JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2011, 62, 2, 151-157
www.jpp.krakow.pl

Review article

R.J. REITER, D.-X. TAN, A. KORKMAZ, L. FUENTES-BROTO

DRUG-MEDIATED OTOTOXICITY AND TINNITUS: ALLEVIATION WITH MELATONIN

Department of Cellular and Structural Biology, The University of Texas Health Science Center, San Antonio, Texas USA

This review evaluates the published basic science and clinical reports related to the role of melatonin in reducing the side effects of aminoglycosides and the cancer chemotherapeutic agent cisplatin, in the cochlea and vestibule of the inner ear. A thorough search of the literature was performed using available databases for the purpose of uncovering articles applicable to the current review. Cochlear function was most frequently evaluated by measuring otoacoustic emissions and their distortion products after animals were treated with cytotoxic drugs alone or in combination with melatonin. Vestibular damage due to aminoglycosides was evaluated by estimating hair cell loss in explanted utricles of newborn rats. Tinnitus was assessed in patients who received melatonin using a visual analogue scale or the Tinnitus Handicap Inventory. Compared to a mixture of antioxidants which included tocopherol, ascorbate, glutathione and N-acetyl-cysteine, melatonin, also a documented antioxidant, was estimated to be up to 150 times more effective in limiting the cochlear side effects, evaluated using otoacoustic emission distortion products, of gentamicin, tobramycin and cisplatin. In a dose-response manner, melatonin also reduced vestibular hair cell loss due to gentamicin treatment in explanted utricles of newborn rats. Finally, melatonin (3 mg daily) limited subjective tinnitus in patients. These findings suggest the potential use of melatonin to combat the ototoxicity of aminoglycosides and cancer chemotherapeutic agents. Additional studies at both the experimental and clinical levels should be performed to further document the actions of melatonin at the cochlear and vestibular levels to further clarify the protective mechanisms of action of this ubiquitously-acting molecule. Melatonin's low cost and minimal toxicity profile supports its use to protect the inner ear from drug-mediated damage.

Key words: *antioxidant, cochlea, free radicals, melatonin, ototoxicity, tinnitus, vestibular system*

INTRODUCTION

Aminoglycosides are bactericidal aminoglycosidic. Aminocyclitols were discovered about 70 years ago when they were the most common treatment for tuberculosis and other bacterial infections (1). Aminoglycosides continue to be cost effective and widely used particularly in developing countries where the prevalence of tuberculosis remains a major threat to human health. In contrast, the use of these drugs in the developed countries has declined in recent decades because of their significant toxic side effects (2). This toxicity is especially manifested at the level of the inner ear (3) and the kidney (3, 4). In the former, the aminoglycosides reportedly cause hearing loss in roughly 33% of the individuals treated with these drugs while vestibular toxicity is roughly 15% (2). The higher rate of toxic side effects in developing countries is believed to be a consequence of their increased use in multidrug-resistant tuberculosis, their over-the-counter availability and an inadequate monitoring of auditory function during the course of what is often long term therapy (5). Included in the superfamily of the aminoglycosides are dihydrostreptomycin, tobramycin, kanamycin, amitracin and gentamicin, among others.

Ototoxicity, *i.e*., cochlear and/or vestibular toxicity, was first seen in individuals who used streptomycin and dihydrostreptomycin for the treatment of tuberculosis (6). After 1963 when gentamicin first became available, it has remained the most widely-used aminoglycoside (7, 8). While gentamicin is a highly effective treatment for idiopathic endolymphatic hydrops (Meniere's disease), a third of the patients who use the drug develop hearing loss (9). The bactericidal actions of the aminoglycosides involve a series of defined steps including their binding to ribosomes and the inhibition of protein synthesis (10).

Cisplatin, a widely-used chemotherapeutic agent but not an aminoglycoside, also has toxic actions, which limits the dose at which it is given. The negative side effects of this drug are similar to those of the aminoglycosides at the level of the inner ear.

