AUTHOR QUERY SHEET

Author(s): Cannizzaro, Cannizzaro, Plesacia, Martines, Soleo, Pira, Lo Coco

Article title: Exposure to ototoxic agents and hearing loss: A review of current knowledge

Article no: 964939

Enclosures: 1) Query Sheet

3) Article Proofs

Dear Author,

Please check these proofs carefully. It is the responsibility of the corresponding author to check against the original manuscript and approve or amend these proofs. A second proof is not normally provided. Informa Healthcare cannot be held responsible for uncorrected errors, even if introduced during the composition process. The journal reserves the right to charge for excessive author alterations, or for changes requested after the proofing stage has concluded.

The following queries have arisen during the editing of your manuscript and are marked in the margins of the proofs. Unless advised otherwise, submit all corrections using the CATS online correction form. Once you have added all your corrections, please ensure you press the "Submit All Corrections" button.

[AQ1] Please review the table of contributors below and confirm that first and last names are structured correctly and that the authors are listed in the order of contribution.

Contrib. No.	Given name(s)	Surname	Suffix
1	Emanuele	Cannizzaro	
1	Carla	Cannizzaro	
1	Fulvio	Plesacia	
2	Francesco	Martines	
3	Leonardo	Soleo	
4	Enrico	Pira	
5	Daniele	Lo Coco	

Page No.	Query Details	Author Reply
1	**[AQ2]** Affiliations are correct? Please check and advise	
3	**[AQ3]** We have inserted a running head. Please approve or provide an alternative.	
7	**[AQ4]** A declaration of interest statement reporting no conflict of interest has been inserted. Please confirm whether the statement is accurate.	
8	**[AQ5]** Editor's initials, please.	

Hearing, Balance and Communication, 2014; Early Online: 1–10

informa
healthcare

REVIEW ARTICLE

Exposure to ototoxic agents and hearing loss: A review of current knowledge

[AQ1] EMANUELE CANNIZZARO[1], CARLA CANNIZZARO[1], FULVIO PLESACIA[1], FRANCESCO MARTINES[2], LEONARDO SOLEO[3], ENRICO PIRA[4] & DANIELE LO COCO[5]

[AQ2] [1]*Department of Sciences for Health Promotion and Mother and Child Care 'Giuseppe D'Alessandro', University of Palermo, Palermo,* [2]*Department of Experimental Biomedicine and Clinical Neurosciences, University of Palermo, ENT Section, Palermo,* [3]*Interdisciplinary Department of Medicine, Section of Occupational Medicine 'E.C. Vigliani', University of Bari, Bari,* [4]*Section of Occupational Medicine, Department of Public Health and Paediatrics, University of Turin, Turin, and* [5]*O.U. Neurology, Department of Neuroscience, Ospedale Civico ARNAS, Palermo, Italy*

Abstract

Several experimental and clinical studies have shown that a variety of ototoxic agents (such as drugs, industrial chemicals and noise) can cause sensorineural hearing loss. The most common ototoxic drugs used in clinical practice include: aminoglycoside and macrolide antibiotics, quinoline anti-malarials, platinum analog antineoplastics, loop diuretics, and acetylsalicylic acid. Among chemical agents with potential ototoxic properties are: organic solvents, heavy metals, organotins, nitriles, asphyxiants, and pesticides/herbicides. Acoustic exposure to high intensity and/or prolonged noise can also cause permanent threshold shifts in auditory perception. Ototoxic agents can influence auditory function by different mechanisms: ROS overload, inhibition of mitochondrial protein synthesis, DNA/RNA damage, activation of the apoptotic pathways, excessive calcium influx, increase of proinflammatory cytokines, interference with fluid and electrolyte balance of the endolymph, atrophy of the stria vascularis, changes in blood-labyrinth barrier and overstimulation of the stereocilia of the ear cells. Since noise exposure and many drugs or chemical compounds frequently share the same ototoxic mechanisms, this may explain why hearing loss can be potentiated by combined exposure to these agents. However, a great variability in the individual's response to a given xenobiotic exists and depends on a complex interplay between endogenous and exogenous factors.

Key words: *ototoxicity, hearing loss, pharmacological injury, reactive oxygen species*

Introduction

Robust evidence from a large number of experimental and clinical studies indicates that ototoxic agents such as drugs, chemical agents, and excessive noise exposure can cause permanent hearing damage subsequent to acute or chronic prolonged exposure (Table I). Acoustic damage can manifest as impaired ability to discriminate sounds, hearing loss or balance disorders. These symptoms are caused by functional changes to the inner ear, resulting from the detrimental action on the organ of Corti, vestibular organ, and/or vestibular-cochlear nerve exerted by the xenobiotic or noise (1,2). The different ototoxic agents can damage the inner ear in its entirety, specific cells within the organ, individual components of specific cells within the inner ear, or specific intracellular biochemical pathways. Hearing damage generally appears after exposure to sufficiently high doses of the drug or chemical for a relatively long time. The damage usually develops gradually, starts at the high frequencies and subsequently progresses toward the lower frequencies. Cochlear damage is often initially asymptomatic or it may present with tinnitus (1,3,4). The tinnitus can be preceded by vestibular damage, causing vertigo, headache, nausea, vomiting, ataxia or nystagmus, although, at the beginning, these

Correspondence: E. Cannizzaro, Department of Sciences for Health Promotion and Mother and Child Care 'Giuseppe D'Alessandro', University of Palermo, V. Vespro 129, 90127 Palermo, Italy. Tel: +39 091 655 5812. E-mail: emanuele.cannizzaro@unipa.it

(Accepted 9 September 2014)

ISSN 2169-5717 print/ISSN 2169-5725 online
DOI: 10.3109/21695717.2014.964939

Table I. Principal classes of ototoxic compounds.

