

PHARMACOTHERAPY OF THE INNER EAR

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Introduction

The auditory system seems better equipped to deal with injuries in lower species than in mammals. In fish and amphibians¹, the inner ear will produce new sensory cells (hair cells) throughout their life and, consequently, injured cells can be replaced continuously. Birds lose this ability during embryonic development, but instead possess the capacity to replace the injured sensory cells by regeneration and thus maintain hearing function. In contrast, mammalian hair cell loss has always been considered irreversible.

The mechanism of cell death in the cochlea is produced in two ways; through *necrotic cell death* mediated by very loud noise, or *apoptosis*, mediated by the activation of cysteine protease family within the cells, the caspases (very loud noise can also induce immediate apoptosis[1]). Originally these mechanisms, necrosis vs apoptosis, were thought to operate with different initiators (as extrinsic cellular pathway and intrinsic cellular pathway, respectively), but assumingly these mechanisms are more or less under statistical control in that dependent on stimulus the wealth of cell death and damages are operated with one of these two major mechanisms. Each of these mechanisms provides the possibility to reduce and, in some cases, to prevent cochlear cell death through active intervention with pharmacotherapy.

Recently, many researchers have investigated the role of antioxidant agents in different models of peripheral hearing disorders. It has been found that antioxidants protect the cochlea from noise-induced trauma, as well as cisplatin and aminoglycoside exposure [2-4]. Van De Water et al. recently suggested that protection of auditory sensory cells from cisplatin is carried out at the molecular level by three mechanisms: prevention of ROS formation; neutralisation of toxic products, and blockage of apoptotic pathways [5].

Several genes regulate the differentiation of cochlear hair cells and supporting cells from their common precursor cells during mammalian embryogenesis. Recent experiments have provided new and exciting information about the processes related to inner ear damage. For example, in the mammalian vestibular system, hair cell regeneration has been shown to occur under certain circumstances [6]. The situation in the auditory system is less clear. There is evidence of hair cell regeneration in newborn mice given explants of cochlear duct [7] and in replacing the damaged hair cells by converting the supporting cells [8]. A key gene is *Atoh1* (also known as *Math 1*). This is the mouse homologue of the *Drosophila* gene *atonal*, that encodes a basic helix-loop-helix transcription factor [9]. Overexpression of *Atoh 1* in nonsensory cells of the normal cochlea generates new hair cells, both *in vitro* and *in vivo*. *Atoh 1* has been shown to act as a "pro-hair cell gene" and is required for the differentiation of hair cells from multipotent progenitors. Recently Izumikawa et al (2005) demonstrated that in mammals by using gene therapy the lost hair cells will regenerate and that hearing may be returned to the profoundly deaf mammalian ear [10]. This finding opens new perspectives for the treatment of hearing loss and justifies the efforts to encapsulate nucleotides encoding the *Math 1* gene within the nanostructures for the treatment of deafness.

In addition, a moderate degree of spontaneous recovery of hearing after noise trauma has been observed in humans, implying that humans may also have the capacity to regain hearing function [11]. However, the mechanisms behind the recovery have not yet been fully delineated. There is, however, substantial evidence that cochlear damage induced by noise can be prevented by the application of different pharmacologically active substances [12]. Thus, there are grounds to expect that hearing disorders in mammals may, under certain circumstances, be successfully treated.

Drugs can reach the inner ear by systemic application (orally, intravenously or via the cerebrospinal fluid (CSF)) or locally (from the middle ear over the round window membrane (RWM) through permeation, direct injection through the RWM or the oval window and also with an osmotic pump by passing through the lateral wall of the cochlea). However, not all of these approaches are clinically possible.

Mechanisms of Noise-induced hearing loss.

Normal auditory stimulation elicits pressure differences across the cochlear partition causing a number of mechanical events within the organ of Corti: vibrations, shearing motion, deflection of the stereocilia [13]. The end-result is excitation of the outer and inner hair cells and, following release of transmitter substances, increased activity in the cochlear nerve. The outer hair cells are activated and react in a linear manner to sinusoidal sound stimulation with one impulse to one sinusoid up to 1000 Hz. At higher frequencies other mechanisms are involved in coding the amplification of the signal. These are not known in detail.

Assisting the tight coupling between the tectorial membrane and the basilar membrane, the tips of the stereocilia of the outer hair cells are buried within the tegmentum. The contractions of the outer hair cell-bodies amplify the basilar membrane vibration and transduce the vibration to shear forces that will activate the inner hair cells. The perceived and actively enhanced basilar membrane vibration is transmitted into the central auditory system and is perceived as sound. The role of the supporting cells is not clear yet but they may serve as a supporting organ to provide stability and damping of excessive vibration. Damage to the cochlea may also lead to hyperacusis and we hypothesize that this symptom may be linked to supporting cell damage.

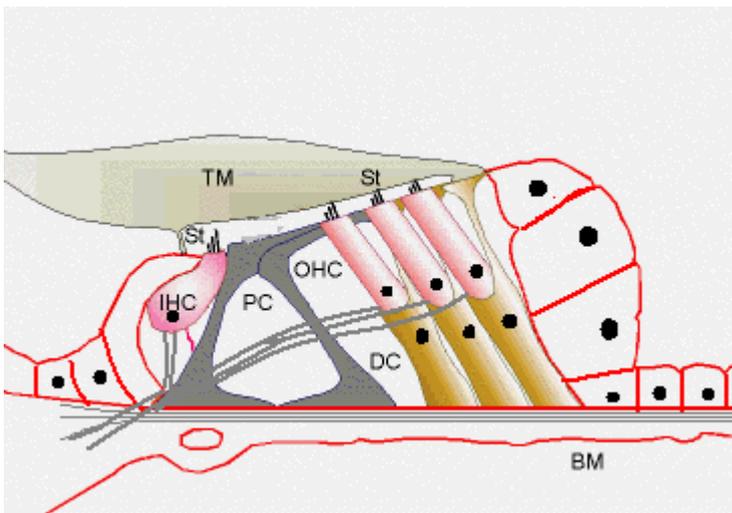


Fig. 1. Schematic drawing of organ of Corti. TM, tectorial membrane; ST, stereocilia; IHC, inner hair cells; PC, pillar cell; OHC, outer hair cells; DC, dieters cells; BM, basilar membrane.

Obviously, noise or excessive auditory stimulation will elicit shear forces in the cochlea but at much larger amplitudes. There are two fundamentally different ways that overstimulation may lead to cochlear injury: mechanical or metabolic [14]. Intense noise exceeding 125 dB SPL in animal experiments leads to large amplitude vibration that may mechanically alter or disrupt cochlear structures causing mechanical damage to cell membranes, nerve endings and disturb the blood circulation. Cellular distortion, disorganisation of the stereocilia and possible rupture of cell membranes disable the cochlear fluid barriers and

will cause immediate reduction of auditory sensitivity [15].

At sound pressure levels of less than 125 dB, sound-induced overstimulation and overactivity of the cochlea can result in disturbed cochlear homeostasis and subsequent functional impairment in the absence of direct and immediate mechanical damage. Experimental evidence suggests a critical level about 125 dB SPL, at which the cause of damage changes from predominantly metabolic to mechanical [16]. Thus, at moderate sound pressure levels damage would mainly be caused by metabolic mechanisms while at higher levels mechanical mechanisms would predominate. As changes in homeostasis may also occur in mechanical trauma and the effects of metabolic stress are also likely to be expressed as mechanical damage, it is not meaningful to make a strict separation between metabolic and mechanical causes of noise-induced hearing loss.

When the metabolic and/or mechanical stress is too large, the cells will die and a permanent hearing loss results. Cell death is either a result of apoptosis or necrosis. Apoptosis is a strictly controlled process to eliminate dysfunctional cells without affecting the surrounding tissue. It can be viewed as a counterbalance to cell division, and a disturbance may, for example, result in degenerative disorders or tumour growth. Necrosis on the other hand, is a more passive type of cell death, involving a rapid and disorganised breakdown of a cell, often as a consequence of acute trauma (toxic substances, ischaemia etc.). As the cell contents are released directly into the surrounding tissue, an inflammatory reaction usually follows. Thus, for the organism, apoptosis is the preferred method when it is necessary to eliminate cells. In the auditory system there is no conclusive evidence that apoptosis does play a significant role. Structural observations of DNA fragmentation may suggest the involvement of either apoptotic or necrotic mechanisms during peri-and post-

natal development of the inner ear [17]. A recent study on autopsy materials from subjects with no history of acoustic trauma suggests that apoptosis does not contribute significantly to the regulation of the cell population in the normal adult inner ear [18]. Nevertheless, apoptosis may be involved during noise-induced trauma, although there is to date no direct evidence in humans.

Changes in cochlear blood flow have generally been suggested as contributing to noise-induced hearing loss [19]. Recent findings have clearly demonstrated noise-induced alterations in the cochlear microcirculation causing local ischaemia [20]. The effect varies with the intensity and duration of the exposure, but when vascular insufficiency is manifest, the reduced oxygen and energy supply to the cochlea and the accumulation of metabolites will be accompanied by severe functional alterations. It has been shown experimentally that applying drugs blocking vasoconstriction prevents a noise-induced microcirculatory disorder, and maintains normal hearing [21]. However, the exact role of local blood flow alterations is unclear and it should be noted that it has been observed that hearing loss and cochlear hypoxia may actually precede changes in cochlear blood flow [22].

