Otology Seminar: Drug delivery for treatment of inner ear disease

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Introduction

- Therapeutic strategies of inner ear diseases
  - Delivery of medications (systemically and locally)
  - Surgical intervention
  - Sound amplifications (with hearing aids)
  - Physical therapy
Introduction

- Systemic treatments for inner ear diseases
  - Streptomycin for vertigo
  - Steroids for SSNHL
  - Diuretics for Meniere’s disease
  - Biphosphonates for otosclerosis

- Drawbacks of systemic route of delivery
  - Variable penetration into the inner ear (Blood-cochlea barrier)
  - Undesirable systemic side effects
Introduction

- Intratympanic drug delivery:
  - Routine strategy

- Potential drawbacks
  - Anatomic barriers (Round window membrane)
  - Loss of drug down the Eustachian tube
  - Variable or unknown pharmacokinetics profiles of medications
Introduction

- Newer drug application systems
  - Cochlear implantation
  - RNA interference
  - Stem-cell therapy
  - Microsystems of drug delivery (Osmotic pump, reciprocating perfusion system)
Rationale of local applications

- Locally given medications to RWM with higher concentrations in inner-ear fluid than systemic route
- Technical difficulties
  - Which parts of the ear? Concentration? Time course?
  - Different delivery methods or applications v.s. drug levels at each time point and different locations
- Major sources of variation?
General principles of drug distribution in the inner ear

- ST (Scala tympani) and SV (Scala vestibuli): perilymph: similar to extracellular fluid
- ELS (endolymphatic space): unique, high-potassium composition
- Inner ear fluids do not move or flow appreciably
- From RWM to inner ear → Passive process
- Active transport process: not confirmed yet
- RWM: a semipermeable membrane
Principles of substance distribution in the inner ear

Radial process

Longitudinal process
Clearance v.s. Diffusion

- Clearance
  - Removal of substance in cochlear fluids from lateral wall and modiolus
- Clearance rate increased → Decreased concentration at the apical regions of cochlea
- Greatest technical problem
  - Large gradients of drug concentration
  - Predicted by computer simulation, ion-electrode measurements, histological methods
Preclinical study of pharmacokinetics in the inner ear

- Animal studies: variable results
- Withdrawal and sampling fluids from the inner ear → technical challenge
- Extremely small volume (Guinea pig: < 10 μl)
- When fluid aspirated from basal turn of the ST: CSF enters ST through cochlear aqueduct → contaminates the perilymph
- A 1 μl sample taken through the RWM → contaminated with 20 % CSF
Equivalent Tube Diameter of Cochlear Scalae

Scala Vestibuli

Scala Media

Scala Tympani

Cochlea: Guinea pig (Salt et al.)
Potential misinterpretation of fluid sample measurements

![Graph showing the concentration of RW drug over time](image)
Microdialysis

- An alternative for sampling
- Drawback:
  - the leakage of drug from perilymph into the dialysis probe → a non-physiological clearance of the drug from cochlea fluid spaces
- High rates of drug clearance in previous studies → underestimation of
  - Drug concentration
  - The time the drug remains in the perilymph
Cochlea of the Guinea Pig

Round Window Membrane

Gentamicin Bolus (0.5mg) or Gentamicin Solution (5mg) was administered to this site.

Microdialysis Probe

Scala Tympani

Adhesive

Syringe Pump
Artificial Perilymph
2 to 3 μl/min

Dialysate

Fig. 1. Schema of the study.
In vivo study

- Only few pharmacokinetic studies fluids samples in humans.
- Patients with labyrinthectomy (Becvarovski et al., 2002)
  - Perilymph from the vestibulum at various times following local delivery of gentamicin to the middle ear
  - Valuable in the extrapolation of pharmacokinetic studies in animals to the situation in humans.
Methods for intratympanic delivery

