DOCTORAL (Ph.D.) THESIS

Diagnostics and rehabilitation of hearing loss with inner ear origin in animal models and humans

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2016

I. Introduction

Structure of the inner ear

The inner ear consists of the bony and membraneous labyrinth as well as the peripheral processes and ganglia of the vestibulocochlear nerve. The bony labyrinth is a complex cavity embedded in the petrosal part of the temporal bone. The fragile membranous labyrinth is stretched inside this bony cavity. The peripheral end structures of the 8th nerve are located in the wall of the membraneous labyrinth.

The vestibulum is the central structure of the bony labyrinth, from which the 3 semicircular canals and cochlea originate. In humans, the cochlea consists of 2 and 3/4 turns. The base of the cochlea starts from the vestibulum, the apical part is called the cochlear apex or the cupula. In its axis, in the modiolus, the vestibulocochlear nerve runs. On a cross section there are 3 compartments to be distinguished: the scala vestibuli and tympani, and the scala media or cochlear duct, which is actually the membranous cochlea. The organ of Corti is located in the cochlear duct and contains the receptor cells of the hearing system, the so called hair cells. The cochlear nerve starts with the peripheral processes of the spiral ganglion neurons, which are in synaptic contact with the hair cells.

Malformations of the inner ear

Development of the bony cochlea terminates on the 8th gestation week. Malformation of the bony structures occurs if the organogesis is blocked before this. The hearing rehabilitation is determinated by the severity of the deformity. For the diagnosis of different malformations, high resolution computed tomography of the temporal bone as well as inner ear MRI is required to perform.

Physiology of hearing

Humans can perceive sound in a range of 16 and 20000 Hertz. The signal is conducted and amplified by the outer and middle ear and finally reaches the inner ear. Here, as a function of the hair cells, the mechanical stimulus is translated to an electrical signal, which runs across the hearing pathway.

Hearing loss

Types of hearing loss

Based on the hearing measurements, hearing loss can be conductive, sensorineural and mixed. In the conductive type, damage of the conducting system can be located anywhere from the pinna until the stimulus reaches the hair cells. In the sensorineural form, either lesion of the inner ear (cochlear) or the hearing pathway (retrocochlear) can occur.

Etiology of sensorineural hearing loss

Sensorineural hearing loss can be present even at birth or may develop during later stages of life. The cause of congenital sensorineural hearing loss remains unclear in 57 %, with 18% of acquired origin and 25% with genetic background. Usually, functional failure of the hair cells can be found in these patients, but in 20% of the cases, bony malformation of the cochlea can be detected as well.

Hearing loss due to environmental factors can develop in any stage of life. Among these factors, usage of drugs with ototoxic side effects like aminoglycoside antibiotics (kanamycin, gentamicin) or diuretics (furosemide, ethacrynic acid) is very common.

Degree of hearing loss

Hearing loss can also be classified according to its severity. Mild, moderate, severe and profound hearing loss as well as complete deafness can be distinguished. The type of rehabilitation is determined by the degree of hearing loss.

Diagnostics of hearing loss, hearing measurements

Subjective measurements

Pure tone and speech audiometries are the most common subjective hearing measurements. For proper result, active attention of the person is required. No measurement for children or non-cooperating subjects can be achieved.

Objective measurements

No active attention of the patient is needed in objective measurements. Brainstem evoked response audiometry (BERA) or electrocochleography (ECoG) are mainly used for children or in animal studies. With these methods, objective hearing threshold or place of damage along the pathway can be defined.

Rehabilitation of hearing loss

The way of hearing rehabilitation is determinated by the type or degree of the hearing loss. Hearing aid is the most frequently used method in sensorineural hearing loss, amplifying the signal to replace the non-functional outer hair cells. Furthermore, implantable hearing devices are also available on the market. In our human studies, results with bone conducting hearing aid as well as cochlear implantation have been analyzed.

Bone conducting hearing aid

Bone conducting hearing aids and bone anchored hearing aids (BAHA) have been widely used for decades in conductive or mixed hearing loss. New generation of these devices are already transcutaneous systems (BAHA Attract, Bonebridge). These are free from any wound healing problems, which were regarded as the most common disadvantage of the former percutaneous devices. Newly, these types of implants are also used in single sided deafness.

Cochlear implantation

Cochlear implantation is an ideal method for individuals with severe or profound hearing loss, where rehabilitation with hearing aid does not lead to acceptable hearing. The outer part of the implant contains the microphone and the sound processor. The electrode, as end of the inner part, is placed inside the cochlea. The spiral ganglion neurons are electrically stimulated directly by the device without any need of functioning hair cells.