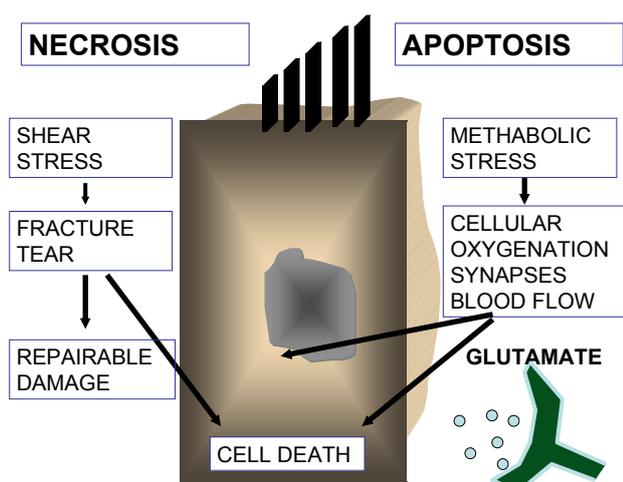


Fig. 2. Schematic drawing representing the necrotic and apoptotic cell death mechanisms, as excitotoxicity caused by glutamate.

There are several mechanisms leading to cellular damage after acoustical overstimulation (Fig. 2. above). The damages can be repaired or they can be irreversible leading to cell

death. Some of the mechanisms are mainly related to metabolic changes, e.g. oxidative stress, synaptic hyperactivity and altered cochlear blood flow, while others are predominantly mechanical. It is likely, however, that the resulting damage to the auditory system is partly mediated by similar mechanisms irrespective of the cause. Although definite evidence of a common final pathway' is missing, experimental data suggest that free radicals and other highly reactive endogenous substances play a significant role in noise-induced hearing loss.

The mechanisms causing cell death through necrosis are fundamentally different from those in apoptosis. Table 1 summarizes the differences. The apoptotic mechanism is in the developmental stages and in some disease stages, such as in cancer or granulomatous infection (for example in tuberculosis), a normal and vital part of life. With these mechanisms the body shelters from infection and eliminates small tumours and controls the growth of larger tumours.

Table 1. A comparison between apoptosis and necrosis

Apoptosis	Necrosis
1. may happen under both physiological and pathological conditions	1. only happens under pathological conditions
2. a gene-directed process	2. not a gene-directed process
3. an energy-dependent process	3. not an energy-dependent process
4. protein synthesis is increased	4. protein synthesis is decreased
5. ATP content is normal	5. ATP content is decreased
6. single cell involved	6. several cells involved
7. a delayed degeneration process	7. an immediate degeneration process
8. cellular shrinkage	8. cellular oedema
9. organelles are intact	9. organelles are destroyed
10. chromatin condensation	10. chromatin destruction
11. late membrane damage	11. early membrane damage
12. does not cause inflammation	12. causes inflammation

Apoptotic mechanisms and free radicals

It is well known, from other biological systems, that reactive oxygen metabolites (ROM) are important mediators of cell injury. ROM are free radicals or other molecules, which have a chemical structure, making them extremely reactive. As they react very easily with cellular components such as lipids, proteins and DNA, they are potentially cytotoxic. ROM are produced continuously as part of normally occurring reactions, e.g. in the mitochondria. However, protection is offered by several endogenous antioxidants. These are either enzymes catalysing reactions to neutralise the ROM, or scavengers binding them. When there is an imbalance between the production of ROM and the endogenous protective mechanisms, the tissue is under oxidative stress. Increased ROM production can cause cell death, whereas overactive protective mechanisms may lead to tumour growth. In the auditory system there are several reports demonstrating both elevated levels of either ROMs or antioxidants following noise exposure [23], and reduced hearing loss by treatments increasing the antioxidant level [24].

The key element in apoptosis is the caspase-induced cell death pathway. Caspases consist of a family of cysteine proteases that are present in the cells in an inactive form. In short, when the cell is damaged, a lethal chain reaction occurs that is triggered by activation of Bax gene. In the reaction, apaf-1 interacts with cytochrome c that is located on the mitochondrial surface, the complex interacts with procaspase 9 (a complex called to apoptosome) that cleaves and results in the caspase 9 that finally activates the caspase 3 through cleavage of some other procaspases. The "killer" caspase 3 reacts with the mitochondrial membrane and causes membrane lysis by liberating the lysosome enzymes from the cell leading to degradation of DNA and the proteins and disintegration of the cell

(fig 3).

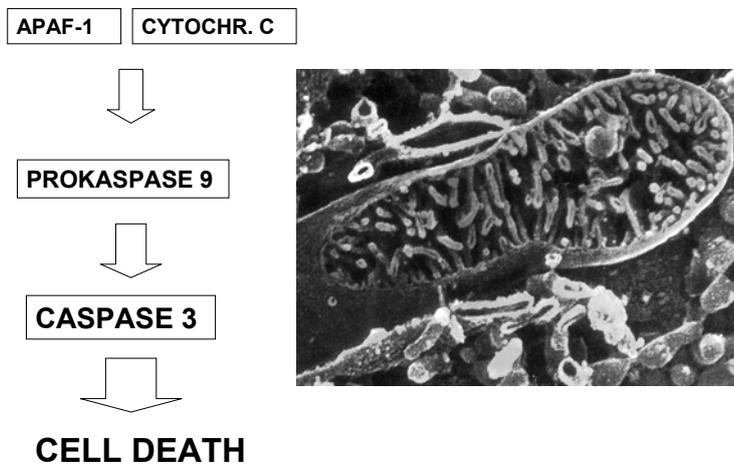


Figure 3. A diagram showing activation of caspase pathway in the final apoptotic pathway leading to disintegration of the cell. For details: see text

There are today 14 members of caspase family, but not all members of caspase family participate in the apoptosis, as caspase 1 and 11 functioning in the regulation of cytokines. The initiator includes caspase 9 and 8 and the effector includes caspase 3, 6 and 7. The initiation of the caspase reaction can be regulated by the external cell death receptor pathway through the Fas ligand – receptor activation or through the intrinsic cell death pathway (the MAPK/JNK pathway). Both these pathways trigger responses that lead to final stage activation of caspase -3 that acts as the executioner molecule for the cell. Nevertheless, caspase-3 appears to participate in the normal development and maturation of the membranous labyrinth and its cochleo-vestibular ganglion, so that a loss of function mutation of the gene for caspase-3 could result in maldevelopment of the inner ear and a hearing deficit.

Radical oxygen species and the caspase induced cell death pathway

Oxidative stress is a key factor in apoptosis with the creation of reactive oxygen species (ROS) and other free radicals (e.g., hydroxyl radical) which activate the apoptotic pathway through cellular mechanisms that are linked to caspase activation [5]. These ROS and

other radicals damage the affected cell's organelles and internal membranes resulting in mitochondrial membrane damage and a loss of the membrane potential. This loss of mitochondrial membrane integrity results in a release of cytochrome C from the damaged mitochondria into the cytoplasm. Once cytochrome C enters the cytoplasm, it combines with a facilitating molecule termed apoptotic protease-activating factor-1 (APAF-1), dATP (an energy-supplying molecule), and procaspase-9 to form the apoptosome (also known as the aposome), which cleaves procaspase-9 and generates activated caspase-9 [25]. A small mitochondrial "proapoptosis molecule" facilitates apoptosis of an affected cell by inhibiting some of the damaged cell's naturally occurring caspase inhibitory molecules (e.g., NIAP inhibitory protein in neurones). Once procaspase-9 has been activated, its downstream targets are effector caspases, e.g., caspase-3, -6, and -7 [26]. The naturally occurring cellular apoptosis inhibitory proteins (IAPs) are thought to target activated effector caspases such as caspases-3 and -7 for deactivation [27].

The activated effector caspases can interact with a large number of targets within an affected cell to bring about its destruction by apoptosis. Some of the cellular molecules targeted by the caspases are summarized by van de Water et al. [5] as: PARP-1 ; DNA within the nucleus and a DNA repair enzyme; nuclear lamin molecule A, B, and C; DNA fragmentation factor 45; inhibitor of caspase-activated DNase; receptor interacting protein (RIP); DNA topoisomerase; signal transducer and activator of transcription-1; Rb; X-linked inhibitor of apoptosis (XIAP); U1 small nucleoprotein; fodrin; vimentin; and procaspase-2, -6, and -10. Caspase-3 has been suggested as being the primary executioner in most cellular apoptosis during both normal developmental cell death and the removal of damaged cells after injury (i.e., apoptosis) [25] .

Hu et al., examined sound trauma-initiated apoptosis of cochlear outer hair cells in the chinchilla [28]. In a double labeled study the authors localized the activated caspase-3 to the cell bodies of damaged hair cells undergoing apoptosis. The results show a relationship between post-noise exposure progression of hair cell loss, apoptosis of damaged hair cells, and activation of caspase-3. The study also demonstrated that activation of caspase-3 persists for at least 2 days after the initial sound trauma exposure. There was a correlation between post-exposure loss of noise-damaged outer hair cells, apoptotic changes in the outer hair cell nuclei, and the presence of activated caspase-3, -8, and -9 in the cell bodies of damaged sensory cells. The finding also indicates that the treatment window for noise-induced apoptosis of cochlea lasts at least 2 days.