Tinnitus, also commonly referred to as "ringing in the ears", is distracting and occasionally debilitating to the extent it may cause sleep loss. Many agents are believed responsible for causing tinnitus, *e.g*., excessively loud noise, medications, *etc*., and the currently-available treatments are less than optimally effective in reducing tinnitus.

Each of these subjects will be discussed in the current survey. The amount of information related to the protective effects of melatonin in these conditions varies widely with the bulk of data describing the benefits of the indoleamine against aminoglycoside toxicity.

AMINOGLYCOSIDE TOXICITY: MECHANISMS

Aminoglycoside molecules per se are not inherently toxic; rather, they require the redox capacity of a transition metal ion to become destructive (1). Thus, an aminoglycoside chelates a metal ion to generate chelated metal complexes (11, 12) which are redox active and, therefore, give rise to reactive oxygen (ROS) including free radicals. Once produced, these oxidizing agents have the capability of mutilating nearby biomolecules (13). There is direct evidence that at least some of the damage inflicted by aminoglycosides involves ROS and reactive nitrogen species (RNS) (14-16). The generated toxic agents not only cause molecular damage but also initiate cell death *via* apoptosis and necrosis.

There is experimental evidence supporting the role of free radicals and related reactants in ototoxicity that results from the interaction of an aminoglycoside with cellular molecules. Hence, animals over expressing cytosolic superoxide dismutase (SOD), the enzyme that metabolizes the superoxide anion radical (O_2•-) to the non-radical product hydrogen peroxide (H_2O_2), exhibit less ototoxic damage when given the aminoglycoside, kanamycin, than do control animals having normal SOD activity (17). Likewise, the inhibition of c-Jun N-terminal kinase (JNK), one of the signaling pathways activated by aminoglycosides *via* free radicals, protects the cochlear hair cells from damage due to neomycin treatment (18, 19). This finding indicates a central role for JNK in mediating the cellular implosion that occurs when cells are exposed to an aminoglycoside antibiotic. The transcription factor, nuclear factor kappa-β (NFκB), may also play a role in hair cell cytoprotection against kanamycin. When mouse outer hair cells were destroyed by kanamycin administration, a loss of p65 and p50 labeling in the nucleus was observed; immune labeling of these two submits of NFκB was preserved in the surviving hair cells, suggesting a yet undefined role for NFκB in rescuing hair cells from aminoglycoside-mediated toxicity.

Since aminoglycosides react with transition metals, *e.g.*, iron, to generate destructive ROS/RNS, it was presumed that metal chelators may be effective in ameliorating loss of hair cells caused by these drugs. Indeed, the use of iron chelators, *i.e.*, desferoxamine or dihydroxybenzoate, do reduce ototoxicity that results from aminoglycoside treatment; this is accomplished without interfering with the antibiotic activity of the drug (2).

There is also a modicum of evidence indicating that aspirin (sodium salicylate) attenuates gentamicin-mediated ototoxicity (5). In a double-blind study in which gentamicin treatment was coupled with aspirin administration, a reduced incidence of hearing loss was observed. Aspirin usage, however, was associated with gastric bleeding in a small percentage of the patients. Mechanistically, it was presumed that either the metal chelating or antioxidant activities of aspirin accounted for its protective actions against gentamicin in this clinical trial. The use of other antioxidants in an attempt to alleviate the loss of inner ear hair cells during aminoglycoside administration has been attempted with variable success. The antioxidants include *d*-methionine and lipoic acid.

MELATONIN AS AN ANTIOXIDANT

Melatonin, N-acetyl-5-methoxytryptamine, is an endogenously produced indoleamine that is best known for its ability to synchronize circadian and circannual rhythms (21, 22). While it was initially discovered as a secretory product of the pineal gland of vertebrates (23), it is in fact produced in all organisms of the animal kingdom from unicells to humans (24) and also in plants (25-27). Also, although discovered as a synthetic product of tryptophan metabolism in the mammalian pineal gland, it is now known to be produced in a variety of different cells/tissues (28), including in the cochlea (29). During evolution, melatonin's original function seems to have been as an antioxidant with its other functions being later additions to its physiological repertoire (28).

