REVIEW

# Pathogenesis of Tinnitus: Any Role for Oxidative Stress?

**Andrea Ciorba , Chiara Bianchini, Antonio Pastore,  Manuela Mazzoli**

*ENT and Audiology Department, University Hospital of Ferrara, Italy*

**Background:** Tinnitus is one of  the most prevalent disabilities in developed countries, nonetheless understanding the mechanisms of its generation still remains a challenge for physiologists, otolaryngologists and audiologists. Several authors have already claimed the possible role of oxidative stress in its pathogenesis and have therefore advocated the use of antioxidants in its treatment.

**Methods:** A systematic review of the English literature among tinnitus and oxidative stress has been performed through Medline searches.

**Results and Conclusions:** While oxidative stress could have an implication in the pathogenesis of tinnitus secondary to some cochlear pathologies, its role in idiopathic tinnitus is still unclear. Consequently, treatment with antioxidants might be suitable for tinnitus secondary to cochlear pathology induced by oxidative stress; instead concerning idiopathic tinnitus, it is still necessary to understand the etiopathologic mechanism underlying this disease, in order to then develop a causal approach.

## Introduction

Deafness and tinnitus are between the most prevalent disabilities in developed countries, and there is a considerable social and economic demand for the development of new therapeutic approaches for these conditions [1]. The reported prevalence of tinnitus in adults is estimated estimated to be in the range of 10% and 15% n developed countries [1].

It is well known that the loss of hair cells within the human inner ear results in hearing disorders that significantly reduce quality of life. The mammalian cochlea is unable to replace lost hair cells (inner and outer) and this is the cause of an irreversible hearing impairment. Tinnitus is a perception of sound in the absence of a corresponding external sound. It is reported to be a very common symptom, nonetheless understanding its generation still remains a challenge for physiologists, otolaryngologists and audiologists. Tinnitus may be present as the only symptom and in a situation of normal hearing (idiopathic tinnitus), or it can be an accompanying symptom of sensorineural hearing loss. In this case, several authors have already claimed the possible role of oxidative stress in its pathogenesis [1,2]. Thus, from the studies on ototoxicity, it is proposed that antioxidants could be used for the prevention or the repair of damage to the labyrinth[1,2].

It is well established that reactive oxygen species (ROS) are generated in hair cells exposed to several insults including cisplatin, aminoglycosides, or noise, and that antioxidant drugs can reduce the amount of intracellular damages mediated by ROS. In highly oxidative

**Corresponding address:**
Andrea Ciorba
ENT & Audiology Department
University Hospital of Ferrara
C.so Giovecca 203, 44100 Ferrara, Italy
Tel.: +39-0532-237669  Fax: +39-0532-236887

conditions, endogenous antioxidant pathways can become overwhelmed, and free oxygen radicals may become abundant. Particulary, aminoglycosides, such as gentamicin, can activate inducible nitric oxide synthase (iNOS) in inner ear tissues, triggering an increase in nitric oxide [3,4]. As a result of the increased intracellular free radical production, apoptosis (active cell death) begins, and G proteins, such as Ras, and GTPases, such as Rac are triggered. These events result in the activation of a family of stress-activated protein kinases, such as mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK). The increased activity of these enzymes is accompanied by increased intracellular Ca2+ concentrations and the release of cytochrome c from mitochondria. Cytochrome c release (usually mediated by Bax, a protein that enhances apoptotic cell death), causes mitochondrial membrane damage mainly by pore formation [3,4]. The release of cytochrome c from mitochondria activates caspase 3 and/or 8 (caspase dependent pathway); to degrade chromatin, caspase-3 acts on a set of DNAses and nucleases (PARP 1). To degrade the cell membrane and the cytoskeleton, caspase-3 acts on other molecules such as gelsolin and fodrin [5-9]. Also another class of calcium-dependent proteases, so called calpains, can be activated as a consequence of cytochrome c release from mitochondria and can lead to HCs disruption (calpain cascade) [4, 10, 11].

As a result of the above mechanisms triggered by ROS, the inner ear hair cells are damaged and then destroyed.