The MAPK/JNK induced cell death pathway

The extrinsic cell pathway involves the binding of cell death receptors that are members of tumour necrosis factors- α pathway. In this, there are two receptors in the cell that are both activated in the shear stress injury by TNF- α , the type I (p 55 receptor pathway) and type II (p 75 receptor pathway). As in most instances in up-regulation of cellular function, one leads to cell death and the other tries to rescue the cell. The TNF- α type I receptor pathway is the apoptotic pathway. TNF- α through its two types of receptors, activates two signaling pathways within cells [29]. One, linked to receptor type I leads to programmed cell death (apoptosis), whereas the other, linked to receptor II, counters the death signal and leads to survival. When both receptors are expressed, the type II receptor of the TNF- α may enhance the receptor I-mediated death pathway. The final consequence may depend on the level of type I expression. The survival pathway activates a transcription factor, NF- κ B, which works by turning on a set of anti-apoptotic genes.

NF-κ

hyperactivity and an excessive release of transmitter substance. The afferent neurotransmitter is most likely to be glutamate which, like other excitatory amino acids, has toxic effects when released in large amounts. The resulting overstimulation of the glutamate receptors elicits an inflow of calcium ions which, in combination with other ions, brings about the entry of water and subsequent swelling of the nerve endings. The result may be a total disruption of the synapses between the inner hair cells and the afferent nerve fibres in the cochlear nerve [32]. A dorsal root acid sensing ion channel (DRASIC) has been detected in the spiral ganglion cells and the organ of Corti including the nerve fibres innervating the organ of Corti [33]. It is known that opening of the acid sensing ion channel may flux Ca^{++} and induce cell death [34]. This mechanism may also be involved in noise induced hearing loss and ischaemia-induced hearing loss because both shear stress and ischaemia can result in a low pH extracellular homestasis.

In addition to the accumulation of ROM seen following metabolic and/or mechanical stress it has been demonstrated that acoustical overstimulation leads to a significant rise in intracellular calcium levels in the outer hair cells [35]. A sustained increase in the intracellular calcium concentration is known to result in severe cell injuries, such as cytoskeletal break-down, membrane defects and DNA damage [36]. One probable consequence of the increased calcium concentration in the outer hair cells is the loss of cell body stiffness observed after intense acoustical stimulation [37]. Moreover, a structural reorganisation of the organ of Corti has recently been demonstrated following acoustical overstimulation [15]. The noise-induced changes in cellular stiffness and structure of the hearing organ seem to be, at least partly, reversible and the results may thus contribute to knowledge of the mechanisms involved.

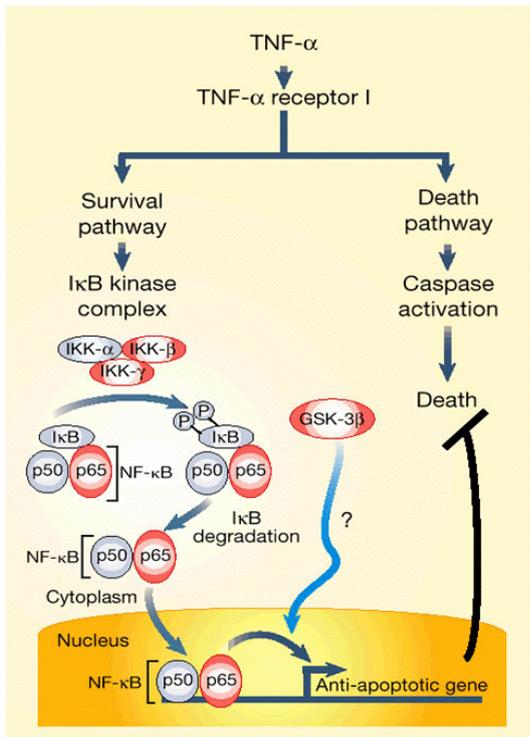


Figure 4. Life-and-death decision in the cells (see reference [38], with permission of Nature). Cellular stimulation with tumour-necrosis factor- α (TNF- α , top) simultaneously activates survival (left) and death (right) signalling pathways. The survival pathway leads to the activation of NF- κ B, which induces the expression of anti-apoptotic genes in the nucleus. NF- κ B (subunits p50 and p65) is normally held captive in the cytoplasm by the I κ B protein. Cellular stimulation with TNF- α leads to activation of the I κ B kinase (IKK) complex, which phosphorylates I κ B. The phosphate tag (circled 'P') singles out I κ B for destruction. NF- κ B is then free to move into the nucleus and activate its target genes. Hoeflich *et al.* have revealed an unexpected requirement for glycogen synthase kinase-3b (GSK-3b) in the NF- κ B-mediated activation of genes needed for survival [39]. It is not yet clear how GSK-3b works in this pathway, but it probably involves a critical step following the movement of NF- κ B to the nucleus. Targeted disruption in mice of any of the molecules coloured red leads to death of the embryo, accompanied by TNF- α -induced apoptosis of hepatocytes.

Necrosis induced by sound stimulation

Very loud sound leads to mechanical damage of the organ of corti with fractures of the cellular membrane, liberation of lysosome content and exposing the cell content to extracellular fluids. In necrosis there is abundance of proinflammatory cytokines, such as IL-1beta, IL-6, TNF- α and migration of inflammatory cells [40].

TNF- α is involved in cellular survival/damage mechanism especially through the TNF receptor I (p55), The survival pathway can induce activation of c-Jun NH2-terminal kinases (JNK) and NF κ B [41]. There is also another TNF- α mediated pathway that acts through receptor II (p75), the necrosis stimulating pathway. This pathway is also self feeding, as the receptor II pathway is activated it enhances production of TNF- α . The upregulation of receptor type II is leading to inflammation and cytotoxic effects.

Upregulation of growth factors in noise trauma

The role of upregulation of growth factors is not yet well known. One of the growth factors the NFG1 is expressed in the cochlea during traumatizing noise leading to PTS but not during non-traumatic noise leading to TTS [41]. One of the immediate responses by NFG1 gene production is activation of c-fos. Loud noise at a damaging level also upregulates genes producing GDNF in rats. The upregulation starts after 4 h, peaks after 12 h and levels out after 12 h from cessation of the noise exposure [42]. GDNF has protective effect on noise induced cellular damage but the exact mechanism has not yet been delineated. It has been hypothesized that GDNF is involved in the consolidation of recovery function from noise damage. It is also possible that GDNF has a function that is related to protection from additional noise-induced stress, rather than recovery from the first stress. GDNF upregulation may be related to the training effect, toughening or conditioning of the organ of Corti [42].

VEGF is also upregulated during shear stress that leads to traumatic changes [43]. The expression of VEGF is limited to the hair bundles and spiral ganglion cells after traumatic

vibration. VEGF receptor 1 is not detected in the vibrated cochlea, whereas VEGF receptor 2 expression is present in the lower part of the outer hair cells, Dieters' cells, Hensen's cells, Claudius cells, the basal membrane of the organ of Corti, the internal sulcus cells, nucleus of the spiral ganglion cells, the lateral wall of scala tympani and the spiral ligament (figure 6). No expression of VEGF receptor 2 was observed in the stria vascularis.

It is well accepted that shear movement exists in the organ of Corti, but there is no documentation of shear stress within the spiral ganglion cells. Shear forces within the bone matrix stimulate bone cells and mechanically transform them causing up-regulation of genes in the cells [44]. The spiral ganglion cells are surrounded by perilymph and bone matrix. The shear force produced by the transcranial vibration is conducted to the spiral ganglion cells and is able to cause shear stress. In our study VEGF and VEGF receptor 2 gene expression in the spiral ganglion cells supports this hypothesis.



Figure 5. Expression of VEGF in the hair bundles of the outer and inner hair cells after shear stress induced cochlear trauma in guinea pig. It is quite possible that this staining covers the tip links.

Vibration induces VEGF and VEGF R2 expression in the cochlea, but not VEGF R1. Our results confirm the biological significance of a previous *in vitro* study, which indicated that in vascular endothelial cells, high shear stress induced an increase in VEGF R2

expression. This up-regulation reached its maximum and was in a linear relationship to the stress strength within a range of 2 to 40 dyne/cm² [45]. The authors interpreted this as showing that an increase in the shear stress in the vasculature by post-ischaemic reperfusion stimulates VEGF R2 expression, resulting in an increase in vascular permeability and leading to neo-vascularization. After myocardial infarction the newly formed myofibroblasts express VEGF and VEGF R2 that seem to play a significant role in tissue repair/remodelling [46]. When the cochlea is exposed to mechanical vibration, a shear stress presented in the various cell types of the cochlea with concomitant increase of expression of VEGF and VEGF receptor 2. Thus, VEGF may contribute to tissue remodelling and angiogenesis at the site of damage in an autocrine manner and may be important in preventing further damage to the cochlea. The most enhanced expression was located in the spiral ganglion cells, stereocilia, supporting cells, the internal sulcus cells and epithelial cells of the lateral wall of the scala tympani. No obvious expression was found in the hair cells. This means that the hair cells are rather stable and not affected by the VEGF induced reaction, and seem not to be able to be remodelled/repared by VEGF when they are damaged. Conversely, the spiral ganglion may be repaired with the assistance of VEGF because both VEGF and VEGF R2 are expressed there.