Drugs	
Aminoglycoside antibiotics	streptomycin, gentamycin, neomycin, tobramycin, kanamycin, amicacin, netilmycin
Macrolid antibiotics	eritromycin, azitromycin, claritromycin
Quinoline anti-malarials	choroquine, hydroxychloroquine, quinine
Platinum analog antineoplastics	cisplatin, carboplatin, oxaliplatin
Loop diuretics	furosemide, bumetranide, ethacrynic acid
Acetyl salicylic acid	
Chemicals	
Organic solvents	toluene, styrene, xylene, ethylbenzene, chlorobenzene, trichloroethylene, n-exane, n-heptane, carbon disulphide
Heavy metals	lead, mercury
Asphyxiants	carbon monoxide, hydrogen cyanide, acrynitrile
Other agents	pesticides (organophosphates, paraquat, pyrethroids, hexochlorobenzene)

symptoms can be compensated and masked by central mechanisms such as visual stimuli and deep proprioceptive sensations. However, the tinnitus is not always the expression of organic lesions of the cochlea or of the acoustic nerve, but could also be induced by an increase of labyrinth fluid (endolymph and perilymph), thus causing excessive stimulation of cochlear hair cells (5). Various biological mechanisms responsible for the hearing damage have been proposed, including oxidative stress and increased formation of highly reactive free radicals, the so-called reactive oxygen species (ROS), lipid peroxidation, inhibition of mitochondrial protein synthesis, DNA and RNA damage, activation of the pro-apoptotic pathways, and interference with fluid- and electrolyte-balance within the endolymph. Interestingly, some of these mechanisms are shared by both ototoxic agents and noise (6–16).

However, an individual's response to a given ototoxic agent is highly variable, and relies on a complex interplay among several endogenous and exogenous factors (Table II). Thus, the effects of the ototoxic agents are influenced by several pharmacokinetic parameters, and, in particular, by their clearance, a measure of the body's efficiency in eliminating endogenous and exogenous substances. This variable is highly dependent on some demographic parameters such as gender and age; thus, dosing adjustment of the drug becomes critical for safe therapeutics. In particular, most drug-metabolizing enzymes are expressed at low level at birth; therefore, their elimination is reduced in the neonatal period. On the other hand, in the elderly, gradual changes in body mass, serum albumin and body water, and decline in renal and hepatic function can alter drug distribution and elimination, and therefore increase inter-individual variation in the response to the ototoxic agent (17,18). Drug metabolism is also influenced by disease induced alterations in pharmacokinetic properties, producing great variations in the level of the drug or chemical within the organism (Tables III, IV). Impaired renal and hepatic clearance, hypothyroidism, circulatory insufficiency secondary to cardiac failure, and altered drug-binding to plasma proteins are all pathological determinants of inter-individual variations of drug metabolism. As a consequence, in these pathological conditions, dose regimens for many drugs must be reduced to avoid drug accumulation and, hence, ototoxic effects (17,18). In older patients, changes in the endogenous sensitivity to many drugs that may further impair renal blood flow, must also be considered. For example, non-steroidal anti-inflammatory drugs can decrease the production of vasodilating renal prostaglandins, which are essential to maintain optimal renal perfusion, thus influencing the elimination of a co-administered ototoxic drug. Furthermore, in the elderly, the physiological response to an administered drug may change, because of a dynamic and time-dependent expression of specific cellular receptors and ligands, which may be temporarily up- or down-regulated by many

Table II. Endogenous and exogenous factors that can contribute to potentiation of the ototoxic effect.

Genetic polymorphisms	Exercise
Age	Heavy alcohol intake
Gender	Heavy smoking
Immunological function	Co-exposure to drugs, chemicals, noise
Diet	Stress

Table III. Conditions that can induce accumulation of the drug following multiple exposure.

Impaired hepatic clearance that reduces the inactivation of the drug
Circulatory insufficiency owing to cardiac failure that reduces renal and hepatic blood flow
Modified drug binding to plasma proteins
Hypothyroidism

Table IV. Mechanisms of interaction between drugs that can induce accumulation in the site of action during maintenance therapy.

Inhibition of ototoxic drug-metabolizing enzyme induced by co-exposed drug
Reduction of ototoxic drug-binding to plasma proteins induced by co-exposed drug
Inhibition of drug transport into cells induced by co-exposed to drug

endogenous and exogenous factors (17,18). Genetic variants may also modify the susceptibility of the individual subject to the ototoxic effect of the drug. Candidate genes for the mediating effect of the ototoxic response can be divided into two categories: pharmacokinetic and receptor/target. In particular, germline variability in genes which encode factors that determine the pharmacokinetics of the compound, such as enzymes and transporters, are the major determinants of the ototoxic response, since they can modify drug levels in the organism. However, several genetic polymorphisms in drug targets can influence not only the responsiveness to the therapeutic effect and the occurrence of adverse effects, but also the overall risk of the underlying otological disease (19–22). Furthermore, several mitochondrial RNA mutations have been associated with drug induced hearing loss, especially in preterm infants (23–25). Finally, another interesting mechanism that has been shown to influence the degree of hearing loss is the synergistic interactions between drugs and chemical compounds given previously or concurrently. Moreover, ototoxicity of specific agents can also be enhanced as the result of a preceding or concurrent noise exposure to a level not usually pathological.

A number of drugs have been associated with ototoxicity, and some are both ototoxic and nephrotoxic. The most known ototoxic drugs are: aminoglycoside antibiotics, macrolide antibiotics, quinoline anti-malarials, platinum analog anti-neoplastics, loop diuretics and acetylsalicylic acid (26,27).

Aminoglycoside antibiotics

Aminoglycosides (AG) are a group of natural products such as streptomycin, gentamycin, neomycin, tobramycin, kanamycin and semisynthetic derivatives such as amicacine netilmycin. AG antibiotics are rapidly bactericidal, interfering with bacterial protein synthesis. After exposure to these agents, high concentrations of AG are found in the renal cortex and in the inner ear, thus explaining the high propensity for nephrotoxicity and ototoxicity of these drugs. As a consequence, vestibular and auditory dysfunction can follow the treatment of any of the AG. Streptomycin and gentamycin are those with the most pronounced and harmful effects on vestibular function, whereas neomycin, kanamycin and amikacin are the most likely cause of hearing loss. AG rapidly enter the cells of the cochlea via endocytosis or non-selective cation channels and, following continuous treatment, they accumulate in the inner ear because of slow plasmatic retro-diffusion (28,29). As a consequence, persistent elevated plasma concentrations of the drug above critical levels correlate with ototoxicity. Cochlear cells can retain AG for six months or longer. This finding may explain the increased susceptibility of some patients to AG induced ototoxicity in the presence of a medical history of previous AG treatment. Because almost 90% of AG are excreted by glomerular filtration they can also damage the kidney. In a downward spiral, nephrotoxicity can further reduce the excretion of the drug, which in turn predisposes to ototoxicity (30). Therefore, in the patient treated with AG it is advisable to frequently monitor auditory function, the plasma levels of the drug, and creatine excretion. Additional care has to be taken with children and elderly people treated with AG, since they are at increased risk of ototoxicity. In these cases, dose regimen, duration of the treatment, concomitant use of other drugs or chemical agents, and level of noise exposure in occupational or recreational places should be taken into consideration. In particular, it has been shown that loop diuretics, such as ethacrynic acid and furosemide, can potentiate the ototoxic effect of AG; exposure to sub-damaging doses of AG can aggravate noise induced cochlear damage, and previous exposure to high levels of noise enhances subsequent AG ototoxicity (31,32). Preterm infants are especially at risk (23–25).

