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## AN OVERVIEW OF PHARMACOLOGY AND CLINICAL ASPECTS CONCERNING THE THERAPY OF COCHLEO-VESTIBULAR SYNDROMES BY INTRATYMPANIC DRUG DELIVERY

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### Abstract

*Intratympanic drug delivery refers to drug administration in the middle ear, the main advantage being direct diffusion of substances in the inner ear through the round window membrane with subsequent high intralabyrinthine drug concentration and very low systemic side effects. The article is a review of literature concerning the inner ear barrier systems, the physiology of inner ear fluids, intralabyrinthine pharmacokinetics and the commonest drugs applied in the middle ear for the treatment of cochleo-vestibular syndromes.*

**Keywords:** intratympanic drug delivery, hemato-labyrinthine barrier, intralabyrinthine pharmacokinetics, intratympanic corticosteroids, intratympanic gentamicine.

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Topical treatment is a unique opportunity for the therapy of inner ear diseases. Systemic drug administration is limited by the low cochlear blood flow and the blood-labyrinthine barrier, which is similar, from an anatomical and functional point of view, to the blood-brain barrier [1,2]. Very tight junctions between cells prevent substances from passing from the bloodstream into the labyrinth; therefore, many therapeutic molecules fail to reach target structures in the inner ear. Moreover, the cochlea, which is a closed space, will be influenced by small changes of fluid volume.

The blood-cochlear barrier (i.e. the blood-labyrinthine barrier) protects the structures of the cochlea in a similar way the blood-brain barrier does. From a pharmacological point of view, extracellular fluid spaces of the inner ear comprise four compartments: (1) systemic blood flow - including the vascular stria and fluids in the capillaries and blood vessels; (2) perilymph, a fluid similar to the CS fluid, which is an optimal environment for functioning neurosensory structures; (3) endolymph, rich in potassium, which provides electrochemical drive for sensory cells activity; and (4) extracellular fluid from the cochlear bone, which is a transitional region between systemic circulation and perilymph [3].

A small area of the inner ear, the vascular stria, can be reached directly from the systemic circulation. It is situated on the lateral wall of the cochlea, within the scala media, and is surrounded by cells connected by tight junctions which provide a part of the blood-cochlear barrier [4]. Drugs acting upon vascular stria can be delivered systemically, their most probable mechanism of action being a reduction of the endocochlear potential. Because the endocochlear potential provides the energy for the transduction of sound into a nervous impulse, its reduction leads to a decrease of hearing capability. The vascularisation of the rest of the cochlea is relatively sparse, except for the modiolus and the scala tympani. Endothelial cells lining the blood vessels are connected by means of tight junctions, without fenestrations, and provide another component of the blood-cochlear barrier [5].

Perilymph, the primary fluid of the inner ear, is in continuity with the CS fluid through the cochlear aqueduct in most of the mammals, although the transfer of liquid between the two compartments is quite limited [6]. Commonly, in humans, the aqueduct is not patent [7]. Yet, diffusional continuity is possible, principally, through spaces around and within the auditory nerve [8].

Perilymph is produced mainly in the vascular system of the cochlea. Vascular networks from the modiolus and the walls of scala tympani, with a possible minor CS fluid contribution, are suggested [9]. In humans, there are

about 70  $\mu\text{L}$  of perilymph, of which around 40  $\mu\text{L}$  reside in the scala tympani [10]. The perilymph composition resembles that of the CS fluid, due to a similar mechanism of production.

Endolymph, a highly specialised fluid that fills the scala media, bathes the apical surfaces of the sensory cells. It is secreted mainly in the vascular stria by its marginal cells on the endolymphatic side. The endolymph, about 8  $\mu\text{L}$  in humans, has a high concentration of potassium, low concentration of sodium and very low concentration of calcium [10]. Scala media is lined with cells that contain tight junctions. These allow for the scala media maintaining an unusual electrical endocochlear potential, around +100 mV. The endocochlear potential and the low potassium concentration of the scala media provide the electrochemical battery for the transduction of mechanical motion in the hair cells [3].

Cochlea is integrated anatomically within the petrous bone. This bone is poorly vascularized and does not remodel. Perilymph can gain access into the bone through *lacuna canaliculi* which are canals and holes in the bone, communicating freely with the scala tympani [8,11]. The bone is also in contact with the systemic circulation by means of fenestrated blood vessels. Up to now, the bone has not been characterized as a component of the blood-cochlear barrier [3].