- Transtympanic injection or myringotomy
- Silverstein MicroWick
- Microcatheter implantation
- Hydrogel application
- Nanoparticles
Transtympanic injection or myringotomy

- Simplest form with widespread use
- Injection or myringotomy w/ or w/o tympanostomy tube
- Need for repeat procedures

Drawbacks
- Persistent perforation
- Otorrhea
- The need to keep the ear dry
Silverstein MicroWick

- 1x9 mm wick composed of polyvinyl acetate (Silverstein et al. 2001)
- Through a ventilation tube overlying the round window membrane
- Sustained release of medication over time
- Vertigo improved in > 66 % patients with Meniere’s disease (Hill et al, 2006)
- Improved hearing in 8 of 12 patients with SSNHL (Van Wijck et al, 2007)
How to localize the round window membrane?
Drawbacks of Silverstein MicroWick

- Persistent perforation
- Infection of the middle ear or external ear
- Tissues ingrowth in the middle ear such as fibrosis or cholesteatoma
Microcatheter implantation

- Placed in round window niche → tympanic annulus → external auditory meatus
- Continuous infusion for several weeks
- Steroid delivery in 25 patients with SSNHL after failed systemic therapy (19 dB improvement) (Plontke et al, 2005)

- Complications:
  - Dislocations (5 pts), obstruction (2 pts)
  - Granulation (7 pts)
  - Perforation (2 pts)
Surgical techniques of microcatheter
Hydrogel application

- Dissolvable matrix mixed with medications released in a controlled fashion by hydrolysis
- BDNF (Brain-derived neurotrophic factor) and IGF-1 (Insulin-like factor 1)
- Several formulations:
  - Siloxane-based polymer
  - Poly-lactic glycolic acid (PLGA) polymers
  - Gelatin
  - Chitosan glycerophosphate
- Not yet for clinical application in human
A  Siloxane-based polymer

B  PLGA nanoparticle

C  Gelatin hydrogel
Delivery of medications directly to the inner ear

- Cochlear implantation
- Osmotic pump
- Reciprocating perfusion system
Application of direct drug delivery to cochlear implantation

- Coating with a biorelease polymer diffusion of medication
  - Neurotrophin-3 (NT-3, Richardson et al, 2009)
  - Dexamethasone (Dinh et al, 2008)

- Potential drawbacks
  - Greater risk of infection
  - Poorer implant performance
Drug delivery via an osmotic pump

- Direct drug delivery to the inner ear (Kingma et al, 1992)
  - Permit longer drug infusion time and bolus dosing, such as viral vector delivery (Prieskorn, 2000)

- Drawbacks
  - Inability to delivery varying dosages or dosing intervals
  - Inability to start and stop externally
  - Limited duration drug delivery
  - Required surgical access to the device if planned for a sustained period of time
Reciprocating perfusion system

- Zero net volume change within the perilymphatic space (Chen et al, 2005)
  - Continuous infusion associated with spread to the contralateral ear through cochlea aqueduct → CSF contaminated (Borkholder 2008)

- Precise flow rated and delivery volume

- Protocols with programmable manner over time

- Micropumping systems → Decreasing hearing damage
  - Extremely low volumes and flow rates (0.5 μl/stroke)
Recirculation component

- Delivery of constant concentrations of medications
- Extended period of time for years without the need to refill

Potential drawbacks

- Surgical implantation
- Little has been written
Emerging breakthroughs

- RNA interference
- Stem-Cell therapy
RNA interference

- Potentially groundbreaking method for changing the outcome of inner ear disease
- Messenger RNA (mRNA) inactivated by particles called small interfering RNAs (siRNA)
  - Cleavage of double-stranded RNA → 21 base pair duplexes with a two base pair overhang at the 3’ end.
  - Incorporated with a protein complex → active strand used to determine the sequence of the mRNA to inactivate → alter gene expression
RNA interference