In special cases with partially preserved hearing only in the lower frequency range, Electric Acoustic Stimulation (EAS) is an optimal choice. The electrode of this device is shorter. The basal part of the cochlea is stimulated electrically whereas the apical part, sensitive for the low frequencies, is stimulated acoustically.

II. Aims of the thesis

The aim of the thesis was to improve the diagnostics of the hearing loss with cochlear origin and consequently to find and carry out the optimal rehabilitation method.

Our aims according to the animal experiments:

1. To develop a histological method and protocol, which serve a highly preserved morphology and maintain the immunogenicity of the tissue for immunohistochemical staining experiments.

2. To develop a deafening method with intratympanic application of kanamycin and furosemide, which leads to profound hearing loss in guinea pigs without any systemic side effect.

3. To perform cochlear implantation on deafened guinea pigs in order to study the changes of electrical responses over time.

Our aims in the human studies:

1. To perform cochlear implantation in patients with bony cochlear malformation and describe the pitfalls of method with analyzing the hearing results.

2. To describe the application of Bonebridge for patients suffering from bilateral conductive hearing loss or single-sided deafness.

3. To present our first clinical experience with Electric Acoustic Stimulation.

III. Experiments

Animal experiments

Methyl methacrylate embedding to study the morphology and immunohistochemistry of adult guinea pig and mouse cochleae

Materials and methods

Seven adult guinea pigs (BFA bunt, 8-34 weeks old) and 6 adult mice (NMRI, 8 weeks old) were used.

Cochleae of the animals were harvested, decalcified and embedded in Technovit 9100 New® embedding system applying a protocol developed by us. Seven µm sections were cut, stained with Epoxy Tissue Stain (ETS) and preservation of the morphology was studied. Other sections were deplastified and immunostained with antibodies widely used in inner ear research.

Results

The preservation of the morphology was shown on the midmodiolar cross-sections of the cochlea with ETS-staining. The Reissner’s membrane and the connection of the scala media to the lateral bony cochlear wall remained intact. On higher magnification images the highly preserved structure of the cells of the organ of Corti and spiral ganglion neurons in the Rosenthal’s canal were showed. The cell borders, nuclei, stereocilia of the hair cells could be distinguished. On immunostained slides, well known staining patters with Myosin7A, SOX2, NF200 and prestin antibodies could be reproduced. Species specific staining pattern of calretinin and peripherin was also demonstrated.

Intratympanic application of kanamycin and furosemide for animal model suitable for cochlear implantation

Materials and methods

Forty-five pigmented guinea pigs (BFA bunt) were used. Six experiment groups were created according to the concentration and exposure time of the locally applied drugs. In groups 1-3, 200 mg/ml kanamycin and 50 mg/ml furosemide were used with an exposure time of 2, 1.5, 1 hours, respectively. In group 4, half of the concentration of each drug was used for 2 hours. In group 5, Ringer’s solution for 2 hours was applied; no solution in group 6 was used. The different solutions were applied in the middle ear under microscope after opening the bulla.

Electrocochleography was performed, and the hearing loss was established with analysis of CAP responses. Hearing measurements were repeated 1, 3, 9, 14, 22 and 26 weeks after the application. Animals were then sacrificed, cochlea were embedded, cut, and stained with ETS. The survived hair cells as well as the density of the spiral ganglion cells were analyzed. In some slides, immunostaining with Myosin7A was performed to justify the survived hair cells.

Results

It was showed, that the method developed by us, results a stable, irreversible hearing loss for 26 weeks. 94% of the ears treated in group 1 showed deafness with complete hair cell loss in all the half-turns except the apex. Decrease of the spiral ganglion neuron density was also demonstrated with a significant difference already 5 weeks after the treatment. In partly deafened ears, the relationship between hearing loss, hair cell loss and spiral ganglion neuron density was shown.

Cochlear implantation on deafened guinea pig

Materials and methods

Seventeen adult guinea pigs (BFA bunt) were used. Cochlear implantation was performed in 3 normal hearing animals; the other ears were treated with deafening solution. After opening the bulla, the implantation was performed through a cochleostomy created directly below the round window. For hearing measurements, acoustic as well as electric stimuli were presented. At the end of the experiment, animals were sacrificed to analyze the morphological changes caused by the cochlear implantation.

Results

Cochlear implantation does not result any short-term hearing loss, but in some animals hearing loss in a low frequency range could be detected in long-term follow up. Deterioration of eCAP-threshold and amplitude with time could be found in deafened animals. No changes in degeneration of the spiral ganglion neurons due to the implantation could be detected.