## Methods

Pubmed database was searched up to February 2013, Full text articles were obtained when the title, abstract or key words suggested that the study may be eligible for this review. The search was carried out independently. No language restriction was applied. Other papers were also identified from the references in the published literature.

The medical subject heading (MeSH) used included: oxidative stress, inner ear, free radicals and tinnitus.

### Oxidative stress and inner ear diseases

It has been reported that an increase of ROS intracellular level may be responsible for cochlear damage in various pathological conditions which express themselves clinically with hearing loss and tinnitus. Therefore, the

identification of possible sources of cochlear ROS and understanding the implementation mechanisms of ROS-mediated cochlear damage, could help in developing new approaches for the prevention and treatment of inner ear disorders.

As presented above, ROS are responsible for direct intracellular lipids, proteins and DNA damages, triggering apoptosis or necrosis in the inner ear [12,13]. Interestingly, it has been recently observed that the different cellular components of the cochlea, do not share the same vulnerability to the injury induced by ROS, as outer hair cells appear to be more susceptible to ROS damage, and especially those at the base of the cochlea, while supporting cells should have a greater capacity of survival [14,15]. Moreover, researches have found that glutathione (an antioxidant agent) is more expressed in the apical hair cells and NOX3, responsible for the production of superoxide, is more expressed in the basal hair cells and neurons of the spiral ganglion [14,15].

*Aminoglycosides.* Once inside the cell, aminoglycosides induce the generation of ROS due to the formation of an aminoglycoside-iron complex, which catalyses the oxidation of unsaturated fatty acids located in the inner leaflet of the plasma membrane [16, 17,18,19]. ROS subsequently activate apoptotic or necrotic intracellular pathways [16,20]. They promote the opening of the mitochondrial permeability pores and they also activate the JNK pathway leading to hair cell apoptosis [16,21,22].

*Cisplatin.* Some studies have demonstrated the direct cytotoxic mechanisms of cisplatin, including DNA damage, mitochondrial dysfunction, and the formation of ROS [16,23]. Increased intracellular ROS trigger mitochondrial release of cytochrome c, through activation of the pro-apoptotic Bcl-2 family proteins. Subsequent apoptosis is mediated by the activation of pro-caspase-9 and -3 [16,24].

*Noise.* Increasing evidence suggests that ROS have an important role in noise-induced cochlear damage: increased levels of superoxide anion, hydroxyl radical are observed in the cochlea after intense noise exposure. In addition, one of the best established endogenous "fingerprints" of ROS action is the peroxidation of polyunsaturated fatty acids. Markers of lipid peroxidation have been demonstrated in the hair cells, supporting cells,

spiral ganglion neurons and stria vascularis after noise trauma. Thereafter, ROS accumulation initiates a complex cascade of biochemical processes that includes the activation of JNK, the release of cytochrome c from the mitochondria and the activation of procaspase-8, -9 and -3 (intrinsic pathway of apoptosis) [16,25,26,27,28,29].

*Presbycusis*. This is an extremely complex, multifactorial process, implying high frequency hearing loss concomitantly with physical signs of ageing. Indeed, genes that protect against oxidative stress are involved in development of age-related hearing loss. Studies of the aging cochlea showed a decrease of antioxidant defences such as glutathione level in the auditory nerve or antioxidant enzymes in organ of Corti and spiral ganglion neurons [16,30,31,32].

*Sudden sensorineural hearing loss (SSNHL)*. Aetiology and pathogenetic mechanisms of SSNHL are still not fully clarified, even if viral infections, autoimmune processes and especially vascular disorders seem to play an important role. Whatever the cause, impaired cochlea perfusion seems to be a key pathogenetic factor of SSNHL because of cochlea terminal vascularisation and its extreme sensitivity to anoxia or hypoxia. Recent studies have stressed the importance of oxidative stress as risk a factor for microvascular damage and hypooxigenation[33]. Thus, imbalance between ROS and total antioxidant capacity is thought to be a potential pathogenetic mechanism leading to cellular damage also in SSNHL [33,34].

*Meniere Disease (MD)*. Recently Calabrese et al observed that patients affected by MD are under condition of systemic oxidative stress and that in peripheral blood of MD patients are present measurable increased markers of cellular stress response and oxidative stress factors [35,36].