There are multiple labyrinthine barrier systems. The first component is the capillary endothelium lining blood vessels in the cochlea. Endothelial cells are connected by *tight junctions* without fenestrations [4]. Drugs can enter the perilymph from the systemic circulation either transported by carrier systems or dissolved into the capillary endothelial cells [3].

Scala media is also lined with *tight junction* connected cells. Drugs entering the scala media from the bloodstream must get first into the marginal cells of the vascular stria or into the perilymph. Either way, drugs are transported through the cellular lining in order to enter the scala media. The electrical charge of the drugs will be an important factor influencing their entry into the scala media which has a positive charge large enough due to the endocochlear potential. Positively charged molecules are at a disadvantage.

As in the blood-brain barrier, there are other elements that constitute the barrier, too. The high protein concentration of the perilymph [12,13,14] results in binding the drugs, buffering their perilymph concentration. There may be other components of the blood-cochlear barrier such as enzymes and cellular carrier systems which can diminish the concentration of chemical substances entering the cochlea [3].

Chemical features of the drugs influence the crossing of the blood-labyrinthine barrier. As in the case of the blood-brain barrier, large molecular weight drugs and electrically charged molecules will find it difficult to cross

passively. The unusual positive potential of the scala media will oppose the drug crossing, those positively charged having to pass against a constraining electrical gradient [3].

Pharmacokinetic variables similar to those in the bloodstream govern drug distribution within the cochlear fluids. Thus, drugs administered into the perilymph can diffuse into the organ of Corti where the hair cells, nervous fibers and other specialised cells are situated. Perilymph is in continuity with the spiral ganglion and structures of the cochlea's modiolus. Access to these last ones is facilitated by the presence of many *lacuna canaliculi* at the surface of the bony spiral lamella [15]. These perforations measure between 0.2 and 23  $\mu\text{m}$  in diameter [11]. Also, perilymph can diffuse by continuity at the site of the spiral ligament and spiral limbus.

Stria vascularis and surface cells lining the scala media are not in contact with the perilymph. All these structures are lined with *tight junctions* connected cells [16].

The protein composition of the human perilymph is similar to that in the bloodstream, with lower protein concentration in the perilymph. The interaction between proteins and drugs is as important in the perilymph as it is in the bloodstream. High levels of albumin bind acidic drugs and acidic glycoproteins bind basic drugs. Protein-drug partition coefficients influence the concentration of free drugs within the perilymph. Protein bound drugs can be deposited in the perilymph, depending on their affinity [17].

Drug distribution within the inner ear depends on the entry site. During intratympanic administration, there are three possibilities: through or near the membrane of the round window (at the base of cochlea, facing the scala tympani), through or near the oval window (at the base of the cochlea, facing the scala vestibuli) or through the otic capsule. A lacunocanicular system within the otic capsule both in human and animal has been described although bone permeability for drugs and other substances is supposed to be very low [18]. The bony lacunocanicular system provides a ready communication with the perilymph, allowing substances which bathe the otic capsule in the middle ear to enter the perilymph.

In as many as one third of human subjects, the *fossula fenestrae cochleae* may be obstructed by bony septa, false membranes and fatty or conjunctive tissue plugs which can influence the entry of drugs from the middle ear into the inner ear [19].

Drug concentration in the cochlear fluids depends both on the absorption into the cochlea and on the elimination rate into the bloodstream through the blood-labyrinthine barrier or to the middle ear through the round window membrane. Substance concentration in the middle ear diminishes due to both absorption into the cochlea through the round window membrane and the bone of the otic capsule or to deglutitions. Eventually, a concentration

gradient across the round window membrane will result, facilitating the removal of the drug from the perilymph into the middle ear [20].

Anatomically, the cochlea is a 33 mm long coiled tube. The perilymphatic space of the scala tympani begins at the base of the cochlea near the round window membrane, then continues apically to the end of the scala tympani where it communicates through the helicotrema with the perilymph of the scala vestibuli and reaches the base near the oval window, where the footplate is situated. The relative long length compared to the section area of the cochlea, determines a long duration of drug transport by simple diffusion along the length of the perilymphatic space in order to accomplish a semnificative drug distribution within the entire cochlea (it would last hours or days). Pharmacokinetic studies suggest a diffusion from one scala to the other through cochlear tissues, the so called *interscalar* or *radial diffusion*, besides diffusion along the cochlea, called the *longitudinal diffusion* [21,22].