Human studies

Cochlear implantation in patients with inner ear malformation

Materials and methods

Between 2009 and 2012, 7 ears on 6 patients with cochlear malformation were implanted. No hearing on BERA could be detected preoperatively on the selected side. In all cases, high-resolution temporal bone CT scans were requested as well as inner ear MRI was performed. Common cavity deformity was reported in 3 patients, cochlear hypoplasia and incomplete partition type I in 2 patients. Transmastoideal approach was carried out in all cases. The inner ear was opened through posterior tympanotomy in 2 cases; labyrinthotomy was performed in 2 patients. In 3 cases posterior together with anterior tympanotomy was necessary to perform. In 2 cases with incomplete partition type I, intraoperative gusher was detected. The devices were fitted 4-8 weeks after the operation. Hearing performance with sound-field pure tone audiometry was measured.

Results

The best audiological result could be found in a patient with incomplete partition type I with an average of 21.25 dB threshold on speech frequencies 4 years after the operation. The patient was implanted at the age of 3.5 years. The other patient with the same deformity showed the worst audiological performance with the average of 47.5 dB. She was operated at the age of 19. From the 3 cases with common cavity, the worst result was found in the patient operated at the oldest age. Average audiogram of the patients showed a slightly sliding characteristic with constant performance at speech frequency range.

Our first experience with Bonebridge bone conducting hearing device

Materials and methods

Between 2013 and 2014, 4 patients were implanted with Bonebridge, an active bone conduction device. Two patients presented with single-sided deafness, 2 patients had a conductive hearing loss after multiple ear operations in the past. Sound-field pure tone threshold and speech perception (SRT, WRS) measurements were performed preoperatively and 1 and 3 months after the procedure

Results

Improvement in pure tone and speech audiometry could be found in all of the patients postoperatively. Three months follow-up results were even better in some patients.

Our first experience with Electric Acoustic Stimulation

Materials and methods

Between 2012 and 2014, 2 patients were implanted with Electric Acoustic Stimulation. Both patients were suffering from bilateral sensorineural hearing loss with a threshold of the low frequencies ranged 25 and 60 dB. Sound-field pure tone threshold and speech perception (SRT, WRS) measurements were performed preoperatively and at the time of turning on the device.

Results

Residual hearing could be preserved at low frequencies in both cases. The threshold with EAS improved, functional gain in speech audiometry could also be measured.

IV. Discussion

High quality cochlear histological analysis is essential to comprehend cochlear physiology and pathophysiology. In the past, many attempts have been made to establish an optimized histological protocol that preserves both the structure and immunogenicity of the cochlea for morphological and immunohistochemical studies on the same specimen. Difficulties occur due to a very delicate cochlear anatomy. A fragile epithelium of the cochlear duct consists of cells of different sizes and shapes surrounded by fluid-filled spaces. The cochlea is embedded in the hardest bony capsule in vertebrates. Technovit 9100 New® is a resin embedding system that contains methyl methacrylate and catalysators, i.e., N, N-dimethylaniline and benzoyl peroxide. This combination allows the medium to polymerize at low temperatures, that is, between −8°C and −20°C. Our aim was to develop a protocol, which allows both morphological and immunohistochemical analysis. In our study, the maintained structures of the scala media were shown in both species. The preserved immunogenicity was demonstrated with antibodies widely used in the field of inner ear research. Based on our results, this method was found to be sufficient enough to observe the structures of the organ of Corti and spiral ganglion neurons also in non-physiological circumstances like in our locally applied deafening method.

In animal research, deafening is performed usually with ototoxic agents like aminoglycoside antibiotics of chemotherapy drugs. Hearing loss occurs as a side effect of the application of the drugs. Aminoglycosides were first tested in the 50s. It has been shown that outer hair cells are the most sensitive against the drug, and a higher concentration in needed to damage the inner hair cells. Spiral ganglion neurons also show sensitivity against kanamycin, but degeneration of these cells is thought to be secondary and occurs only after hair cells loss. Hearing impairment due to loop-diuretics has also been published. Ethacrynic acid or furosemide influences the endocochlear potential, but this effect is found reversible. Severe ototoxicity of combined application of aminoglycosides and loop-diuretics was first found in patients were given both drugs simultaneously. In animal experiments, subcutaneous administration of kanamycin with combination of intravenous furosemide is widely used for creating deafening models. Unfortunately not only the ototoxic but the nephrotoxic effect of the kanamycin is amplified by the furosemide, which leads to undesirable morbidity or mortality of the animals. We showed a safe and reliable deafening method resulting in almost complete hair cell loss. Applying locally 200 mg/ml kanamycin and 50 mg/ml furosemide for 2 hours, 94% of the ears were found to be deafened. This effect remained stable during our follow-up. Histologically severe hair cell loss was proved and the dynamics of the degeneration of the spiral ganglion neurons was demonstrated. Analyzing the condition of SGNs after manipulation of the inner ear is very important, since they are the target cells of cochlear implantation as well as other animal experiments.