In all these conditions, hair cell injury and loss can also be followed by a retraction of the peripheral processes of the auditory nerve and, later, by a gradual degeneration of spiral ganglion neurons [16,37].

Current evidence of the involvement of reactive oxygen species in the diseases of the inner ear and auditory pathways may therefore represent a possible therapeutic approach (by the use of antioxidants) to tinnitus secondary to hearing loss (i.e due to acoustic trauma) [3,38]. In particular, possible future pharmacological interventions could be aimed to: (i) interrupt the process of lipid peroxidation, thereby preserving the integrity of cell membranes (i.e. lazaroids), (ii) prevent noise induced damage mediated by ischemia / reperfusion (i.e. pentoxifylline and sartran), and (iii) to arrest the mechanisms of cellular apoptosis (ie using JNK inhibitors)[3]. These pharmacological interventions could therefore inhibit the onset/progression of hearing loss mediated by ROS, as well as reduce the perception of tinnitus [3, 39].

## Oxidative stress and idiopathic tinnitus

The pathophysiological mechanisms related to the genesis and the persistence of idiopathic tinnitus is still controversial and therefore a rational pharmacological approach to this disease cannot be proposed yet. Moreover, there are still scarce studies in the literature among the aetiology of idiopathic tinnitus.

Some have hypothized that idiopathic tinnitus could result from an aberrant neural activity in any location along the auditory pathway and that its persistence could be related to the involvement of limbic and emotional network [40]. The genesis of idiopathic tinnitus could then involve numerous etiological factors such as imbalance between excitatory and inhibitory neurotransmitters in the midbrain, hyperactivity of the auditory cortex and the dorsal cochlear nucleus, abnormal ciliar' hair cells activity, irritation of the cochlear or vestibular nucleus, and hyper-excitability of spiral ganglion neurons, which could also be induced by oxidative stress damage [2]. Dysfunction from the central cortex to the inner ear apparatus is increasingly thought to be related to biochemical pathway abnormalities and to free radical–induced oxidative damage [2]. Also, there is some evidence that psychological stress itself may cause oxidative damage in vivo, both in animal models and in humans. This phenomenon could be linked to biochemical pathway abnormalities in the midbrain and cortex and could then be linked to tinnitus [34,40,41,42,43].

In this way, there are some reports in the literature that assign to oxidative stress a role in the pathophysiology of idiopathic tinnitus. Particularly, Savastano et al (2007) have observed elevated levels of ROS in the cerebral venous blood (internal jugular vein) of patients suffering

by idiopathic tinnitus; a therapy with antioxidant agents administered orally (beta-carotene, vitamin C and E) resulted, in their study group of 31 patients, in a significant reduction of circulating ROS level, just after 48 hours from the beginning of the therapy, as well as in a reduction of the subjective tinnitus intensity (no change in auditory threshold has been observed) [44].

According to other authors, idiopathic tinnitus may be due to endothelium dysfunction within the cochlear microcirculation. They observed high levels of oxidative stress markers (as malondialdehyde, 4-hydroxynonenal, myeloperoxidase, glutathione peroxidase, nitric oxide, L-arginine, L-ornithine, thrombomodulin and von Willebrand factor) in the cerebral venous blood of patients with idiopathic tinnitus; they concluded that oxidative stress may then be responsible for endothelial damage within the cerebro-vascular district and therefore within the inner ear microcirculation [45].

Since the aetiopathogenesis of idiopathic tinnutus is still controversial, more studies are necessary in order to improve our knowledge about the pathophysiology of this condition in near feature, and therefore to develop a causal therapeutic approach.

## Conclusions

To date, oxidative stress has been implicated in the pathogenesis of some inner ear ototoxic lesions, and therefore several attempts have been made to protect cochlear cells from reactive species with antioxidants. Nonetheless, since the few experimental data available, , there is still need to more studies and of randomized controlled trials to determine which could be advantages of antioxidant therapy in humans.

Concerning idiopathic tinnitus, it is still necessary to understand the etiopatogenetic mechanism underlying this disease, in order to then develop a causal approach.

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