Reported incidences of ototoxicity vary widely, depending upon subject groups, treatment parameters, assessment methods, and definitional criteria of hearing impairment. The estimated incidence of ototoxicity, including both cochleotoxicity and vestibulotoxicity, ranges from 15% to 50%, although such data include all measurable hearing and balance deficits and are not indicative of disabling conditions (33,34). AG ototoxic effect results from a progressive destruction of vestibular and cochlear sensory cells. The degree of dysfunction is directly proportional to the dose of the drug and correlates with the number of damaged sensory hair cells. The damage progresses from the base of the cochlea, where high frequency sounds localize, to the apex, where low frequencies are detected. Once they are damaged, these cells cannot be replaced so the impairment is permanent.

Several studies indicate that AG antibiotics have a wide sphere of action, and might interfere with DNA, RNA, protein synthesis, energy metabolism, calcium transport, synthesis and degradation of

prostaglandins, mucopolysaccahrides and lipids (10, 23,35–37). As a consequence, a wide variety of mechanisms has been associated with AG ototoxicity. It has been suggested that AG once entered into the outer hair cell can induce cell death by either caspase-dependent and caspase-independent mechanisms (30,38). In particular, it has been reported that AG might form AG-iron complexes within the cells, which can react with electron donors to form ROS. ROS, in turn, might activate a number of downstream metabolic signalling pathways that can trigger apoptosis via caspase activation. In line with this, deferoxamine, an iron chelant frequently used in clinical practice, partially protects the cochlea from the ototoxic effect of AG forming an inactive iron-AG complex. On the other hand, the scavenger tocopherol reduces AG induced ototoxicity preventing the production of free radicals. In line with this, other antioxidants too, such as aspirin, have been shown to protect against aminoglycoside induced hearing loss and, importantly, they do so without compromising drug serum levels or antibacterial efficacy (39). Genetic factors might also modify the sensitivity to AG ototoxicity. In particular, transitional mutations in the mitochondrial small ribosomal RNA gene, namely *A1555G* (and less frequently *C1494T*), have been identified as primary genetic traits in aminoglycoside induced deafness (40–42). The availability of genetic testing for the determination of the A1555G mutation allowed the screening of people at potential risk of AG induced ototoxicity. Recently, it has been suggested that genetic deficiency in megalin, an endocytic receptor that bonds and internalizes within the cochlea a number of substances, including AG, may play a crucial role in AG induced hearing loss (43).

Macrolide antibiotics

Macrolide antibiotics including erythromycin, azithromycin and clarithromycin represent the gold standard therapy in respiratory tract infections and otitis media (44–48). The anti-bacterial effect of macrolides is due to inhibition of bacterial protein synthesis. The ototoxic effect of macrolides appears when they are given by intravenous injection at high doses. The symptomatology is characterized by an accentuated hearing loss, particularly at the beginning of therapy, and tinnitus. These symptoms, however, disappear after treatment suspension. The mechanism of action of macrolide ototoxicity is still unclear.

Quinoline anti-malarials

Quinoline anti-malarials, chloroquine and hydroxychloroquine, initially employed in the prevention and treatment of malaria, have been used subsequently for the treatment of rheumatoid arthritis and other connective tissue diseases (49–52). Besides the well-known gastrointestinal, neuronal and retinal toxicity, prolonged exposure to high cumulative doses of these drugs frequently induces irreversible ototoxicity that is manifested by sensorineural hearing loss, tinnitus, sense of imbalance and cochlea-vestibular symptoms (52). These effects are associated with deposition of the drug in the internal ear and with several different types of injury to the cochlear sensory hair cells, decrease in neuronal population, loss of supporting hair cells, and atrophy of the stria vascularis (12,53). Brainstem auditory evoked potentials appear to be a sensitive method for detecting early manifestations of cochlear injury caused by these drugs when they are still reversible. Anti-malarial quinine, when it is given in full therapeutic or excessive doses, can also be associated with auditory functional impairment, presenting with tinnitus, vertigo and high-frequency deafness. Fortunately, although these symptoms occur very frequently, they disappear soon after drug withdrawal. The auditory effects probably reflect a direct neurotoxicity of the eighth nerve, although secondary vascular changes may also play a role. On the other hand, tinnitus after small doses of quinine usually results from drug hypersensitivity (54).

Platinum analog antineoplastics

Platinum analogs, cisplatin, carboplatin, and oxaliplatin, are effective and widely used antineoplastic agents for the treatment of many types of cancer. These drugs enter the malignant cells and inhibit DNA replication and transcription; cell death is primarily through apoptosis. Side-effects of platinum analogs include ototoxicity, nephrotoxicity, and neurotoxicity. Ototoxicity is manifested by otalgia, tinnitus, and severe, bilateral, and irreversible sensorineural hearing loss (11, 55). High-frequency audiometric thresholds are often affected first; progression to low frequencies may occur with prolonged treatment regimens. Elderly and paediatric patients are particularly sensitive to platinum analog ototoxicity. High cumulative doses, concomitant noise exposure, co-administration of other ototoxic drugs and/or chemicals, depleted nutritional condition, renal and hepatic insufficiency, anaemia, hypoalbuminaemia and prior cranial irradiation usually play a relevant role in the development of ototoxicity for this class of drugs (56).