- Difficulties
  - Delivering selectively to target tissues
  - Suited for dominant-negative forms of hearing loss by reducing aberrant mRNA available for translation
    - GJB2-related hearing loss caused by autosomal dominant nonsyndromic form of hearing loss in a mouse model (Maeda et al, 2005)
    - Reduce cisplatin-related hearing loss in a rat model (Mukherjea et al, 2008)
A cell can censor the expression of an individual gene inside it by interfering with the mRNA transcribed from the offending gene, thus preventing the RNA from being decoded by ribosomes into active protein. The censorship machinery is triggered by small, double-stranded RNA with ragged ends. An enzyme called Dicer chemically snips such short interfering RNAs (siRNAs) from longer double-stranded RNAs produced by self-copying genetic sequences (a) or viruses (b). Regulatory RNA sequences known as microRNA precursors (c) are also cleaved by Dicer into this short form. And scientists can use lipid molecules to insert artificial siRNA into cells (d).

HOW RNAi SUPPRESSES GENE EXPRESSION

Cell unwinds RNA strands

Single strand of siRNA or microRNA

RNA-induced silencing complex (RISC)

If the two RNA strands are highly complementary, the mRNA is cleaved

Cleaved mRNA

NO PROTEIN IS MADE

If the two strands are somewhat mismatched, the RISC sticks to mRNA

Ribosomes stall on the mRNA
Stem-cell therapy

- Neural stem cells from rat hippocampus survived with morphologic changes in the inner ears of neonatal rats (Ito et al., 2001)

- Challenges to clinical applications
  - Deliver the cells to organ of Corti in humans
  - Control of differentiation to desired end cell line
  - Develop surrounding support structure
  - Innervation within a given ear
Endogenous stem cells

- Stem cells isolated from cochlea of neonatal mouse (Oshima et al, 2007)
- Decreased stem cell number while aging in mammalian cochlea → uncertainty of potential utility
  - A loss of stem cells?
  - Repression of stem-cell activity by the maturing cochlea endorgan?
Challenge for hearing restoration

- Regenerate both hair cells and spiral ganglion
  - Achieve a functional neural synapse
- Embryonic stem-cell derived progenitor cells (both the mouse and human) into cochlea nerve of gerbil (Corrales et al. 2006)
  - In vivo neurite outgrowth to the organ of Corti
- Functional synapses between stem-cell-derived neurons and hair cells demonstrated (Wei et al., 2008)
Transdifferentiation

- One type of differentiated cell changing into another type of differentiated cell w/ or w/o mitosis (usually related developmentally, Batts and Raphael, 2007)

- Nonsensory cells within the mammalian inner ear → functional hair cells by transinfection of the transcription factor Atoh1 (Izumikawa et al, 2005)
Transdifferentiation

- **Obstacles**
  - Selectively target supporting cells within the organ of Corti → avoid generating ectopic hair cells
  - Generation of hybrid cells with dual differentiation
  - Lack of closely related cells (Complete replacement of the organ of Corti with simple epithelial cells in longstanding severe hearing loss)
Conclusions

- Two most widely used systemic therapy
  - Intratympanic gentamicin for Meniere’s disease
  - Intratympanic steroids for idiopathic SSNHL
- Limitations of middle ear applications:
  - Anatomic obstruction of round window membrane
  - Loss of medication through eustachian tube
  - Unclear pharmacokinetics
Conclusions

- Direct delivery to inner ear
  - Cochlear implant → closest to the clinical applications
  - Osmotic pump
  - Reciprocating perfusion system → broadest clinical applicability
- Novel systems: promising and very likely to change treatment of human inner ear disease
  - RNAi
  - Stem-cell therapy


21. Wei D, Levic S, Nie L, et al. Cells of adult brain germinal zone have properties akin to hair cells and can be used to replace inner ear sensory cells after damage. Proc Natl Acad Sci U S A. 2008 Dec 30; 105(52): 21000-5

Thank You for your attention!