Shear stress induced VEGF expression seems to be time-dependent. After acute shear stress within 6 hours of the exposure, it is not expressed [47]; whereas after longer shear stress, its expression is increased up to 14 days [48]. In the cochlea we observed expression of VEGF and VEGF R2 1-3 days after vibration. This is in accordance with time limit of previous reports. In pathological states, especially at the acute phase of brain ischaemia and myocardial infarction, VEGF was expressed and could induce oedema which is deleterious. This happened within 6 hours [49]. The naturally occurring

upregulation of VEGF at a later phase (6 h later) means that VEGF and receptor 2 responses are protective responses in the individual. There is no evidence to show whether VEGF and receptor 2 are expressed in the spiral ganglion within 6 h of cochlea shear stress. It is worth investigating this response to provide reference data for clinical treatment.

PHARMACOTHERAPY OF THE INNER EAR

Free radical scavengers

At least three important ROS are generated in the reduction of O_2 to H_2O : superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-). It has been demonstrated that ROS are involved in noise trauma [50-52], cisplatin ototoxicity [53-55], and aminoglycoside ototoxicity [56]. Direct evidence of ROS ototoxicity has been demonstrated using isolated outer hair cells and by intra-perilymphatic infusion [57]. ROS ototoxicity is believed to be mediated by deleterious effects at multiple sites including lipid peroxidation, DNA strand breaks, and alterations in carbohydrate and protein structures.

Increased knowledge of the processes leading to cellular injuries is of fundamental importance in order to develop clinical means for protection and repair. Many recent reports on the protection against noise-induced hearing loss offered by drugs such as antioxidants and neurotrophins are promising. Table 2 shows the antioxidants for which this has been experimentally demonstrated, and some are currently in use.

In addition to these agents there are several other compounds that have been tried and some may be useful, but there are insufficient data on their efficacy in preventing or healing cochlear injury.

There are several different pharmacologically active agents that have been tried or are in use to treat sudden acoustic trauma. In general, only few experiments have been prospective with relevant control material. The experiments carried out in military camps with use of Mg^{++} are effective and usable, but the limitation in their use is that Mg^{++} should be administered before exposure to inner ear trauma. The efficacy seems to be limited to preventative action by alleviating the accumulation of excessive Ca^{++} in the cochlea.

Table 2. Possible ROM scavengers

- (1) Glutathione, is a nucleophilic scavenger and an electron donor via the sulfhydryl group of its business residue, cysteine.
- (2) N-acetylcysteine (NAC), the acetylated variant of the amino acid L-cysteine, is an excellent source of sulfhydryl (SH) groups, and is converted in the body into metabolites capable of stimulating glutathione (GSH) synthesis, promoting detoxification, and acting directly as free radical scavengers.
- (3) Ascorbic acid and its sodium, potassium, and calcium salts are commonly used as antioxidant food additives. These compounds are water soluble and thus cannot protect fats from oxidation: for this purpose, the fat-soluble esters of ascorbic acid with long-chain fatty acids (ascorbyl palmitate or ascorbyl stearate) can be used as food antioxidants.
- (4) Salicylic acid, is able to absorb hydroxyl ions and thus impede a main step in the process of membrane lipid peroxidation.
- (5) Melatonin, once oxidized, cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant.
- (6) Tocopherols, are the most abundant and efficient scavengers of hydro peroxy radicals in biological membranes.
- (7) The iron chelator (desferrioxamine) forms a stable complex with ferric iron, decreasing its availability for the production of reactive oxygen species. Desferrioxamine is a powerful inhibitor of iron-dependent lipid peroxidation and hydroxyl radical formation.
- (8) Mannitol, is a free radical scavenger of the hydroxyl radical to which the aldehyde moiety of mannitol reacts and binds. This forms a mannitol radical that undergoes disproportionation or dimerises, and thus becomes less cytotoxic than the former hydroxyl radical, causing less damage to the cellular ultrastructure.

An iron chelator and free radical scavengers have been shown to attenuate cochlear damage caused by noise [58]. Also the antioxidant D-methionine has proved to be useful in preventing gentamicin-induced ototoxicity. [4] N-acetylcysteine (NAC) is metabolized to cysteine (among other molecules) and may provide cellular needs for glutathione (GSH) in the presence of ROS. Several recent articles demonstrate that L-NAC or related drugs could reduce noise-induced hearing loss [59]. ROS-induced damage may occur in vibration induced hearing loss, and ROS scavengers such as NAC may prevent vibration induced hearing loss. In animal model, NAC could not prevent vibration induced hearing

loss although different administration approaches have been tested {Zou, 2003 #153}. Figure 6 shows the results of NAC on vibration induced hearing loss. In fact, NAC appears to have synergistic neurotoxic effects in combination with glutamate, which may be the primary afferent neurotransmitter of the cochlea {Puka-Sundvall M, 1995 #234}{Nordang L, 2000 #235}.

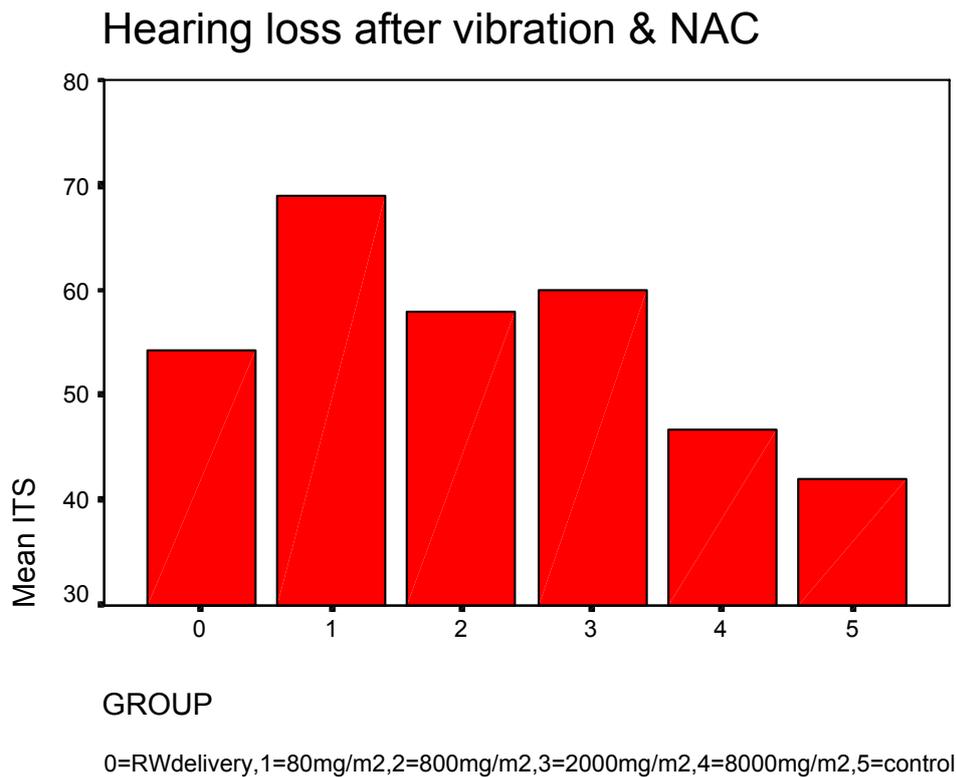


Figure 6. Mean hearing threshold change after exposure the guinea pigs on traumatizing vibration causing shear stress, which was administered with NAC before vibration.

Several compounds have been tried in the prevention or treatment of noise induced hearing loss in man (see table 3). Few of these experiments have control group or are randomized and prospective. So far, based on evidence in man, only Mg^{++} seems to be effective in prevention on noise induced hearing loss. The study of Attias was carried out in Israeli army forces and included controls, indicating that replacement of Ca^{++} ions in

body led to protection from noise damage [60].

Table 3. Clinical trial of drug treatment of acute acoustic trauma.

drugs	sample number	method	efficacy	authors
Vitamin A		Re	no	Ward&Glorig,1960
Dextran	72	Re,Co	yes	Martin&Jakobs,1977
	209	Re,Co	no	Eibach&Borger,1980
Dextran+Pentoxifylline	147	Pr,Ra	no	Probst et al.,1992
Bencyclan	50	Pr,Co	no	Eibach&Borger,1979
Xantinol nicotinate	85	Re,Co	no	Eibach&Borger,1980
ATP	85	Re,Co	no	Eibach&Borger,1980
Vitamin A,B,E	136	Re,Co	no	Eibach&Borger,1980
Methionine	85	Re,Co	no	Eibach&Borger,1980
Cinnarizine	85	Re,Co	no	Eibach&Borger,1980
Betahistine	57	Re,Co	no	Eibach&Borger,1980
	122	Pr,Ra	no	Pilgramm&Schumann,1985
Magnesium				
4g,drink	320	Pr,Ra	yes	Joachim et al.,1993
10mg/kg, infusion	80		no	Pilgram et al.,1993
167mg,drink	300	Pr,Co	yes	Attias et al.,1994
Diltiazem	100	Pr,Ra	no	Maurer et al.,1995

note: Pr, prospective study; Re, retrospective study; Ra, randomized study; Co, control group.

References: Ward&Glorig (1960)[61]; Martin&Jakobs (1977) [62]; Eibach&Borger (1980) [63]; Probst et al.(1992) [64]; Eibach&Borger (1979) [65]; Eibach&Borger (1980) [63]; Pilgramm&Schumann (1985) [66]; Joachim et al. (1993) [67]; Pilgram et al. (1993) [68]; Attias et al. (1994) [60]; Maurer et al. (1995) [69].