The magnitude of interscalar diffusion depends largely on the physico-chemical characteristics of the therapeutic substance. Highly liposoluble drugs, such as corticosteroids, present a larger interscalar diffusion than drugs which necessitate carrier systems [23].

Intratympanically administered drugs diffuse through the round window membrane into the scala tympani at the base of the cochlea. The round window, a true Achilles heel of the fluid compartments of the cochlea, is permeable to water and many drugs [24,25]. The fact that the drugs reaching the scala vestibuli in the wake of intratympanic delivery, will distribute into the cochlea by simple diffusion in a fluid space with very low turnover, is a pharmacokinetic challenge of this kind of therapy. Cochlear electrode and ion tracer measurements have revealed a negligible longitudinal flow in the scala tympani, apically directed, of about 1.6 nL/min [26]. The varied thickness of the round window membrane determines a varied absorbtion of drugs in the scala tympani [27].

The administration of drugs into the scala vestibuli does not provide ready access to the structures in the organ of Corti such as scala tympani administration does. Theoretically, drugs could reach auditory neurons through *canaliculae perforantes* of the bony spiral lamela on the scala vestibuli side. Drugs could also reach scala tympani through the modiolus and spiral ligament [8].

The metabolism and the clearance of intratympanically administered drugs are specific for each drug. The same barrier systems limit both the entry and the clearance of medicine. Uptake systems that remove drugs from perilymph into the bloodstream have been described. The chemical structure of drugs in the perilymph can be altered by enzymes similar to the esterases of the bloodstream. Drug removal can occur through the membrane of the round window, in the opposite way of the absorbtion, depending on the concentration gradient.

By the time perilymphatic concentration exceeds the intratympanic concentration, the concentration gradient reverses, favouring the removal of drugs from scala tympani into the middle ear, when fluid solutions are administered intratympanically.

The access of the drugs into the cochlea for inner ear disease therapy have variable efficiency during common methods of administration. Most often, drugs are administered systemically with the expectation they will reach structures of the middle ear in the active form and efficient concentration, without deleterious side effects. This is the case for systemically administered corticosteroids for the treatment of sudden onset neurosensorial hearing loss and autoimmune inner ear diseases. Their clinical use is limited by unwanted side effects due to high bloodstream concentrations that provide therapeutic concentrations in the cochlea [3,28].

Topical therapy of inner ear afflictions comprises: otoprotection, treatment of sudden onset neurosensorial hearing loss, treatment of vertigo related to Meniere disease and treatment of autoimmune inner ear diseases. Sensory cells of the cochlea need protection from noise and surgical trauma, ototoxic drugs like cisplatin and aminoglycoside antibiotics and from radiation of the head and neck. Intratympanically administered drugs are also used for mitigating the hearing loss due to sudden events of unknown etiology or secondary to immune reactions within the inner ear.

There is a wide range of strategies of topical treatment of inner ear diseases, each providing perfusion of the inner ear with intratympanically or intracochlear administered medicine. These include: 1) single or repeated intratympanical injections, with or without volume stabilisation, with or without visualisation of the round window membrane, 2) continuous or intermittent administration by means of partial or totally implantable pump systems, 3) biodegradable polymers, and 4) direct intracochlear administration at the time of the cochlear implant operation [29].

Corticosteroids and aminoglycoside antibiotics are the most frequent intratympanically administered drugs for the perfusion of the cochlea during therapy of diverse cochleo-vestibular syndromes. Promising outcomes were provided by experimental administration of antioxidants, antagonists of glutamate, calpain inhibitors, neurotrophic growth factors, interference RNA, gene therapies with viral vectors and cell therapies [30]. All expect further evidence-based proof.

### Corticosteroids

The precise mechanism of the action of the corticosteroids within the inner ear is scarcely known. Multiple pathways are suggested.

Corticosteroids bind to glucocorticoid receptors on the cell membrane surface, then are transferred into the

cell nucleus where transcription is initiated or modulated, influencing cell signalling pathways [31].