Melatonin also is an uncommonly potent direct free radical scavenger and indirect antioxidant (30, 31). Moreover, in the process of scavenging toxic free radicals, melatonin is converted to metabolites that seem to be as least as effective as melatonin itself in neutralizing free radicals and associated toxic agents. These melatonin metabolites include cyclic 3-hydroxymelatonin (32), N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) (33), N1-acetyl-5-methoxykynuramine (AMK) (34) and perhaps

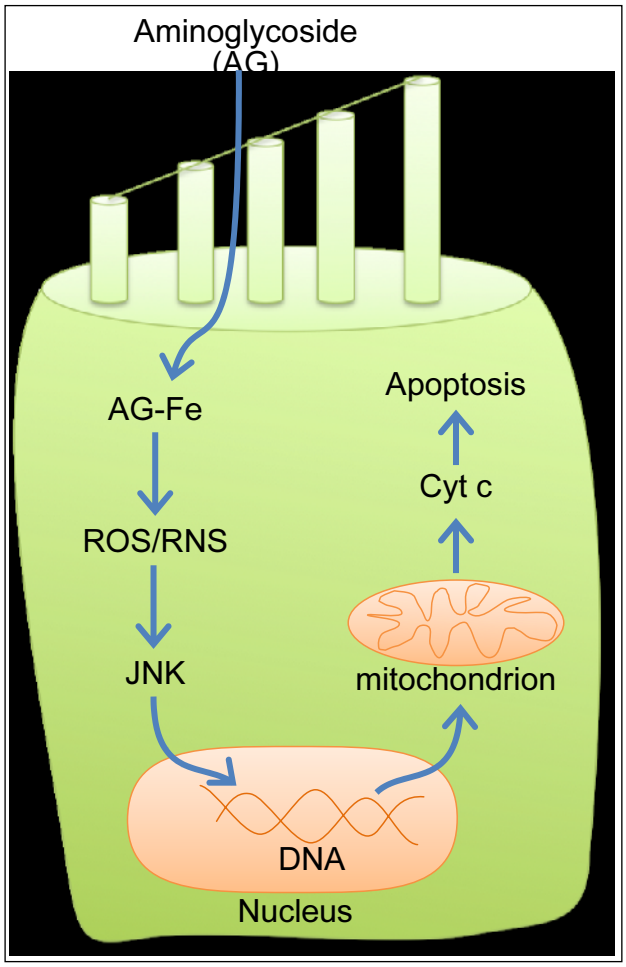

Fig. 1. Proposed mechanism of aminoglycoside (AG) toxicity at the level of the hair cell in the cochlea and utricle of the inner ear. After the AG enters the hair cell through mechano-electrical transducer channels, it forms a complex with a transition metal, in this case iron (Fe). These redox active complexes generate ROS/RNS including the superoxide anion radical, hydrogen peroxide, hydroxyl radical, peroxynitrite anion, *etc*. These reactants activate JNK (c-Jun N-terminal kinase) which then translocate (straus locates) to the nucleus to activate genes in the cell death pathway; these products are then transferred to the mitochondria (mito) where they promote the release of cytochrome c (Cyt c). In the cytosol, Cyt c triggers the activation of a series of caspases followed by apoptosis (programmed cell death) *via* what is referred to as caspase-dependent cell death. In addition to this caspase-dependent apoptotic pathway, AG may also kill cells *via* caspase-independent mechanisms.

others (35). The functional diversity of melatonin and its metabolites is emphasized by its ability to neutralize toxic derivatives of oxygen which include the most highly reactive products, the hydroxyl radical (•OH) and the peroxynitrite anion ($ONOO^-$), as well as O_2•-, H_2O_2 and singlet oxygen ($^1O_2$) (36-38). Additionally, melatonin's direct free radical scavenging activity extends from plants to unicellular animals and up to and including humans (24, 27, 28, 37-39).