Unfortunately, there is at present no effective treatment to prevent ototoxicity, which can be severe and disabling. However, adequate hydration and increased diuresis are used to prevent renal insufficiency, which increases the chances for ototoxicity

of these drugs (57). In clinical situations, up to 100% of patients may sustain some degree of hearing loss with prolonged treatment. Various species of experimental animals are likewise susceptible to this drug and the incidence of hearing loss is generally high (57).

Mechanisms of action of platinum analog ototoxicity have been only partially understood. Several studies suggest that these drugs react with the cochlear tissues to generate ROS. ROS overload induces depletion of the cochlear antioxidant defensive enzyme system, preventing scavenging and neutralization of the superoxides generated. Moreover, ROS may lead to excessive calcium influx in the cell, and to an increase of proinflammatory cytokines. The uncontrolled increase in ROS generation within cochlear cells may also activate the pro-apoptotic pathways, both caspase-dependent and independent, leading to death of the outer hair cells (11,55,57). The cell death is time- and drug concentration-dependent. Antioxidants have been used to decrease platinum analog ototoxicity in animal models with some success, including glutathione, superoxide dismutase, vitamin C, vitamin A, vitamin E, and transferases (58). However, clinical studies of antioxidant-based amelioration of cisplatin ototoxicity are minimal (59). Moreover, a potential drawback of the administration of antioxidants is the potential reduction in anti-tumoural efficacy of the drug (60).

There is substantial variability in susceptibility to the ototoxic effect of platinum analogs. Many studies suggest that several genetic variants can contribute to increased sensitivity for platinum analogs' ototoxicity (19). In particular, differences in functional polymorphisms of glutathione-S-transferases, and in two genetic variants in thiopurine-S-methyltransferase and catechol-O-methyl transferase were found to be highly associated with cisplatin induced hearing loss (20,61). Moreover, recent studies suggest that polymorphisms of *megalin* gene, a multifunctional receptor involved in the transport of several substances including platinum analogs, may play a crucial role in susceptibility to the ototoxic effect of these drugs (21).

Loop diuretics

Loop diuretics, furosemide, bumetanide and ethacrynic acid, are used in the therapy of oedema, heart failure, hypertension and, sometimes, in the management of severe hypercalcaemia. The diuretic effect depends on the inhibition of the Na-K-2Cl cotransporter (NKCC2) in the thick ascending loop of Henle (12). On the other hand, the mechanism of loop diuretics' ototoxicity is due to interference with fluid and electrolyte balance induced by NKCC2 inhibition, expressed at the base of the marginal and dark cells of the stria vascularis of the cochlea. Since these cells are responsible for endolymph secretion, it follows that there is a consequent drop in the endolymphatic potential (26,62,63). Ototoxicity for this class of drugs manifests as tinnitus, hearing impairment, deafness, vertigo and a sense of fullness in the ears. Hearing impairment may appear a few minutes after drug administration and regresses in parallel with its elimination. The ototoxic effect results usually after elevated parenteral doses or rapid intravenous administration, and is especially evident in patients with renal failure. Ethacrynic acid appears to induce ototoxicity more frequently than other loop diuretics.

The variations observed in the incidence of ototoxicity with different loop diuretics can be partially explained by the changing balance between ototoxic and diuretic potency. Other possible explanations include differences in drug metabolism, protein binding capacity, and different ability of penetration of the drug into the cochlea. Synergism of ototoxicity may occur when loop diuretics are co-administered with AG, platinum analogs, or when noise exposure and chemical agents are present in the environment. Genetic or acquired defects in several proteins in both renal and ear tissues can potentiate the loop diuretic ototoxicity (63).

Acetylsalicylic acid

Acetylsalicylic acid (ASA) is one of the most used drugs worldwide, with therapeutic effects on fever, pain and phlogosis. Besides the well known side-effects on the gastrointestinal tract, blood and kidney, ASA may induce moderate hearing loss, alteration of sound perception and tinnitus. However, these effects are always reversible after discontinuation of the treatment. Several studies have shown a large inter-individual variability in the susceptibility to ASA ototoxicity (27). The usual targets for ASA ototoxic effects are the outer hair cells and their motility mechanism, the cochlear blood flow, and the spontaneous activity in the cochlear nerve. It has been shown that ASA accumulates in the extracellular fluid, modifies ionic equilibrium and reduces prostaglandin synthesis in the stria vascularis, thus inhibiting the cyclooxygenase, an enzyme that catalyses the synthesis of prostaglandins. This inhibition leads to vasoconstriction of stria vascularis, and inhibits the action potential of the cochlear nerve (27,64). The ototoxic effect of ASA occurs when high doses of the drug are used, e.g. 6–8 g/day. Side-effects usually disappear 48 h after the interruption of treatment. ASA can potentiate the ototoxic effects of several drugs and chemical agents (65).

Chemical agents

The class of chemical agents investigated as potential ototoxic compounds includes organic solvents, heavy metals, nitriles, asphyxiants, pesticides/herbicides. Robust evidence from a large number of animal studies has demonstrated that many of these compounds are potent ototoxic agents (66–69). Addition of other stressors, such as exposure to impact or continuous noise, and other chemicals or ototoxic drugs, can reduce the threshold needed to elicit the auditory damage (70–78). However, there are no regulations that require monitoring of the hearing of workers who are employed at locations in which occupational exposure to potentially ototoxic chemicals occurs in the absence of noise exposure (79). A few human studies, conducted mainly over the last three decades, have brought attention to the risk of acoustic damage following exposure to chemical agents, and their interaction with noise exposure in the workplace. Unfortunately, results of these studies were not always consistent and showed limited generalizability, because of the elevated number of existing industrial substances, and because of the great individual variability due to several endogenous and exogenous factors. In addition, it is not easy to establish a causal relationship between exposure to chemicals and hearing loss, because of insufficient information about exposure history and a lack of comparability between study and control groups (80–84). Nevertheless, several researches suggest that the association between industrial chemical exposure and hearing impairment is biologically plausible. Human data support the evidence that structure and toxic properties of the chemical agent, past occupational exposure to excessive noise, history of heavy smoking, physical exercise, personal life-style, age of the subject, genetic individual variability, pharmacokinetic and pharmacodynamic subjective variability and pathological associated conditions are responsible for the wide differences in susceptibility to the hearing damage observed (12,18,66,85,86). Evidence from a large number of experimental and clinical studies showed that most of the chemical agents have many different targets for injury within the auditory system, and may affect both the cochlea and the central auditory pathways, depending on the compound. For chemicals such as n-hexane and n-heptane, metals such as lead and mercury and organophosphate pesticides, the auditory effects are especially connected to an intrinsic neurotoxic action of these substances. These compounds exhibit more central neurotoxic effects than pure ototoxic effects (87–91), so that exposure to these agents may impair not only the detection but also the discrimination of sounds. Accumulating data link ROS production to cochlear damage for both chemical agents and noise trauma (92). Histological studies on specific chemical agents and concomitant noise exposure have demonstrated that, during stressful conditions, damage to hair cells is caused by a disruption of the intrinsic antioxidant defenses, following overproduction of ROS. Moreover, reduced blood flow seems to be another important ototoxic mechanism shared by both chemicals and noise exposure (7,67,92,93). This can explain why additional stressors, such as noise or drugs, can reduce the chemical exposure threshold needed to elicit a hearing damage, and why a single environmental and/or occupational exposure to a specific chemical agent may not elicit an ototoxic response, whereas the same exposure in the presence of a high level of noise can lead to oxidative stress and to the death of cells in the inner ear (77,78,94–96).