In animal studies, the control of noise dose and environmental factors can be minimized. There has been much research conducted in animals with several pharmacological compounds. In general all seem to work in animal experiments that have been tried for prevention, but their clinical value needs to be documented. For treatment of sudden deafness there are several substances suggested for use. These are listed in table 4.

Table 4. Animal studies of drug treatment of acute acoustic trauma.

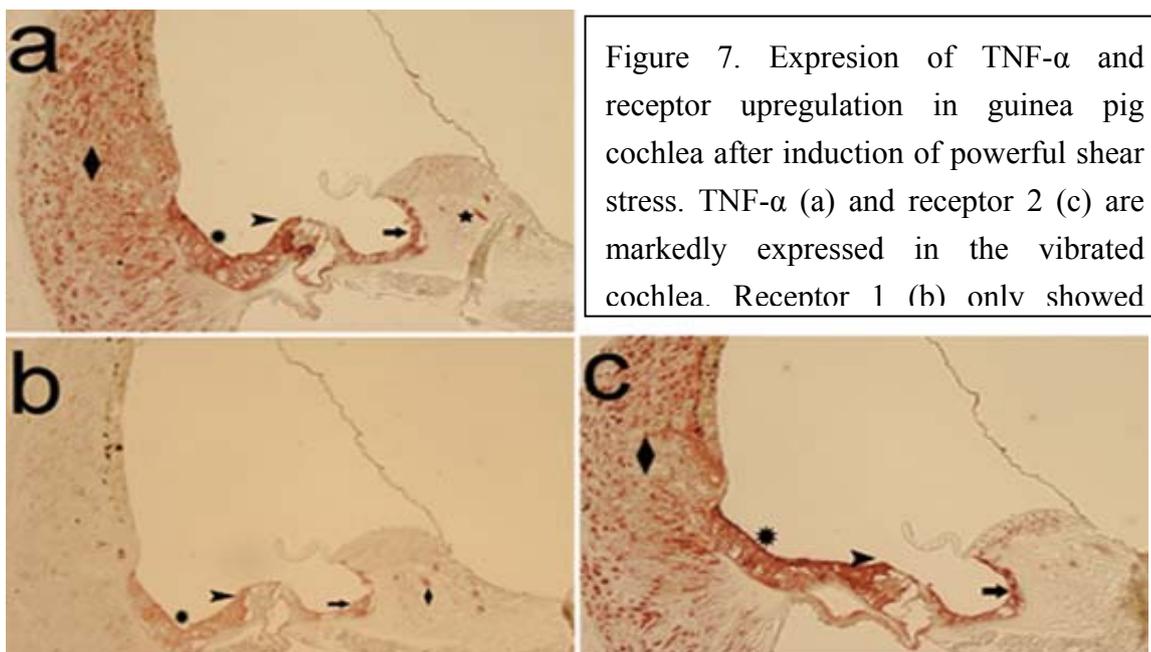
drugs	animal species	efficacy	parameters	authors
NAC	Guinea pig	++	ROS, ABR, Hair cell loss	Ohinata et al.,2003
NAC+acetyl- salicylic acid	Chinchilla	+++	ABR, cytochleo- gram	Kopke et al.,2000
HES70, ES200, Pentoxifylline, Gingo biloba, Betahistidiini	Guinea pig	HES+++, betahistid.+ others-	pO2, CAP, ABR, CM	Lamm & Arnold, 2000
Allopurinol	Guinea pig	+	ROS,ABR	Atanasio et al.,1999
Allopurinol, SOD-PEG	Rat	+	ABR, CAP	Seidman et al., 1993
Dipyridamol, Allpurinol	Guinea pig	+	CAP	Bergmann,1976
DFO, Mannitos, GDNF	Guinea pigs	++	Cytochleogram, ABR	Yamasoba et al., 1999

References: Ohinata et al. (2003) [70]; Kopke et al. (2000) [59]; Lamm & Arnold (2000) [21]; Atanasio et al. (1999) [71]; Seidman et al. (1993) [72]; Bergmann (1976) [73]; Yamasoba et al. (1999) [58].

TNF- α and its antagonists

After shear stress of the cochlea Zou et al demonstrated a weak TNF receptor 1 staining mainly in Hensen's cells, Claudius cells, the internal sulcus cells and the capillaries of the spiral ganglion (figure 7) [43]. Much stronger expression of TNF receptor 2 was found mainly in the spiral ganglion cells, Henson's cells, Claudius cells, the internal sulcus cells, Dieters' cells, the basal membrane of the organ of Corti, the spiral ligament, the spiral vascular prominence; with weaker staining in the lower part of the out hair cell [43]. No TNF receptor expression was detected in the normal cochlea.

Although TNF- α , TNF receptor 1 and receptor 2 were observed in the vibrated cochlea, the expression of TNF receptor 2 was more prominent in the cochlea. The combination of TNF- α with TNF receptor 2 is capable of activating Jun N-terminal kinases (JNK) and nuclear factor (NF)- κ B [41]. The activation of JNK and NF- κ B has the function of an anti-apoptotic agent [41]. On the other hand, the activation of TNF receptor 1 induces apoptosis [74]. When both receptors are expressed, the activation of TNF receptor 2 enhances the effects of receptor 1 activation. The final fate of the cells should be related to the expression ratio of both receptors. Shear stress inhibits TNF- α induced apoptosis by activating phosphatidylinositol 3 (PI3)-kinase and inhibiting Caspase-3 [75].



Recently anti-cytokine therapies have become a common treatment in diseases of autoimmune origin such as rheumatoid arthritis and Crohn's disease [76]. Treatment with monoclonal antibodies against TNF- α suppresses inflammation and improves patient well-being [76]. TNF- α is a proinflammatory cytokine released during infection or inflammation, which calls the immune system to action [38]. Anti-TNF- α antibody administration in vivo

results in the rapid down-regulation of a spectrum of cytokines, cytokine inhibitors, and acute-phase proteins [76].

Etanercept and infliximab are drugs that potently and selectively bind TNF- α in the cellular microenvironment, thereby preventing TNF- α from interacting with membrane-bound TNF receptors on target cells. Etanercept is a recombinant fusion protein of the soluble type II TNF receptor on a human IgG1 backbone, whereas infliximab is a chimeric anti-TNF- α monoclonal antibody containing a murine TNF- α binding region and human IgG1 backbone.

Both etanercept and infliximab are reported to have a positive effect on hearing loss or hearing fluctuation in Menière's disease and idiopathic sensorineural hearing loss [77]. In the animal model in which KLH was used to induce autoimmune hearing loss in guinea pigs, etanercept could effectively alleviate the hearing loss and cochlear damage in the animal model [78]. The findings were confirmed in later study in the same animal model [79]. However, a multicentre study on immunomediated cochleovestibular disorders by Matteson et al. could not demonstrate that etanercept was effective in alleviating vertigo and tinnitus or improving hearing in these patients [80]. Zou et al. studied the effect of infliximab on the prevention of hearing loss after shear stress induced cochlear trauma (unpublished data). In this trauma TNF- α and its receptor I and II are upregulated in the cochlea. Infliximab was administered through different approaches in the experiments; intravenously, intraperitoneally and transtympanically. None of the administration methods could prevent the animals from developing hearing loss. In a subsequent trial 4 patients with vertigo and bilateral severe sensorineural hearing loss were followed for 3 months and infliximab with azathioprine were administered intravenously according to protocol used

for treatment of severe rheumatoid arthritis. In none of the patients was hearing improved or preserved. Noteworthy was that one of the subjects responded to corticosteroids with an improvement of hearing of 50 dB, but did not show a similar responsiveness to infliximab.

Infliximab may cause severe adverse effects, the main being hypersensitivity reactions, development of antinuclear antibodies, possibly lymphoproliferative disorders and reactivation of latent tuberculosis. Also a case has been reported with severe neutropaenia and thrombocytopenia associated with infliximab [81]. Infliximab infusions are accompanied by acute reactions in approximately 5 % of infusions [82].

To summarize the findings of TNF- α , it seems well documented that in a damaged cochlea there is upregulation of TNF type I and II receptors but the efficacy of the blocking agents have not yet been demonstrated, so that neither etanercept nor infliximab can be recommended for treatment of hearing loss in man.

Neuroprotection; Calpain, Nitric oxide (NO) and glutamate receptors

The accumulation of free radicals severely damages the inner ear and other tissues. Through a complex chain of events, this damage can then cause a release and accumulation of glutamate and calpains. Nitric oxide (NO) plays a role in a great range of important functions in the organism, such as vasodilatation, relaxation of muscles, neurotransmission, neuromediation. Nitric oxide has been found to cause ototoxicity. Ruan et al. demonstrated that sodium nitroprusside, a NO donor, produced both outer hair cell

and inner hair cell damage when it was applied at the cochlear round window [83]. Nitric oxide synthase (NOS) has been shown to play an active role in the initiation of degeneration of the spiral ganglion cells of the rat cochlea [85]. It has been suggested that noise-induced hearing loss is partly due to excessive release of the excitatory amino acids such as glutamate and consequently exciting the post-synaptic receptors leading to swelling of the nerve endings [86]. It has also been suggested that the ototoxicity of noise trauma and aminoglycoside may result from the same excitatory process at the glutamate receptor [87]. Nitric oxide mediates the effects of excitatory amino acids in the central nervous system and may play a similar role in the peripheral auditory system, since glutamate is considered to be the afferent neurotransmitter at the inner hair cell synapse. NO plays an important role in kainic acid-induced ototoxicity [88]. A study demonstrated that 7-NI, a competitive inhibitor of neuronal nitric oxide synthase, could attenuate the compound action potential threshold shift caused by kainic acid, suggesting that NO is coupled to a glutamate receptor [89]. Indeed, Amaee et al. suggested that NO might be involved in sensorineural hearing loss induced by bacterial meningitis [90].