In addition to its ability to directly detoxify radicals, melatonin has a variety of other means by which it quells oxidative damage. Notably, melatonin stimulates antioxidative enzymes which convert free radicals to innocuous non-reactive products (31, 40, 41), it may chelate transition metals which normally would participate in redox reactions (42), it reduces election leakage from mitochondria thereby preventing free radical formation (38, 43-45) and it promotes the synthesis of another important antioxidant, glutathione (46). Finally, melatonin and/or AMK inhibit the prooxidative enzyme, nitric oxide synthase (47). This enzyme converts arginine to nitric oxide making it available to couple with the O_2•- to form the highly aggressive $ONOO^-$.

The combination of actions of melatonin, as described above, in resisting oxidative/nitrosative stress presumably make the indoleamine an important antioxidant. In comparative studies with other well-known free radical scavenges, *e.g*., vitamins C and E, *etc*., melatonin has often been found to be better in terms of its ability to protect against molecular destruction by toxic oxygen derivatives (48). One of melatonin's advantages is seemingly its ability to enter both the lipid (for example, membranes) and aqueous (for example, cytosol and nucleoplasm) environments of the cell where it scavenges radicals and their derivatives. Often the unique solubility of antioxidants (for example, vitamin E works exclusively in the lipid portions of cells) limits their efficacy in neutralizing radicals that are produced in all subcellular compartments. Additionally, the actions of melatonin at the level of the mitochondria (43, 45, 49-51), which are normally the major site of intracellular free radical generation, may well contribute to its high efficacy in protecting against free radical damage.

ISCHEMIC AND AMINOGLYCOSIDE-INDUCED COCHLEAR TOXICITY

In the organ of Corti of the cochlea, the outer cells are crucial elements of the transduction mechanism by which environmental sound is converted to an electrical signal that is sent to the brain *via* the cochlear division of cranial nerve VIII. The hair cells, when transducing environmental sound waves, produce what is referred to as otoacoustic emissions (OAEs) (52). The OAEs are recorded by placing an external probe in the auditory canal of the temporal bone. This technique allows the detection of electrical signals representing sound using an easy and non-invasive method (53). OAEs are subclassified according to the type of acoustic stimulation used to produce them; one subcategory of OAEs is distortion products, or DPOAEs (54).

Realizing that the antioxidant melatonin is present in the cochlea (55) and that excessive free radical generation compromises the function of cochlear outer hair cells and, therefore, the recordable OAEs, Lopez-Gonzalez and colleagues (56) tested whether melatonin administration would alter the DPOAEs from hair cells experiencing excessive oxidative stress due to ischemia. In this study, melatonin (250 μg) or a mixture of other antioxidants consisting of 0.25 mg α-tocopherol, 3 mg ascorbic acid, 1 mg glutathione and 65 mg of N-acetylcysteine were injected 30 minutes in advance of death of anesthetized rats due to either decapitation or chloroform inhalation. The total antioxidant dose of the mixture was roughly 150 times greater than that of melatonin. In the control animals, DPOAEs were recorded for 2 minutes when the rats were killed by decapitation and 3 minutes when death was due to chloroform inhalation. Both melatonin and the antioxidant mixture were effective in prolonging DPOAEs after death by either means. Thus, they prolonged the recording of DPOAEs 3.5-fold and 7-fold when death was due to decapitation or chloroform exposure, respectively.