Noise exposure

Exposure to high intensity and/or prolonged noise and vibrations causes temporary or permanent threshold shifts in auditory perception reflected by reversible or irreversible, often bilateral, sensorineural damage that starts within the outer hair cells and progressively spreads over the entire cochlea. As mentioned before, many studies have shown that hearing loss produced by excessive noise exposure can be added to the effects induced by co-exposure to chemical agents. However, intense noise or vibrations are often present in many occupational workplaces (e.g. industrial, manufacturing, construction, and military) where exposure to chemicals can also occur. Therefore, in the majority of cases, the hearing loss observed in these settings is not solely imputable to a single agent.

The damaging properties of noise exposure depend in part on the characteristics of the sound reaching the sensory structures in the inner ear. The characteristics of noise regarded as critical (harmful) are: intensity, sound spectrum, duration and temporal distribution during the day, week, or month. However, wide variations in the subjective response may be present, due to genetic susceptibility, young and elderly age, pathological comorbidities, preceding exposure to ototoxic drugs or chemical agents, vibrations, and personal life-style. Gender and race seem to be also associated with susceptibility to noise induced hearing loss (96–100). Exposure to damaging levels of sound occurs in two forms. High intensity sounds can physically damage hair cells stereocilia, disrupt the permeability of the strial blood-labyrinth barrier, and induce a reduction or loss of the electrical endocochlear potential. Moreover, high intensity sounds can

induce physical disruption of the organ of Corti, increased cellular endocytosis, elevated calcium intracellular concentrations, and mitochondrial lesions with release of mitochondrial pro-apoptotic factors into the cytosol (6,17,66). Long-term exposure to lower intensity noise generates high levels of metabolic activity and formation of ROS coupled with physiological changes in the blood-labyrinth barrier, resulting in temporary auditory dysfunction and often permanent hearing loss. High levels of metabolic activity and formation of free radicals may continue for several days after cessation of the sound exposure (6,9,14–16,101,102). In the presence of drugs or chemical compounds that interfere with intracellular calcium regulation in the outer hair cells, these can be more vulnerable to excessive levels of noise. This interaction is imputable to outer hair cells being electromotile, i.e. the cells change their length in response to sound stimulation, and this process is controlled by the calcium concentration within the cell.

Concluding remarks

The examples presented in this review illustrate the potential for many commonly used drugs and chemical agents, as well as noise exposure, to contribute significantly to ototoxicity in man. However, although aminoglycoside and macrolide antibiotics, quinoline antimalarials, platinum analog antineoplastics, loop diuretics, and acetylsalicylic acid are well characterized molecules and their clinical adverse effects are well established, the exact mechanisms by which they may induce their toxic effects and auditory impairment are not fully established. The contribution of oxidative stress is emerging as one of the most important mechanism in the pathophysiology of hearing loss, but it is clear that more data are required to provide insight into individual susceptibility to specific ROS-dependent mechanisms of toxicity. Understanding individual differences of this type and the potential for redox effects to manifest as toxicities is increasingly valuable, not just for existing therapies, but for tailoring clinical drug development. More research is also needed to address the complex interplay between endogenous and exogenous factors underlying ototoxicity and the tangled net of interactions among drugs, chemicals and noise exposure. Investigation of the ototoxic properties of different compounds and the underlying pathophysiologic variables is important, not only for medical progress and researches purposes, but also to establish recommendations for good health in the workplace, and to identify best practices for hearing loss prevention.

Acknowledgement

The authors thank Mauro Gagliano for his excellent help in the collection of the bibliographic references for the manuscript.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. **[AQ4]**

