegradation
both in vitro (Muller et al. 1990) and in vivo (Sullivan
and Birkinshaw 2004). Core-shell NP may provide
advances since they are composed of a resistant poly-
styrene core and a PBCA shell allowing a ‘burst
release’ of encapsulated drugs aiming at targeting
beta-amyloid in Alzheimer’s disease (Härtig et al.
2003).

Neuron targeting
To target neurons of the inner ear (and Schwann
cells) is a challenge. Efforts are ongoing in our labora-
tory to uncover techniques to selectively target neu-
rons in the inner ear. Townsend et al. (2007) devel-
oped NPs from PLGA-PEG-biotin polymers and used
biotin-binding proteins (avidin, streptavidin, or neutral-
vidin) as crosslinkers for protein conjugation. The
tetanus toxin C (TTC) fragment was modified and con-
jugated to NPs, allowing targeted binding to neuroblas-
toma cells, while not targeting liver or endothelial cells.
TTC is the neuronal binding portion of the native
tetanus toxin. TTC demonstrates extremely high
affinity binding to the neuronal ganglioside GT1b that
is the tetanus receptor, which is located selectively on
the surfaces of neurons. TTC selectively targets neu-
ronal cells in vitro and was found to be endocytosed
and efficiently carried via retrograde transport from
the distal axonal terminus to the neuronal cell body.
This system may have applications for delivering ther-
apeutics to neurons in the ear affected by neurodegen-
erative diseases. In our studies of the uptake of NPs
in growing auditory neurons we could not yet observe
an uptake in the distal terminal zone of the axons, only
in the cell body. TTC mediated uptake may prove
useful and these studies are under way.

In the current study we used fluorescent loaded
block-copolymer NPs, liposomes and lipid core
nanocapsules to study uptake within the SG cells of
both the guinea pig and human ear. Such NPs are
well tolerated by the cells and may be a suitable carrier
for compounds that are otherwise difficult to deliver
to the inner ear. Hydrophilic drugs, which may be of
benefit, are currently limited in their use within all
compartments of the central nervous system as they
have a low tendency to cross the blood-brain barrier
after i.v. infusion. The identification of an efficient
carrier in conjunction with the development of suitable
targeting system, if successful, will prove invaluable in
the future treatment of inner ear diseases.

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