When the organ of Corti is rendered ischemic as a result to the death of the animal, the cells generate free radicals due to hypoxia and DPOAEs can be recorded. The fact that both melatonin and the antioxidant mixture prolonged the duration of the DPOAEs indicates that they successfully scavenged the radicals produced and slowed the death of the hair cells. Lopez-Gonzalez *et al*. (56) surmised, based on the doses of melatonin (250 μg) and antioxidant mixture (64.25 mg) administered, that melatonin was roughly 350 times more effective than the mixture in terms of their abilities to reduce free radical formation. Since both antioxidant concoctions seem to have been maximally effective in limiting free radical generation, however, more studies are required to determine the relative efficacies of these antioxidants.

In a related study, Lopez-Gonzalez and co-workers (16) also examined whether melatonin would influence the recorded DPOAEs measured after rats were treated with 5 daily intramuscular injections of either gentamicin (160 mg/kg body weight (BW)) or tobramycin (200 mg/kg BW). Melatonin was either given in the drinking water (10 mg/L) or as a daily subcutaneous (250 μg) or intramuscular (250 μg) injection. The ototoxicity measured as DPOAEs which accompany the administration of the aminoglycosides was maximal at 3-5 days during treatment; the DPOEAs disappeared 9 days after aminoglycoside administration was discontinued. By contrast, normal OEAs reappeared within 5 days when either gentamicin or tobramycin were given in combination with melatonin. Also, importantly, melatonin did not interfere with the antibiotic activities of the aminoglycosides as measured using antibiograms after the inoculation of either *Escherichia coli* or *Pseudomonas aeruginosa*.

Since it appears that excessive free radical generation is, at least in part, responsible for the ototoxicity of gentamicin and tobramycin, it is reasonable to assume that the ability of melatonin to function as an antioxidant may well account for its efficacy to reduce DPOEAs generated at the level of the cochlear hair cells. Considering the multiple means by which melatonin functions (as summarized above) to overcome free radical production and the associated molecular damage, which of melatonin's effects explain its protective actions in hair cells remains unknown.

AMINOGLYCOSIDE-INDUCED VESTIBULAR TOXICITY

At least as devastating as cochlear hair cell loss resulting from the use of aminoglycoside antibiotics, is the irreversible damage to the vestibular hair cells. The vestibular dysfunction that arises is sometimes unrecognized as being related to aminoglycoside use due to the sudden onset of this serious adverse side effect. Moreover, the emotional distress and financial impact related to an episode of vestibular malfunction due to aminoglycoside use may be substantial. Hence, damage to the vestibular apparatus may prolong hospitalization as well as physiotherapy and rehabilitation, but has even more long-range consequences in terms of difficulty in securing or maintaining employment and inability to operate a vehicle (57).

The hair cells of the vestibular apparatus of the inner ear are a major target for the damage inflicted by aminoglycosides. There is general agreement in the published literature that vestibulotoxicity, like ototoxicity, related to aminoglycosides

stems, at least in part, from the excessive generation of toxic free radicals in the hair cells (58); radicals not only damage these cells but often cause them to undergo apoptosis (59). Once lost, these cells cannot be replaced so the damage and associated vestibular dysfunction are permanent.

The initiation of free radical generation presumably involves the natural action of aminoglycosides to chelate iron which greatly enhances the likelihood of the hair cells to produce toxic oxygen-based radicals in excess (14, 56, 60, 61). Considering the processes described, Song and colleagues (62) found that the administration of an iron chelator to animals receiving aminoglycosides limited vestibular toxicity without changing the antibacterial properties of the drug.

Within the vestibular apparatus, the damage caused by the aminoglycosides is not uniformly distributed. Rather, damage initially develops at the top of the cristae and at the striolar regions of the maculi; it then progresses to the type I hair cells and later to the type II cells (63).

While all currently-used aminoglycosides have been found to cause vestibulotoxicity by destroying hair cells in the ampullae cristae (58, 61-63), there does seem to be some organ specificity with gentamicin seemingly being more destructive to the hair cells of the vestibule than those of the cochlea while the actions of amikacin are opposite to this (64). It was initially thought that the concentration of the specific drug within the fluid of the vestibular apparatus was a contributor to the resulting toxicity. Research has not borne this out, however, with the degree of damage to the hair cells being independent of the concentration of the aminoglycosides in the vestibular fluid (14, 65).