References

1. Martines F, Maira E, Ferrara S. Age related hearing impairment (ARHI): a common sensory deficit in the elderly. Acta Medica Mediterr. 2011;27:47–52.
2. Martines F, Dispenza F, Gagliardo C, Martines E, Bentivegna D. Sudden sensorineural hearing loss as prodromal symptom of anterior inferior cerebellar artery infarction. ORL. 2011;73:137–40.
3. Martines F, Bentivegna D, Martines E, Sciacca V, Martinciglio G. Assessing audiological, pathophysiological and psychological variables in tinnitus patients with or without hearing loss. Eur Arch Otorhinolaryngol. 2010; 267:1685–93.
4. Martines F, Bentivegna D, Martines E, Sciacca V, Martinciglio G. Characteristics of tinnitus with or without hearing loss: clinical observations in Sicilian tinnitus patients. Auris Nasus Larynx. 2010;37:685–93.
5. Martines F, Pangaro A, Martines E. Ménière's disease and neurovascular cross-compression: case report. Otorinolaringologia. 2009;59:65–70.
6. Henderson D, Bielefeld EC, Harris KC, Hu B. The role of oxidative stress in noise-induced hearing loss. Ear Hear. 2006;27:1–19.
7. Chen GD, Chi LH, Kostyniak PJ, Henderson D. Styrene induced alterations in biomarkers of exposure and effects in the cochlea: mechanisms of hearing loss. Toxicol Sci. 2007;98:167–77.
8. McNeil PL. Cellular and molecular adaptations to injurious mechanical stress. Trends Cell Biol. 1993;3:302–7.
9. Waters C. Molecular mechanisms of cell death in the ear. Ann N Y Acad Sci. 1999;884:41–51.
10. Hutchin T, Cortopassi G. Proposed molecular and cellular mechanism for aminoglycoside ototoxicity. Antimicrob Agents Chemother. 1994;38:2517–20.
11. Rybak LP, Whitworth CA, Mukherjea D, Ramkumar V. Mechanisms of cisplatin-induced ototoxicity and prevention. Hear Res. 2007;226:157–67.
12. Hadi U, Nuwayhid N, Hasbini AS. Chloroquine ototoxicity: an idiosyncratic phenomenon. Otolaryngol Head Neck Surg. 1996;114:491–3.
13. Jiang H, Sha SH, Forge A, Schacht J. Caspase-independent pathways of hair cell death induced by kanamycin in vivo. Cell Death Differ. 2006;13:20–30.
14. Ohlemiller KK, Wright JS, Dugan LL. Early elevation of cochlear reactive oxygen species following noise exposure. Audiol Neurootol. 1999;4:229–36.
15. Suzuki M, Yamasoba T, Ishibashi T, Miller JM, Kaga K. Effect of noise exposure on blood-labyrinth barrier in guinea pigs. Hear Res. 2002;164:12–8.
16. Henderson, DH, Hu B, Bielefeld EC. Patterns and mechanisms of noise induced cochlear pathology. In: Schacht TS, Popper AN, Fay RR, editors. Auditory trauma, protection and repair. New York: Springer; 2008. p. 195–217.

17. Cannizzaro G, Cannizzaro C, Cannizzaro E, Gagliano M, Giammona G, Martire M, Provenzano G. Variabilità nella risposta ai farmaci. In: Idelson-Gnocchi, editor. Principi di Farmacologia Generale. Napoli, Italy: 2002. p. 93–107.
18. Annunziato L, Di Renzo G. Interazioni farmacologiche. In: Idelson-Gnocchi, editor. Trattato di Farmacologia. Napoli, Italy: 2010. p. 145–55.
19. Mukherjea D, Rybak LP Pharmacogenomics of cisplatin induced ototoxicity. Pharmacogenomics. 2011;12:1039–50.
20. Ross CJ, Katzov-Eckert H, Dubé MP, Brooks B, Rassekh SR, Barhdadi A, et al. Genetic variants in TPMT and COMT are associated with hearing loss in children receiving cisplatin chemotherapy. Nat Genet. 2009;41:1345–9.
21. Riedemann L, Lanvers C, Deuster D, Peters U, Boos J, Jürgens H, et al. Megalin genetic polymorphisms and individual sensitivity to the ototoxic effect of cisplatin. Pharmacogenomics. 2008;8:23–8.
22. Tang HY, Hutcheson E, Neill S, Drummond-Borg M, Speer M, Alford RL. Genetic susceptibility to aminoglycoside ototoxicity: how many are at risk? Genet Med. 2002;4:336–45.
23. Zimmerman E, Lahav A. Ototoxicity in preterm infants: effects of genetics, aminoglycosides, and loud environmental noise. J Perinatol. 2013;33:3–8.
24. Salvago P, Martines E, Martines F. Prevalence and risk factors for sensorineural hearing loss: Western Sicily overview. Eur Arch Otorhinolaryngol. 2013;270:3049–56.
25. Martines F, Martines E, Mucia M, Sciacca V, Salvago P. Prelingual sensorineural hearing loss and infants at risk: Western Sicily report. Int J Ped Otorhinolaryngol. 2013;77: 513–8.
26. Tabuchi K, Nishimura B, Nakamagoe M, Hayashi K, Nakayama M, Hara A. Ototoxicity: mechanisms of cochlear impairment and its prevention. Curr Med Chem. 2011; 18:4866–71.
27. Arslan E, Orzan E, Santarelli R. Global problem of drug induced hearing loss. Ann N Y Acad Sci. 1999;884:1–14.
28. Marcotti W, van Netten SM, Kros CJ. The aminoglycoside antibiotic dihydrostreptomycin rapidly enters mouse outer hair cells through the mechano-electrical transducer channels. J Physiol. 2005;567:505–21.