Fluid and ionic transfer within the inner ear is influenced by modulation of Na/K-ATPase activity [32].

Hydro-ionic homeostasis of the inner ear is influenced by means of mineralocorticoid receptor regulated genes induction [33,34] and control of aquaporin channels [35].

Dexamethasone has been proved to modulate ionic homeostasis by means of ENaC epithelial channels in a semicircular membranous channel in primary cultures. This results in the cationic transport in the vestibular labyrinth and osmotic coupled aqueous flow [36].

A decrease of free radical species production within the inner ear has been shown alongside with the protective effect against proinflammatory cytokines such as the  $\alpha$  tumor necrosis factor, after glucocorticoids administration [37].

Inner ear disease glucocorticoid therapy is based most frequently on the supposed immunosuppressive and antiinflammatory effects within the cochlea. Thus, a nuclear transcription factor regulates the synthesis of several proinflammatory cytokines involved in the glucocorticoids inhibited general immune response.

Systemic inoculation of lipopolisaccharides in mice induces the expression of transcription factor mRNA in the mouse cochlea. The process has been suggested to be inhibited by glucocorticoids leading to a reduction of the nuclear factor in different compartments of the inner ear and recovery of hearing loss [38].

Topical administration of corticoids improves the cochlear blood flow [39,40].

Among multiple factors influencing corticoids access to the inner ear structures, the administration modality (systemic or intratympanic) seems to be the most important.

An animal study has compared the concentrations of hydrocortisone, methylprednisolone and dexamethasone in the perilymph, blood and CS fluid after oral, intravenous and intratympanic administration in mice [28]. Perilymph hydrocortisone concentration was higher than that of methylprednisolone and dexamethasone after intratympanic instillation. Plasma concentrations of methylprednisolone and dexamethasone exceeded those in perilymph after oral and intravenous administration. The study missed to compare blood and perilymph concentrations after intratympanic administration.

There are many deleterious side effects of systemic corticotherapy. They include: hydro-ionic disturbances, hyposaline retention, heart failure in susceptible patients, hypotatemia, hypertension, muscular atrophy, osteoporosis, vertebral fractures, aseptic necrosis of humeral or femoral heads. Gastrointestinal side effects include: peptic ulcer, bowel perforations, pancreatitis and reflux esophagitis. Dermatologic side effects include alteration of the healing processes, thin and brittle skin, bruises and hyperhidrosis. Neurologic side effects include

convulsions, intracranial hypertension with papilledema, headaches, psychosis. Endocrine problems associated with corticosteroids are menstrual irregularities; development of cushingoid state; suppression of growth in children; secondary adrenocortical and pituitary unresponsiveness, particularly in times of stress; decreased carbohydrate tolerance; latent diabetes mellitus; increased requirements for insulin or oral hypoglycemic agents in patients with diabetes; and hirsutism. Possible ophthalmic complications are posterior cataracts, increased intraocular pressure, glaucoma, and exophthalmos. Myocardial rupture after recent myocardial infarction has been reported with corticosteroid use. Other adverse effects include thromboembolism, weight gain, increased appetite, nausea, and malaise.

The wide range of adverse effects favors the intratympanic corticosteroids administration as a direct pathway to obtain high therapeutic intracochlear concentrations, devoid of systemic side effects [41].

The indications for intratympanic corticosteroid therapy include sudden sensorineural hearing loss, Meniere disease resistant to salt restriction and medical treatment with diuretics and vasodilators, tinnitus and autoimmune inner ear disease.

Outcomes of treatment are heterogeneous. Nowadays, there is a strong recommendation for treatment only for salvage therapy in the case of sudden neurosensory hearing loss after systemic corticosteroid failure [42,43].

The treatment strategies, doses and the volume of drug injected intratympanically have been empirically devised. Intratympanic injections are recommended sometimes daily for five days or weekly for up to four consecutive weeks. The drug solutions are variable, providing a minimal and short-lived exposure of inner ear to corticosteroids [44].

Modern polymer compounds, like dexamethasone based poloxamer OTO-104, seem to have several advantages such as: prolonged exposure of inner ear after single injection, low injection volume, avoidance of a second myringotomy for aeration, few postinjections sequelae due to filling of the middle ear with solution, lack of restriction of the orthostatism, speech and deglutition after injection [45].