Since vestibular hair cell loss may involve the generation of ROS and apoptosis after gentamicin administration, Kim and colleagues (66) reasoned that the antioxidant melatonin may be useful in combating the destruction of the hair cells of the utricles of the rat. For this *ex vivo* study, utricles were obtained from the temporal bones of 2-4 day old Sprague-Dawley rats; thereafter, the explants were cultured in Dulbecco's Modified Eagle's Medium containing 1 mM gentamicin and melatonin at concentrations of either 10, 50 or 100 μM. Following the various treatments, the number of phalloidin-fluorescein isothiocyanate (phalloidin-FITC)-stained hair cells were counted in the utricles. In a dose-response manner, melatonin reduced live hair cell loss although even at the dose of 200 μm, it did not totally reverse the toxicity of 1 mM gentamicin. With the aid of the fluorescent indicator, hydrofluorescent diacetate acetyl ester, the generation of ROS was estimated and found to be significantly elevated in the utricles that were treated with gentamicin only. When the drug was combined with 50 μM melatonin, the fluorescent signal identifying ROS was weakened indicating fewer radicals being formed. Likewise, caspase-3 (estimated using CaspGLOLO fluorescein) activation was elevated in the utricles treated with gentamicin alone and reduced when the aminoglycoside was given in combination with 50 μM melatonin. Western blot analysis confirmed caspase-3 activation by gentamicin and its reduction following treatment with melatonin. In this study, Kim and co-workers (66) did not test the efficacy of the highest dose (100 μm) of melatonin in altering ROS production or caspase-3 activation.

The results of this report, which used explants of newborn rat utricles, documented the ability of melatonin to attenuate loss of hair cells, ROS generation and activation of caspase-3. The implication of these findings is that melatonin may have utility in preventing utricle hair cell loss that results from aminoglycoside toxicity *in vivo*. Kim *et al*. (66) concluded that melatonin does prevent oxidative damage and apoptosis in utricular hair cells. Since the toxicity of aminoglycosides may rely on their ability to generate excessive ROS, it is assumed that melatonin could be effective against each of these drugs in reducing utricular hair cell loss.

CISPLATIN-MEDIATED TOXICITY

There is ample justification for the assumption that melatonin may also be capable of overcoming hearing loss due to cisplatin treatment. Cisplatin, a common cancer therapeutic drug, is not an aminoglycoside but it has some similar side effects. Also, like the aminoglycoside, cisplatin is often dose-limited by its toxicity which includes damage to the inner ear (67, 68); one of the underlying mechanisms of the auditory damage that accompanies the ototoxicity of cisplatin is the excessive generation of free radicals although it also has the ability to crosslink DNA and proteins.

Since free radicals may be involved with the toxicity of cisplatin, Lopez-Gonzalez and co-workers (69) examined whether melatonin would also limit the auditory damage caused by this chemotherapy. As in their studies of aminoglycoside toxicity, these workers recorded DPOAEs from the external auditory canal of rats after their treatment with cisplatin alone or in combination with antioxidants. To produce the DPOAEs, the animals received a single intraperitoneal dose of 10 mg/kg cisplatin. Additional animals were given the cancer chemotherapeutic agent in combination with melatonin (10 mg/L in the drinking water or a daily subcutaneous dose of 250 μg) or with a mixture of antioxidants, also injected subcutaneously, that included 0.2 mg alpha-tocopherol, 3 mg ascorbic acid, 1 mg glutathione and 60 mg N-acetylcysteine.