Recently Barkdull et al used cochlear microperfusion to treat sensorineural hearing loss caused by inflammation, in a guinea pig model [91]. The microperfusion was effective in the acute phase that is associated with elevations in cytokines, nitric oxide, and cellular infiltrates and the breakdown of the blood-labyrinthine barrier. The chronic phase leads to irreversible ossification of the labyrinth demanding other kinds of treatment to facilitate removal of inflammatory cells and their byproducts. The benefit of microperfusion may be sustained when combined with local delivery of immuno-suppressive agents to the inner ear.

Studies have shown that excessive glutamate may play a role in the production of tinnitus. They also show that glutamate antagonists can have a protective effect on the inner ear and possibly be a treatment for peripheral tinnitus, that which is generated by the inner ear. Several such drugs are currently under investigation for hearing loss and tinnitus as for example memantine, caroverine and magnesium. Caroverine has been shown to restrict the activity of glutamate receptors and protect the hearing of guinea pigs. Its safety and tolerance have been demonstrated in some clinical studies. In one study 63% of patients treated with intravenous caroverine reported a significant improvement in their tinnitus immediately after intravenous infusion [92]. Over 48% of patients remained stable after one week. No severe adverse effects were identified for the majority of patient. However, a few patients experienced mild transient side effects. There is, however, conflicting data that suggest the placebo effect may have been responsible for the reduction in tinnitus. More clinical studies need to be conducted to resolve the controversy.

Glutamate receptor antagonists have been found to protect the cochlea from noise trauma and aminoglycoside ototoxicity. Excitotoxicity can be prevented by a non-NMDA receptor antagonist [93]. Swelling of the dendrites under the inner hair cells induced by the glutamate agonist AMPA can be partly prevented by the non-NMDA receptor antagonist DNQX. Noise-induced swelling of the dendrites under the inner hair cells has been found to be prevented by either MK 801, a NMDA receptor antagonist, or kynuretic acid, a wide glutamate receptor antagonist for both NMDA and non-NMDA receptors [94]. Aminoglycoside-induced hearing disorders could be prevented by the NMDA receptor antagonist MK 801 [95]. This suggests that the glutamate receptor plays an important role in noise and drug-induced hearing loss.

Puel et al.(1991) observed total disruption of all synapses between the inner hair cells and spiral ganglion neurone dendrites, together with the disappearance of cochlear potentials after applying AMPA, a glutamate agonist, to the cochlea [96]. In addition, recovery of both the normal pattern of inner hair cell innervation and the physiological responses has been observed within five days.

Treatment of cochlear with nerve growth factors

Neurotrophins, including nerve growth factor (NGF), brain-derived nerve growth factor (BDNF), neurotrophin-3 (NT-3) and glial cell line-derived neurotrophic factor (GDNF), are known to play a role in the survival of injured cochlear neurones both *in vitro* and *in vivo*. Schindler and colleagues found that NGF significantly prevented damage to spiral ganglion neurons from neomycin *in vivo* [97]. BDNF and NT-3 have been shown to protect spiral ganglion neurons from ototoxicity of cisplatin and aminoglycoside both *in vitro* and *in vivo* [98]. BDNF and GDNF have also been found to protect the cochlea from noise-induced damage [99]. In addition, Pirvola et al. found that FGFR-3 mRNA was present in the organ of Corti following acoustic over-stimulation and suggested that FGFR-3 could be involved in protecting the cochlea from noise-induced damage [100].

Recent findings that GDNF, BDNF, NT-3, and transforming growth factor- α (TGF- α) can protect the auditory hair cells from acoustic trauma or aminoglycoside ototoxicity *in vivo* raise the question of whether other neurotrophic factors can also protect the hair cells *in vivo* [101-104]. Fibroblast growth factor-2 (FGF-2) can protect hair cells from neomycin ototoxicity *in vitro*, and an *in vivo* study has shown upregulation of FGF receptor-3 in the

cochlea following noise exposure, suggesting that some FGF family members might play a role in protection or repair of the cochlea from damage [105]. However, no significant difference in threshold shifts was observed between the treated and untreated ears in any of the groups [106]. The extent of hair cell damage was also comparable among the different treatment groups. These findings indicate that exogenous FGF-1 or FGF-2 does not influence noise-induced hair cell damage under the experimental conditions used in this study, suggesting that these FGFs are not good candidates as auditory hair cell protectors in vivo

Zou et al. demonstrated, in the guinea pig, that after damage under shear stress in cochlea the hearing loss could be alleviated by combining BDNF and CNTF [107]. Because BDNF+CNTF can improve the survival of spiral ganglion cells while affording no protection to hair cells from noise, protection from hearing loss with BDNF+CNTF suggests that ganglion cell damage may be important in vibration induced hearing loss [108]. Apparently we need to protect the hair cells, ganglion cells and possibly other structures such as supporting cells and strial cells from vibration induced hearing loss. Because of severe side effects from BDNF+CNTF, it is still too risky to give neurotrophins systemically but, in the future, local application may be useful in preventing inner ear trauma.

The development, within the mammalian cochlea, of neurite sprouting and integrity of spiral ganglion cells (SGCs) is influenced by members of several growth factor families. Among these NGF, BDNF, NT-3 and NT-4/5 are important [109]. NGF, BDNF, NT-3 and NT-4/5 can promote the survival of postnatal mammalian SGCs in culture [110]. Delivery of exogenous BDNF, NT-3 and NGF to the mammalian inner ear can prevent loss of SGCs following administration of ototoxic drugs [98]. Neurotrophins have been associated

with regenerating neurones in avian cochleae [111]. CNTF and leukaemia inhibitory factor (LIF) are members of the neurotrophic cytokine family and can also promote the survival of SGCs [112]. These cytokines and neurotrophins act in concert upon mammalian SGCs. For example, the combination of CNTF and NT-3 is more effective in promoting the survival of dissociated SGCs *in vitro* than either factor alone [112].

In recent years, studies on antioxidants and/or neurotrophins show promise in protecting the inner ear (hair cells, spiral ganglion cells) from trauma such as noise [58]. The next question, of clinical relevance, is to assess such pharmacological treatment in the prevention of surgically induced trauma to the human inner ear. In a previous experimental study we showed that vibration resulted in significant hearing loss [31]. Therefore, experimental studies mimicking the clinical situation are required to provide information on the mechanism underlying this kind of damage.

Based on the evidence of animal experiments on the neurotrophic factors, it is possible in the future, when targeted drug therapy will become feasible, that the neurotrophins may be the key molecules used in hearing preservation.

Treatment of cochlear trauma with corticosteroids

The inner ear contains both glucocorticoid and mineralcorticoid receptors providing substrate for the biological action of corticosteroids. The normal cortisol production in human plasma is 14-70 $\mu\text{mol/day}$ (5-25 mg/d) and peaks in the early morning. Injuries, infections, cold, and pain result in a 10-fold and a greater increase in the rate of production

of cortisol. Dexamethasone has a biological half-life in plasma of about 2-5 hours (Baxter Dexamethasone data sheet. see also [113]). For cortisol, this is about 80 min. The anti-inflammatory effect lasts longer than the half time in the plasma.

Bachmann et al. evaluated the Prednisolone level in perilymph after round window application [114]. They applied 0.1 ml of 50 mg/ml prednisolone solution directly to the round window niche. Samples were taken from the apex of the cochlea. The steroid concentration in the scala tympani reaches a peak of 1 mg/ml after 1 hour, which is equal to 2% of the steroid concentration of the solution applied to the round window membrane.

The dexamethasone concentration found by Parnes in the cochlea was about 10 times higher than that found by Chandrasekhar et al., although Parnes used a lower dexamethasone concentration applied on the round window membrane (4.4 mg/ml) than Chandrasekhar (10 mg/ml), who completely filled the bulla [115, 116]. Parnes used a much slower perilymph sampling technique. Since both Parnes and Chandrasekhar took 10 µl samples from the cochlea base, and Bachmann from the apex, the latter data are the most reliable. Also, Bachmann put the steroid solution directly onto the round window niche (the others just filled the bulla) and left the animal in anesthesia for a longer time. In addition, his periods of sampling cover a longer total period. The other authors rinsed the bulla with saline 30 min after the steroid injection.