29. Dulon D, Hiel H, Aurousseau C, Erre JP, Aran JM. Pharmacokinetics of gentamycin in the sensory hair cells of the organ of Corti: rapid uptake and long-term persistence. C R Acad Sci III. 1993;316:682–7.
30. Rybak LP, Ramkumar V. Ototoxicity. Kidney Int. 2007; 72:931–5.
31. Collins PW. Synergistic interactions of gentamycin and pure tones causing cochlear hair cell loss in pigmented guinea pigs. Hear Res. 1988;36:249–59.
32. Ryan AF, Bone RC. Non-simultaneous interaction of exposure to noise and kanamycin intoxication in the chinchilla. Am J Otolaryngol. 1982;3:264–72.
33. Xie J, Talaska AE, Schacht J. New developments in aminoglycoside therapy and ototoxicity. Hear Res. 2011; 281:28–37.
34. Martines F, Martinciglio G. VEMPs: clinical applications and personal experience. Otorinolaringologia. 2008;58: 137–40.
35. Hirose K, Hockenbery DM, Rubel EW. Reactive oxygen species in chick hair cells after gentamycin exposure in vitro. Hear Res. 1997;104:1–14.
36. Ward DT, McLarnon SJ, Riccardi D. Aminoglycosides increase intracellular calcium levels and ERK activity in proximal tubular OK cells expressing the extracellular calcium-sensing receptor. J Am Soc Nephrol. 2002;13:1481–9.
37. Forge A, Schacht J. Apoptotic death of hair cells in mammalian vestibular sensory epithelia. Hear Res. 2000;139:97–115.
38. Davis RJ. Signal transduction by the JNK group of MAP kinases. Cell. 2000;103:239–52.
39. Chen Y, Huang WG, Zha DJ, Qiu JH, Wang JL, Sha SH, et al. Aspirin attenuates gentamycin ototoxicity: from the laboratory to the clinic. Hear Res. 2007;226:178–88.
40. Prezant TR, John V, Agapian JV, Bohlman MC, Bu X, Öztas S, et al. Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. Nat Genet. 1993;4:289–94.
41. Zhao H, Li R, Wang Q, Yan Q, Deng JH, Han D, et al. Maternally inherited aminoglycoside-induced and non-syndromic deafness is associated with the novel C1494T mutation in the mitochondrial 12S rRNA gene in a large Chinese family. Am J Hum Genet. 2004;74:139–52.
42. Hobbie SN, Akshay S, Kalapala SK, Bruell CM, Shcherbakov D, Böttger EC. Genetic analysis of interactions with eukaryotic rRNA identify the mitoribosome as target in aminoglycoside ototoxicity. Proc Natl Acad Sci U S A. 2008;105:2088–93.
43. Tauris J, Christensen EI, Nykjaer A, Jacobsen C, Petersen CM, Ovesen T. Cubilin and megalin colocalize in the neonatal inner ear. Audiol Neurootol. 2009;14:267–78.
44. Bentivegna D, Salvago P, Agrifoglio M, Ballacchino A, Ferrara S, Mucia M, et al. The linkage between upper respiratory tract infections and otitis media: evidence of the 'united airways' concept. Acta Medica Mediterranea. 2012;28:287–90.
45. Martines F, Bentivegna D, Maira E, Sciacca V, Martines E. Risk factors for otitis media with effusion: case-control study in Sicilian schoolchildren. Int J Pediatr Otorhinolaryngol. 2011;75:754–9.
46. Martines F, Martinciglio G, Martines E, Bentivegna D. The role of atopy in otitis media with effusion among primary school children: audiological investigation. Eur Arch Otorhinolaryngol. 2010;267:1673–8.
47. Martines F, Martines E, Sciacca V, Bentivegna D. Otitis media with effusion with or without atopy: audiological findings in primary school children. Am J Otolaryngol. 2011;32:601–3.
48. Martines F, Bentivegna D. Audiological investigation of otitis media in children with atopy. Curr Allergy Asthma Rep. 2011;11:513–20.
49. Page F. Treatment of lupus erythematosus with mepacrine. The Lancet. 1951;2:755–8.
50. Johansen PB, Gran JT. Ototoxicity due to hydroxychloroquine: report of two cases. Clin Exp Rheumatol. 1998; 16:472–4.
51. Rynes RI. Anti-malarial drugs in the treatment of rheumatological diseases. Br J Rheumatol. 1997;36:799–805.
52. Scherbel AL, Harrison JW, Atdjian M. Further observations on the use of 4-aminoquinoline compounds in patients with rheumatoid arthritis or related diseases. Cleve Clin Q. 1958;25:95–111.
53. Nielsen-Abbring FW, Perenboom RM, van der Hulst RJ. Quinine-induced hearing loss. ORL J Otorhinolaryngol Relat Spec. 1990;52:65–8.
54. Bortoli R, Santiago M. Chloroquine ototoxicity. Clin Rheumatol. 2007;26:1809–10.
55. Gonçalves MS, Silveira AF, Teixeira AR, Hyppolito MA. Mechanisms of cisplatin ototoxicity: theoretical review. J Laryngol Otol. 2013;127:536–41.
56. Bokemeyer C, Berger CC, Hartmann JT, Kollmannsberger C, Schmoll HJ, Kuczyk MA, et al. Analysis of risk factors for cisplatin induced ototoxicity in patients with testicular cancer. Br J Cancer. 1998;77:1355–62.