The DPOAEs, recorded as DPgrams, were reduced by both melatonin and the antioxidant mixture to an equal amount on days 0, 5 and 15 after cisplatin administration. The difference, however, was that the antioxidant mixture contained a much higher dose of free radical scavengers than did melatonin alone. As a result, the authors concluded that, in terms of ototoxicity as estimated using DPgrams, melatonin was an estimated 350 times more effective than the antioxidant mixture in preserving the integrity of the auditory apparatus. The importance of these findings is that melatonin interferes with the toxicity of cisplatin, it does not counteract its efficacy as a cancer chemotherapy (70).

TINNITUS: REDUCTION BY MELATONIN

Tinnitus is the perception of sound when it is otherwise externally quiet. It occurs as a result of a variety of conditions including allergies, a consequence of medications (*e.g*., aspirin) or a congenital defect and may accompany natural hearing loss that is observed during aging. Tinnitus is often caused by noise-induced hearing impairment (71). When the health care professional can actually hear a sound emanating from the ear of a patient, it is referred to as objective tinnitus (72). Conversely, where no noise can be perceived by anyone other than the patient, it is referred to as subjective tinnitus (73).

Interest in the potential use of melatonin to minimize tinnitus was initiated by observation of Rosenberg *et al*. (74) who found that melatonin, taken at a dose of 3 mg daily for 30 days, caused a perceptible reduction in tinnitus. The improvement was most obvious in patients with bilateral, as opposed to unilateral, tinnitus and in those whose sleep was disturbed because of tinnitus. Melatonin is also a sleep promoting agent, however, which may have reduced the perception of tinnitus. Due to their results, the authors suggested that melatonin should be included in the armamentarium of drugs useful in treating subjective tinnitus.

A double-blind, placebo-controlled prospective study also showed that melatonin alleviated subjective tinnitus (75). In this study, melatonin (3 mg daily) was compared with the D2 dopamine receptor antagonist, sulpiride (50 mg daily), or combined with it with tinnitus perception being subjectively graded by the patients; the duration of treatment was 30 days.

Using the subjective rating scale, sulpiride reduced tinnitus by 56%, melatonin lowered it by 40% and the combined treatment caused an 81% drop in subjective tinnitus. The visual analogue scale evaluation also indicated that the combination of drugs also was the best treatment to reduce tinnitus perception. In this study it was surmised that melatonin's efficacy in limiting the perception of tinnitus stemmed from its ability to reduce the response to dopamine, possibly by inhibiting the release of the tissue biogenic amine which may be mediated by central-type benzodiazepine receptors (76). While Lopez-Gonzalez and co-workers (75) provide a logical flow diagram to illustrate how melatonin may impact tinnitus *via* a dopaminergic mechanism, the paper provides no direct support for this hypothesis.

Similar findings using a combination of melatonin and sulodexide were observed in terms of reducing tinnitus. In the 34 patients receiving this treatment, the combination of drugs improved the quality of life and reduced the subjective evaluation of the severity of tinnitus (77). Sulodexide, a glycosaminoglycan, was used because of its ability to enhance the blood flow in the labyrinthine microcirculation.

In a study that lacked a placebo-treated control group, melatonin also seemingly reduced the subjective interpretation of the severity of tinnitus in patients as assessed using the Tinnitus Handicap Inventory (78). In the same report, the effect of melatonin on sleep was estimated. As with the presumed reduction in tinnitus, melatonin also improved sleep quality as evaluated using the Pittsburgh Sleep Quality Index. There seemed to be some association between the improvements in sleep and tinnitus. The authors of this report feel melatonin may be a safe treatment for tinnitus, particularly in patients where the tinnitus is so severe it disturbs sleep.

In a recent theoretical report, Pirodda *et al*. (79) enumerated the means by which melatonin may relieve tinnitus. The proposed mechanisms include melatonin's ability, a), to reduce sympathetic drive, b), to induce a more steady hemodynamic condition resulting in a regular labyrinthine perfusion, c), to cause a reduction in skeletal muscle tone which would limit tinnitus that results from tonic contraction of the tensor tympani muscle, d), as a result of its antidepressive actions which may, *via* indirect means, reduce tinnitus or the perception of tinnitus severity, and, e), as a direct regulator of inner ear immunity.