Ikeda & Morizono studied possible adverse effects of triamcinolone in the middle ear of chinchillas [117]. Triamcinolone did not influence the compound action potential (CAP) when compared with the saline-treated control animals. Jinn et al. investigated the length changes in outer hair cells from the chinchilla in-vitro [118]. Tobradex, diluted 1:40 resulted

in an in-vitro dexamethasone concentration of 25 µg/ml. Dexamethasone at this concentration had the least effect on the outer hair cell length when comparing with the others. Kroin et al. perfused the subarachnoid space of the spinal cord in rats (intrathecal) with dexamethasone, via an osmotic minipump (0.5 µl/hr) over 14 days [119]. The objective was to evaluate stability, bioavailability, and safety of long-term drug delivery. They concluded that a dose of 300 ng/day (=12.5 ng/hr) is safe. Higher doses resulted in morphological changes. However, the dose without information of the volume of the spinal cord fluid does not give the correct value of drug concentration. In order to transfer this figure into humans, the relevant volume of the spinal cord fluid of the rat should be compared with the scala tympani volume in humans. The rat spinal cord fluid volume could be in the range of 10 µl, as the guinea pig perilymph respecting the human perilymph volume. Nordang et al. investigated morphological changes of the round window membrane in rats, after the instillation of either dexamethasone (1 µg in 20 µl) or hydrocortisone (2%, 20 µl) every 2nd day for either 5 or 10 days, through the tympanic membrane into the middle ear cavity [120]. Control groups received 20µl of saline. In the group treated with dexamethasone, no morphological differences were found between the steroid group and the control group. However, epithelial thickening and inflammatory cells were found in the round window membranes of the hydrocortisone groups, as compared to the controls. The authors suggest that every instillation of fluid into the middle ear causes some swelling, but dexamethasone reduces the symptoms because it is the most potent drug. Unfortunately, hearing was not monitored.

Spandow et al. observed that hydrocortisone instilled into the middle ear cavity of rats caused irreversible threshold shift in the ABR [121] No morphological changes in the inner ear were observed. The hydrocortisone was dissolved in distilled water. Distilled water

served as control in the other ear. It has been suggested that the threshold shift was due to distilled water rather than hydrocortisone [117].

It has been suggested that acute noise trauma can also be treated with corticosteroids or other treatments aimed at improving the microcirculation of the cochlea [122]. The efficacy of corticosteroids has been evaluated in idiopathic progressive sensorineural hearing loss, sudden deafness and Menière's disease. Nevertheless, no conclusive evidence on their efficacy on treatment in any of these diseases has been achieved. See tables 5-7.

Table 5. A comparison between different synthetic corticosteroids on the treatment of hearing loss.

drugs	anti-inflammatory efficacy	biological t1/2 (hours)	human plasma t1/2 (hours)
Hydrocortisone	1	8-12	1-1.5
Prednisolone	4	12-36	2-3
Triamcinolone (Volon A, Kenacort A)	5	12-36	2-3
Dexamethasone	25-30	36-72	3
Methyl-prednisolone	5	12-36	1.5-35

Table 6. Clinical studies on the efficacy of corticosteroids in Meniere's disease.

drugs	n	administr.	dose	positive effect	authors
dexam.	15	intratymp.	8 mg in hyaluron solution	5/15	Arriaga & Goldman, 1998
dexam	21	intratymp.	4 mg/ml, 1/week, 4 weeks	11/21	Barrs et al., 2001
dexam.	17	intratymp.	16 mg/ml, 3x 0.2-0.4 ml, 1 week	0/17	Hirvonen et al., 2000
prednison. +dexam.	12	ES	P: 20 mg, into ES, D: 32 mg/ml, outside ES.	0/12	Kitahara et al., 2001
dexam.	21	intratymp.	1 x 4mg/ml solution	21/21	Sakata et al., 1986
dexam.	24	intratymp.	0.25 mg/0.25 ml, ventilation tube	vertigo, 17/24 hearing, 0/24	Sennaroglu et al., 1999, 2001
dexam	28	intratymp. + i.v.	RWM: 3x 0.2 mg; iv: 16 mg;	hearing, 19/28 tinnitus, 23/28	Shea et al., 1996

dexam.	22	+ oral intratymp.	oral: 0.25 mg, daily, 1 month intratymp., 1 mg/ml intravenous, 4 mg/ml	dizziness, 27/28 tinnitus, 10/22 hearing, 9/22	Silverstein et al. 1996
dexam	17	intratymp.	8 mg/ml	0/17	Silverstein et al., 1998

References: Arriaga & Goldman (1998) [123]; Barrs et al. (2001) [124]; Hirvonen et al. (2000) [125]; Kitahara et al. (2001) [126]; Sakata et al. (1986) [127]; Sennaroglu et al. (1999) [128]; Sennaroglu et al. (2001) [129]; Shea et al. (1996) [130]; Silverstein et al. (1996) [131]; Silverstein et al. (1998) [132].

Table 7. Efficacy of treatment with corticosteroids on tinnitus and sensorineural hearing loss.

Diseases/n	drugs	administ.	dose	positive effect	authors
Tinnitus /1214	dexam.	intratymp.		862/1214	Sakata et al., 1996
Tinnitus /56	dexam.	intratymp.	4mg/ml	40/56	Sakata et al., 1986
Tinnitus /3041	dexam.	intratymp.	2mg, 4 mg 4/week 4-8weeks	2068/3041	Sakata et al., 1997
Tinnitus /24	dexam.	intratymp.	1mg/ml 1/2d 3months	2/24	Sennaroglu et al., 1999, 2001
SNHL and various /37	dexam./20 methyl- prednisolone/17	intratymp.	dexam., 4mg/ml methyl- prednisolone, 40 mg/ml	SNHL, 13/37 AIED, 7/13	Parnes et al., 1999
sudden SNHL/6	methyl- prednisolone	microcath on RWM	62.5 mg/ml, 14d	5/6	Kopke et al., 2001
sudden SNHL/12	methyl- prednisolone	microcath on RWM	62.5 mg/ml, 10d	12/12	Lefèbvre & Staecker, 2002
labyrinthine fistula	Prednisolone	<u>i.v.</u> , single dose	500mg	prevent HL	Milewski et al., 1995

Note: n= number of subjects participating in the study, administration of the drug, dose of the administration, positive effect of drugs studied and authors of the publication. RWM = round window membrane, i.v. = intravenous.

References: Sakata et al. (1996) [133]; Sakata et al. (1986) [127]; Sakata et al. (1997) [134]; Sennaroglu et al. (1999) [128]; Sennaroglu et al. (2001) [129]; Parnes (1999) [115]; Kopke et al. (2001) [135]; Lefèbvre & Staecker (2002) [136]; Milewski et al. (1995) [137].

Novel substances still at an experimental stage

Peptide inhibitor AM-111 (D-JNKI-1)

D-JNKI-1 is a cell-permeable peptide that selectively blocks MAPK /JNK mediated apoptosis of stress injured hair cells and neurones in the cochlea to protect against permanent hearing loss. When administered within a therapeutic window after the incident, D-JNKI-1 can effectively protect cochlear hair cells and neurones that would otherwise undergo apoptosis and be lost [138]. D-JNKI-1 is an efficient inhibitor of the action of all three JNK isoforms produced by linking the 20 amino acid terminal JNK-inhibitory sequence (JNK binding domain) of JIP-1/IB1 to a 10 amino acid HIV TAT transporter sequence [139]. The oto-protective properties of D-JNKI-1 have been tested and confirmed in various animal models so far, including acute acoustic trauma, surgically induced acoustic trauma (cochlear implant electrode insertion) and aminoglycoside ototoxicity. After acute acoustic trauma in guinea pigs, D-JNK-1 was shown to protect against permanent sensorineural hearing loss even if administered only *after* noise exposure (6 kHz pure tone, 120 dB for 30 minutes) and in just one single dose. The therapeutic window was 12 hours.

This pathway appears to be different from that of CEP 1346. The inhibitory action of AM-111 is thought to be fundamentally different from the action of small chemical inhibitors such as CEP-1347 [140, 141]. AM-111 does not interfere with intrinsic JNK activity that might be involved in such physiological activities as cellular differentiation and neuritic

outgrowth, but rather it targets access of MAPK/JNK to substrates within a cell nucleus by a competitive mechanism [142-145].

CEP 1346

Increased JNK activity and c-Jun induction is observed in stressed cells during various conditions, including degeneration and regeneration. Combined with data from tissue-culture experiments dissecting the JNK pathway, this suggests that activation of the JNK pathway via c-Jun induction or other mechanisms is important in cell-death processes involved in hearing loss, and potentially other neurodegenerative disorders.

The development of the neurotrophic molecule CEP-1347 is based on the observations of the survival-promoting and neurotrophic effects of the naturally occurring small molecule K252a [146]. K252a possesses two activities in neurones. At high concentration, K252a inhibits the survival-promoting and neurotrophic effects of neurotrophins, whereas at low concentrations, K252a, by itself, promotes survival and differentiation similar to the effects of the neurotrophins. Compounds based on the K252a structure were synthesized to enhance the neurotrophic effects of K252a while decreasing its ability to inhibit Trk phosphorylation. The conjugation of alkyloxy- or alkylthio-side chains to the outer benzene rings of the indolocarbazole structure increases ChAT activity in both the rat spinal cord and basal forebrain cultures. Compared to other bulkier alkylthio-derivatives, the 3,9-bis-[ethylthio(methyl)]-substituted K252a has the most potent neurotrophic effects. The 3,9-bis-[ethylthio(methyl)]-substituted K252a was named CEP-1347 or KT-7515. Compared to K252a, CEP-1347 is not cytotoxic above 200 nM, as is K252a, and does not possess the nonselective serine/threonine kinase inhibitor property of K252a, having binding affinities

three orders of magnitude lower than K252a to PKA and PKC and myosin light chain kinase. Thus, the semisynthetic derivative of K252a, CEP-1347, has the desired neurotrophic effects while greatly reducing the nonselective inhibitory profile of K252a.