57. Rybak LP, Mukherjea D, Jajoo S, Ramkumar V. Cisplatin ototoxicity and protection: clinical and experimental studies. Tohoku J Exp Med. 2009;219:177–86.
58. Oishi N, Talaska AE, Schacht J. Ototoxicity in dogs and cats. Vet Clin North Am Small Anim Pract. 2012;42:1259–71.
59. Yildirim M, Inançli HM, Samanci B, Oktay MF, Enöz M, Topçu I. Preventing cisplatin induced ototoxicity by N-acetylcysteine and salicylate. Kulak Burun Bogaz Ihtis Derg. 2010;20:173–83.
60. Greenlee H, Hershman DL, Jacobson JS. Use of antioxidant supplements during breast cancer treatment: a comprehensive review. Breast Cancer Res Treat. 2009;115:437–52.
61. Oldenburg J, Kraggerud SM, Cvancarova M, Lothe RA, Fossa SD. Cisplatin induced long-term hearing impairment is associated with specific glutathione S-transferase genotypes in testicular cancer survivors. J Clin Oncol. 2007; 25:708–14.
62. Rais-Bahrami K, Majd M, Veszelovszky E, Short BL. Use of furosemide and hearing loss in neonatal intensive care survivors. Am J Perinatol. 2004;21:329–32.
63. Abbasi AH, Ramadan R, Hoffman A, Abassi Z. Kidney-ear axis. Isr Med Assoc J. 2007;9:814–8.
64. Johnson AC. Auditory sensitivity in rats exposed to toluene and/or acetyl salicylic acid. Neuroreport. 1992;3:1141–4.
65. Cazals Y. Auditory sensorineural alterations induced by salicylate. Prog Neurobiol. 2000;62:583–631.
66. Johnson AC, Morata TC. Occupational exposure to chemicals and hearing impairment. The Nordic Expert Group for Criteria Documentation of Health Risks of Chemicals. Arbete och Hälsa. 2010;44:1–177.
67. Lataye R, Campo P, Pouyatos B, Cossec B, Blachère V, Morel G. Solvent ototoxicity in the rat and guinea pig. Neurotoxicol Teratol. 2003;25:39–50.
68. Gagnaire F, Marignac B, Blachère V, Grossmann S, Langlais C. The role of toxicokinetics in xylene induced ototoxicity in the rat and guinea pig. Toxicology. 2007; 231:147–58.
69. Hoet P, Lison D. Ototoxicity of toluene and styrene: state of current knowledge. Crit Rev Toxicol. 2008;38:127–70.
70. Lataye R, Campo P, Loquet G. Combined effects of noise and styrene exposure on hearing function in the rat. Hear Res. 2000;139:86–96.
71. Cappaert NL, Klis SF, Muijser H, Kulig BM, Smoorenburg GF. Simultaneous exposure to ethyl benzene and noise: synergistic effects on outer hair cells. Hear Res. 2001;162: 67–79.
72. Kopke R, Allen KA, Henderson D, Hoffer M, Frenz D, van de Water T. A radical demise. Toxins and trauma share common pathways in hair cell death. Ann N Y Acad Sci. 1999;884:171–91.
73. Fechter LD, Cheng GD, Rao D. Characterizing conditions that favour potentiation of noise induced hearing loss by chemical asphyxiants. Noise Health. 2000;3:11–21.
74. Muijser H, Lammers JH, Kullig BM. Effects of exposure to trichloroethylene and noise on hearing in rats. Noise Health. 2000;2:57–66.
75. Fechter LD, Chen GD, Rao D. Chemical Asphyxiants and Noise. Noise Health. 2002;4:49–61.
76. Lund SP, Kristiansen GB. Hazards to hearing from combined exposure to toluene and noise in rats. Int J Occup Med Environ Health. 2008;21:47–57.
77. Fechter LD, Klis SF, Shirwany NA, Moore TG, Rao DB. Acrylonitrile produces transient cochlear function loss and potentiates permanent noise-induced hearing loss. Toxicol Sci. 2003;75:117–23.
78. Pouyatos B, Gearhart CA, Fechter LD. Acrylonitrile potentiates hearing loss and cochlear damage induced by moderate noise exposure in rats. Toxicol Appl Pharmacol. 2005;204:46–56.
79. Vyskocil A, Truchon G, Leroux T, Lemay F, Gendron M, Gagnon F, et al. A weight of evidence approach for the assessment of the ototoxic potential of industrial chemicals. Toxicol Ind Health. 2012;28:796–819.
80. Morata TC. Chemical exposure as a risk factor for hearing loss. J Occup Environ Med. 2003;45:676–82.
81. Crawford JM, Hoppin JA, Alavanja MC, Blair A, Sandler DP, Kamel F. Hearing loss among licensed pesticide applicators in the agricultural health study. J Occup Environ Med. 2008;50:817–26.
82. Vyskocil A, Truchon G, Leroux T, Lemay F, Gendron M, Gagnon F, et al. A weight of evidence approach for the assessment of the ototoxic potential of industrial chemicals. Toxicol Ind Health. 2012;28:796–819.
83. Rabinowitz PM, Sircar KD, Tarabar S, Galusha D, Slade MD. Hearing loss in migrant agricultural workers. J Agromedicine. 2005;10:9–17.
84. Chang SJ, Chen CJ, Lien CH, Sung FC. Hearing loss in workers exposed to toluene and noise. Environ Health Perspect. 2006;114:1283–6.
85. Burr H, Lund SP, Sperling BB, Kristensen TS, Poulsen OM. Smoking and height as risk factors for prevalence and five-year incidence of hearing loss. A questionnaire based follow-up study of employees in Denmark aged 18–59 years exposed and unexposed to noise. Int J Audiol. 2005; 44:531–9.
86. Osman K, Pawlas K, Schütz A, Gazdzik M, Sokal JA, Vahter M. Lead exposure and hearing effects in children in Katowice, Poland. Environ Res. 1999;80:1–8.
87. Abbate C, Giorgianni C, Munaò F, Brecciaroli R. Neurotoxicity induced by exposure to toluene. An electrophysiologic study. Int Arch Occup Environ Health. 1993; 64:389–92.
88. Fuente A, McPherson B. Central auditory processing effects induced by solvent exposure. Int J Occup Med Environ Health. 2007;20;271–9.
89. Campo P, Maguin K, Lataye R. Effects of aromatic solvents on acoustic reflexes mediated by central auditory pathways. Toxicol Sci. 2007;99:582–90.
90. Otto DA, Fox DA. Auditory and visual dysfunction following lead exposure. Neurotoxicology. 1993;14:191–207.
91. Discalzi G, Fabbro D, Meliga F, Mocellini A, Capellaro F. Effects of occupational exposure to mercury and lead on brainstem auditory evoked potentials. Int J Psychophysiol. 1993;14:21–5.
92. Evans P, Halliwell B. Free radicals and hearing. Cause, consequence, and criteria. Ann N Y Acad Sci. 1999;884: 19–40.
93. Rao DB, Moore DR, Reinke LA, Fechter LD. Free radical generation in the cochlea during combined exposure to noise and carbon monoxide: an electrophysiological and an EPR study. Hear Res. 2001;161:113–22.
94. Fechter LD, Gearhart C, Fulton S, Campbell J, Fisher J, Na K, et al. Promotion of noise induced cochlear injury by toluene and ethyl benzene in the rat. Toxicol Sci. 2007;98:542–51.
95. Fechter LD, Gearhart C, Fulton S, Campbell J, Fisher J, Na K, et al. JP-8 jet fuel can promote auditory impairment resulting from subsequent noise exposure in rats. Toxicol Sci. 2007;98;510–25.
96. Toppila E, Pyykkö I, Starck J, Kaksonen R, Ishizaki H. Individual risk factors in the development of noise induced hearing loss. Noise Health. 2000;2:59–70.
97. Davis RR, Murphy WJ, Snawder JE, Striley CA, Henderson D, Khan A, et al. Susceptibility to the ototoxic

properties of toluene is species specific. Hear Res. 2002; 166:24–32.
98. Szanto C, Ionesco M. Influence of age and gender on hearing threshold levels in workers exposed to different intensity levels of occupational noise. Audiology. 1983; 22:339–56.
99. Kujawa SG, Liberman MC. Acceleration of age related hearing loss by early noise exposure: evidence of a misspent youth. J Neurosci. 2006;26:2115–23.
100. Mizoue T, Miyamoto T, Shimizu T. Combined effect of smoking and occupational exposure to noise on hearing loss in steel factory workers. Occup Environ Med. 2003;60:56–9.
101. Bohne BA, Harding GW, Lee SC. Death pathways in noise-damaged outer hair cells. Hear Res. 2007;223:61–70.
102. Fridberger A, Flock, Ulfendahl M, Flock B. Acoustic overstimulation increases outer hair cell Ca+ concentration and causes dynamic contraction of the hearing organ. Proc Natl Acad Sci U S A. 1998;95:7127–32.