Cochlear implantation is a routinely performed procedure nowadays, although it was regarded to be contraindicated in malformed cochleae for a long time. The study on the first implanted malformed cochlea was published in 1983. Since then, remarkable development in the field of medical diagnostics and technology has been witnessed. Due to these factors, cochlear implantation has become a standard and successful method in hearing rehabilitation in patient with inner ear deformities. Temporal bone and inner ear imaging plays also an important role in identifying the possible risks preoperatively. Gusher and the concomitant meningitis, the most frequent complications of the surgery, can occur due to the abnormal anatomical situation and possible widen connections between the inner ear structures and CSF spaces. In our patients, intraoperative gusher occurred in 28.5%, which appears to be lower than the values presented in publications. Our number of cases, however, is not comparable with that reported in the literature. In our cases, intraoperative management of the gusher was sufficient enough in both cases to prevent postoperative meningitis and no lumbal drainage was necessary to insert. Difficulties in performing the cochleostomy or finding the round window for implantation can occur because of the anomalies of the facial nerve. Abnormal, mainly anteromedial position of the nerve usually due the cochlear deformity should be detected preoperatively. No peripheral facial nerve palsy could be observed in our cases.

The audiological performance of implanted deformed cochlea is comparable with the results of deaf cochlea radiologically appearing normal. Although our audiological results cannot be analyzed statistically due to the limited number of patients, tendencies can be observed. The later audiological performance is seemed to be influenced more by the age at surgery than the severity of the deformity. Clear difference in the audiological performance could be observed in ears with the same deformity but different age at surgery. With temporal bone HRCT and inner ear MRI imaging, surgical approach and the best fitted electrode can be selected preoperatively.

In our clinical studies, we showed our first experience with Bonebridge. In the recent years, indication for this type of implant has been widened, leading to the development of rehabilitation of patients with single-sided deafness. These individuals have usually difficulties in speech understanding in noise and sound localization. Our audiological functional gain in patients with single-sided deafness implanted with Bonebridge was demonstrated.

The Electrical Acoustical Stimulation is also a special rehabilitation method. In this method, preservation of the residual hearing in low frequency range is critical. We reported no alteration of this remnant with an improved performance in terms of speech understanding especially in noise.

V. Summary

1. We developed a histological embedding protocol, which allows highly preserved morphology. The tissue remains suitable for immunohistochemical studies. Our results were shown both on guinea pig and mouse cochlea, the two most commonly used species in inner ear research.

2. We carried out a locally applied deafening method, which resulted in profound hearing loss without any systemic side effect. An almost complete hair cell loss with secondary degeneration of the spiral ganglion neurons was demonstrated.

3. As an additional experiment, cochlear implantation in guinea pigs was performed. We observed the long-term effects of the implantation on hearing, responses for electrical stimulation and its deterioration over time. Histologically, no effect of the implantation on spiral ganglion neurons was found.

4. We demonstrated that cochlear implantation on malformed cochlea is a reliable option. Significant hearing improvement could be observed. Audiological results were influenced more by the time of surgery rather by the type of malformation.

5. We performed transcutaneous bone conducting device implantation in single-sided deaf patients for the first time. Improved speech understanding on the long run was demonstrated.

6. We performed Electrical Acoustical Stimulation through the round window for the first time in Hungary. Preservation of residual hearing was proved to be possible with EAS, and hearing improvement in both cases was found.

VI. Publications related to the thesis

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VIII. Acknowledgement

I would like to thank to my tutor, Imre Gerlinger for his help and full support. Without him, it would not have been possible for me to fulfil the animal experiments abroad. Moreover, the human studies have been coordinated by him.

I express my thanks to the former directors of the Department, József Pytel and Miklós Bauer, who first showed me the beauty of the otology and audiology. I also thank all my colleagues in the Department of Otorhinolaryngology, especially to Adrienn Németh and László Lujber for their advices and support.

I am grateful to all the members of the research group Regenerative Medicine of the Tübingen Hearing Research Centre, to Hubert Löwenheim, Marcus Müller, Karina, Mohamed, Aurelie, Claudie, Mirko, who helped my work with their experiences and ideas.