Recent studies in disparate species demonstrate that hair cell loss in response to noise or exposure to aminoglycoside antibiotics is associated with activation of the JNK pathway and subsequent apoptosis [147]. CEP-1347 (1 mg/kg) administered to guinea pigs a few hours before and daily for 2 weeks after 6 h of 120-dB, 4-kHz noise exposure, significantly reduces hair-cell death and hearing loss observed 14 days post-noise exposure [147].

Latanoprost

Endogenous production of prostaglandins has been demonstrated in the cochlea, but no information is available on the distribution of the cyclo-oxygenase (COX), or prostanoid receptors in the cochlea. Stjernschantz et al. investigated the localization of the FP, EP1 and EP3 prostanoid receptors as well as the COX-1 and COX-2 in the cochlea of guinea pigs and man [148]. In both the guinea pig and man the FP prostanoid receptor was abundantly distributed in the cochlea, e.g., in the stria vascularis, spiral ligament, spiral ganglion, and organ of Corti. The immunohistochemical staining of the EP1 and EP3 receptors in the same structure was significantly weaker and sometimes lacking altogether (e.g., EP3 receptor in human cochlea). Weak, but mostly consistent immunostaining of COX-1 was found in the cochlear structures. At the same time COX-2 was absent. The abundant distribution of the FP receptor in several important cochlear structures in both the guinea pig and man suggests a physiological function for PGF₂a in the cochlea. COX-1 seems to be expressed in cochlea in contrast to COX-2.

Latanoprost is a selective agonist for the FP prostanoid receptor (receptor for PGF₂α). Many prostaglandins, including PGF₂α, are produced in the inner ear [149]. Rask-Andersen et al. (administered latanoprost by intratympanic injection once daily for 3 days [150]. Before the first injection (day 1) and on day 5 and 15, hearing and tinnitus were determined. The patients assessed vertigo on a visual analogue scale on days 1-15. The study was randomized, doubleblind, and placebo-controlled. Latanoprost reduced vertigo/dysequilibrium by about 30%, and improved speech recognition by about 15%. Tinnitus loudness was not reduced by latanoprost. There were few side effects. It is likely that latanoprost may be a useful agent for acute hearing loss and also environmental noise-induced hearing loss.

Targeted drug delivery – future treatment.

The specific cell targeting treatment of the inner ear disease represents another challenge. The possibility of nanocarrier-based drug targeting is under development. In this nanocarrier, it can be a nanoparticle (NP) the size of less than 200 nm in diameter. The NPs can be produced by different techniques including interfacial deposition, emulsion, micellular structures, or sonication. PLGA and PCL are biodegradable approved polymers, but typically suffer from low drug incorporation and rapid drug release rates for low molecular weight organic drug molecules. Other materials such as chitosan, silica based materials, demonstrate better incorporation and slower release rates, but suffer from poorer biodegradability and biocompatibility. Additionally, physicochemical properties of drugs affect loading and release, thus choice of drugs and compatible polymers will be

important for the development and clinical therapy.

NPs should be appropriately surface modified to reduce toxicity and immunogenicity. Obviously both of these features represent a challenge. Circumventing the immune response may be solved by: 1) using peptide ligands to avoid protein tags on the nanoparticle surfaces and 2) by coating the surface of nanostructures with poly-ethylene-glycol (PEGylating) to avoid nonspecific reactions with inner ear proteins and opsonization or false targeting of the nanostructures. Coating can be created either by the addition of a PEG containing surfactant at NP production, or after NP manufacture. NP coating must also inhibit aggregation and reduce uptake by non-targeted cells. Unless particles demonstrate significant charge stabilization, they will tend to aggregate, due to their hydrophobic action. Proteins and buffering salts may increase aggregation or may adsorb to the particle surface, resulting in non-targeted cell uptake. Hydrophobic particles and positively charged complexes (as in uncoated polyplexes) will also tend to bind to cell surfaces which will lead to a non-specific uptake by macrophages into cells.

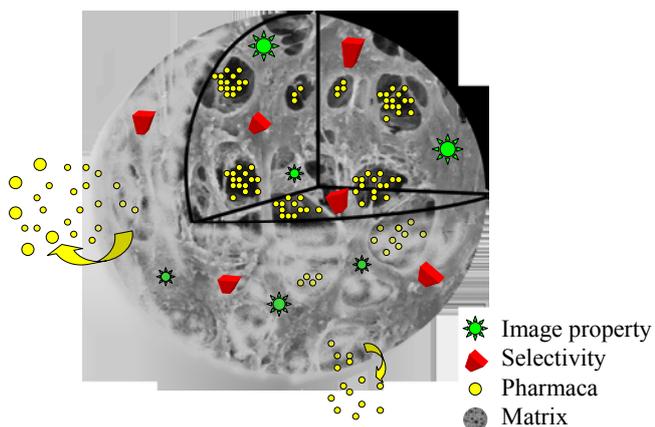


Figure 8. Multifunctional, biocompatible, biodegradable, non-toxic polymer matrix nanoparticle with a matrix integrated “tracer” for magnetic resonance imaging and selective drug delivery

A peptide-based targeting ligand can be attached with covalent bonds to the outer surface

of the NP. The NP will bind with this ligand to specific receptors present, for example, in hair cells, supporting cells and cells in stria vascularis. In hair cells there are several possibilities of targets, including prestin, cadherin, claudin, anion exchanger 2, myosin IVa, among others. On the cochlear nerve the spiral ganglion cells have TrkB and TrkC receptors that can be targeted. Metalloprotein matrix proteins, MMP2, MMP9 can be expressed in the stria vascularis after lesions. Conjugation of ligands may be achieved via PEG-like linkers to ensure that the uptake is specific. Ligands will be identified with a phage display technique. In this technique, 10^9 different DNA sequences coding for a peptide library are cloned to the coat protein gene of the virus, and are displayed in the phage plasmid after protein synthesis. Using immobilized receptors *in vitro* it is possible to select and isolate peptides with different binding specificities. This procedure of ligand screening is called biopanning, and results in highly selective peptides binding to specified receptors, thus allowing accurate targeting.

Several genes regulate the differentiation of cochlear hair cells and supporting cells, during mammalian embryogenesis, from their common precursor cells. A key gene is known to be *Atoh1* (also known as *Math 1*). This is the mouse homologue of the *Drosophila* gene *atonal*, that encodes a basic helix-loop-helix transcription factor. Overexpression of *Atoh 1* in nonsensory cells of the normal cochlea generates new hair cells, both *in vitro* and *in vivo*. *Atoh 1* has been shown to act as a "pro-hair cell gene" and is required for the differentiation of hair cells from multipotent progenitors. Recently our adjunct research institute demonstrated that in mammals by using gene therapy the lost hair cells will regenerate and also return hearing to the profoundly deaf mammalian ear. This finding opens new perspectives for the treatment of hearing loss and justifies the efforts to encapsulate nucleotides encoding the Math 1 gene within the nanostructures in this IP.

Another approach for the inner ear target treatment is to selectively open the passage from blood to perilymph without interference with endolymph because perilymph is essential for the surviving of cochlear hair cells and other cells. When VEGF is delivered to the round window membrane it could significantly enhance the transport of Gd-DTPA-BMA from blood to perilymph without disturbing the blood-labyrinth barrier [151]. This site-specific response might be explained by the different structure of the blood-labyrinth barrier located in the stria vascularis and the blood-perilymph barrier which is located in the spiral ligament and cochlear glomeruli. This response might be used to accelerate the penetration of intravenously administered drug to the cochlea.

FUTURE TREATMENT OF HL

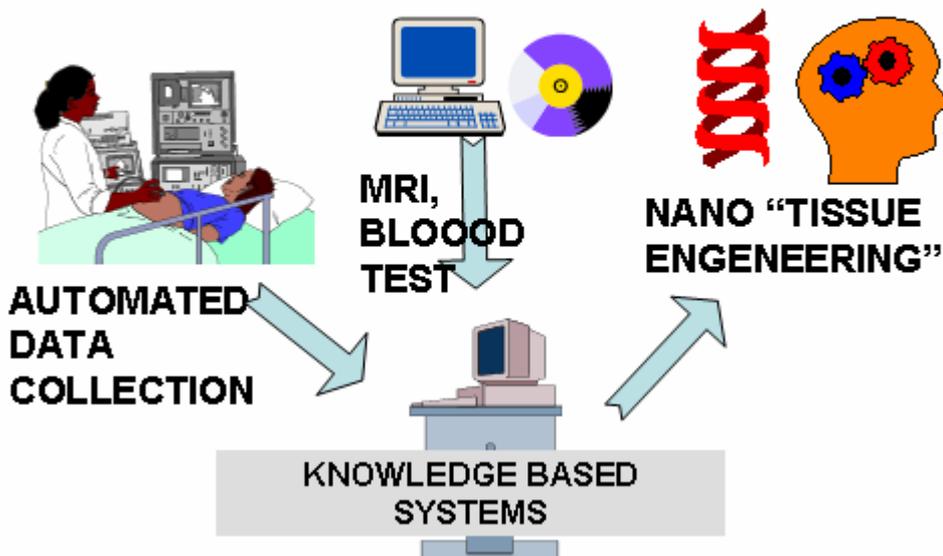


Figure 9. Future aspects of inner ear treatment is based on integrated action with internet based data

collection interface, genotyping with proteomics, data mining and evaluation with artificial intelligence based systems and robotic tissue engineering.

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