### **Aminoglycosides**

Shortly after their discovery, aminoglycoside antibiotics proved their ototoxicity. Although considered an adverse effect of anti-infectious therapy, ototoxicity has been proved to be useful for the therapy of vertigo related to Meniere disease.

Surgery (endolymphatic sac decompression, vestibular neurectomy, labyrinthectomy) and intratympanic therapy are therapeutic alternatives when salt restriction, diuretics and vasodilators fail to decrease the number of vertigo attacks in Meniere disease. Intratympanic therapy

includes corticosteroids, when the auditory function is preserved, and pharmacologic destruction of the labyrinth with aminoglycoside antibiotics when there is no residual serviceable hearing.

Chemical labyrinthectomy with intramuscular streptomycin injections was achieved for the first time in 1948, by Fowler. In 1956, Schuknecht delivered streptomycin injections into the middle ear achieving vertigo control in 63% of the treated patients, with subsequent profound neurosensory hearing loss in all cases. In 1977, Lange delivered gentamicin intratympanically with hearing preservation in 76% of treated patients. Gentamicin becomes the preferred therapeutic compound due to its higher vestibulotoxicity as compared to its cochleotoxicity [46,47].

From the middle ear, gentamicin enters the perilymph of scala tympani by uptake through the round window membrane rather than by diffusion. This is suggested by morphologic features of the membrane. Thus, the outer layer of this trilaminar membrane comprises cubic cells tightly connected by means of *tight junctions*, with numerous mitochondria, endoplasmic reticulum and Golgi apparatus which suggest intense endocytosis [48]. The molecular weight and the positive electrical charge of the gentamicin molecule favors its entry into the scala tympani perilymph from where it reaches the perilymph in the vestibular scala by means of interscalar carrier systems [49]. The positive electrical charge prevents gentamicin from entering passively into the scala media due to scala media's positive endocochlear potential. The aminoglycoside antibiotic seems to reach the cells in the organ of Corti through the cochlear blood flow [50].

Aminoglycosides inflict degenerative injuries in the hair cells of the inner ear. In the organ of Corti, injuries are initially produced in the basal spiral. Outer hair cells are affected predominantly. In the vestibular system type 1 hair cells are more vulnerable than type 2 hair cells and, from an anatomical perspective, hair cells of the ampular crest are affected in the first place, followed by cells of the utricle and saccule [51]. The mechanisms of action by which aminoglycosides inflict cell injuries include thermic shock protein 27 expression, increase of production of reactive oxygen species and nitric oxide [52,53].

Nowadays, *titration* protocols of administration are recommended by most of the clinical studies whenever there is indication for suppression of the vestibular function by intratympanic gentamicin administration for control of vertigo in Meniere disease. Intratympanic gentamicin is administered once a week until either the first symptoms of vestibular hypofunction appear and control of vertigo is achieved or the audition deteriorates. Among these protocols, long term control of vertigo is similar to that achieved by treatment with daily doses or multiple daily doses [54,55].

Titration gentamicin therapy controls vertigo in

87% (between 75 and 100% among different studies) of patients with unilateral Meniere disease, the risk of secondary hearing loss being around 21% (between 0 and 37% among different studies) [54]. Yet, in one third of the patients, the vertigo can recur. Recurrences can also benefit from intratympanic gentamicin with the same risk of secondary hearing loss. The main effect of intratympanic gentamicin would be a reduction of vestibular function due to hair cells injury. Apparently, vertigo control can be achieved by subtotal labyrinthine ablation [47].

## Conclusions

1. Optimisation of strategies for therapeutic inner ear perfusion by intratympanic drug delivery for the therapy of cochleo-vestibular symptoms requires a thorough understanding of the intratympanic administration protocols, knowledge of physico-chemical characteristics of intratympanic administered solutions and of round window normal and pathological anatomy, as well as labyrinthine pharmacokinetics advanced studies.

2. Intratympanic corticosteroid therapy is strongly recommended only as salvage therapy in the case of sudden neurosensory hearing loss after systemic corticosteroid failure. Doses vary among studies.

3. Less frequent dosing regimen of intratympanic gentamicin therapy for control of vertigo in Meniere disease results in better hearing preservation. Titration protocols of administration are recommended by most of the clinical studies.

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