In general, the findings summarized in the reviewed publications suggest the utility of melatonin in treating tinnitus. Considering how common-place this disturbing condition is, any treatment, especially one with the low cost and high safety profile of melatonin, should be considered for use.

CONCLUSIONS

The studies performed to date do not unequivocally establish the mechanisms by which melatonin reduces the toxicity of the aminoglycosides and cisplatin. It is known, however, that the use of these often highly-effective drugs is limited by their negative side effects. If, in fact, melatonin routinely ameliorates their toxicity in humans as it does in animals, then the dose of these drugs could perhaps be increased thereby elevating their effectiveness. To date, there are no studies showing that melatonin, combined with either aminoglycoside or cisplatin treatment, interferes with their action. Rather, the authors of the published reports specifically mention that melatonin does not attenuate the functional efficiency of either the aminoglycosides or cisplatin.

Melatonin, an endogenously-produced molecule, has frequently been used by humans for various purposes (especially as a sleep promoting agent) without evidence of toxicity (80-87). The common doses given to humans usually are in the low milligram range, *i.e*., 1-10 mg/day. Based on animal studies where melatonin has frequently been used as a pharmacological agent, these low doses in humans may be insufficient to reduce the damage done by toxic drugs like the aminoglycosides and cisplatin. Thus, higher doses should be considered in clinical studies. Melatonin has been given to normal humans at the dose of 1 g daily for 30 days with no untoward side effects being apparent (88).

In view of the observations summarized in the review, it would seem worthwhile to consider combining melatonin with toxic pharmaceuticals for the purpose of reducing the damage these latter molecules often promote at the level of the cochlear and vestibular hair cells. The more wide spread use of melatonin in both the experimental and the clinical setting and in clinical trials of several types has been previously proposed (85, 89).

Conflict of interests: None declared.

REFERENCES

1. Schacht J. Biochemical basis of aminoglycoside ototoxicity. *Otolaryngol Clin North Am* 1993; 26: 845-856.
2. Rybak LP, Ramkumar V. Ototoxicity. *Kidney Int* 2007; 72: 931-935.
3. Johnsson LG, Hawkins JE Jr, Kingsley TC, Black FO, Matz GJ. Aminoglycoside-induced cochlear pathology in man. *Acta Otolaryngol* 1981; 381: 1-19.
4. Humes DH. Aminoglycoside nephrotoxicity. *Kidney Int* 1988; 33: 900-911.
5. Chen Y, Huang HG, Zha DJ, *et al*. Aspirin attenuates gentamicin ototoxicity from the laboratory to the clinic. *Hear Res* 2007; 226: 178-182.
6. Guthrie OW. Aminoglycoside-induced ototoxicity. *Toxicology* 2008; 249: 91-96.
7. Minor LB. Gentamicin-induced bilateral vestibular hypofunction. *J Am Med Assoc* 1998; 279: 541-546.
8. Santucci KA, Krieger JN. Gentamicin for the practicing urologist: a review of the efficacy, single daily dosing and "switch therapy." *J Urol* 2000; 163: 1076-1084.
9. Nakashima T, Teranichi M, Hibi T, Kobayashi M, Umemura M. Vestibular and cochlear toxicity of aminoglycosides. *Acta Otolaryngol* 2000; 120: 904-911.
10. Davis BD. Mechanisms of bactericidal action of aminoglycosides. *Microbiol Rev* 1987; 51: 341-350.
11. Jezowska-Bojczuk M, Lesniak W. Coordination mode and reactivity of copper (II) complexes with kasugamycin. *J Inorg Biochem* 2001; 85: 99-105.
12. Yang M, Tomellini SA. Non-derivatization approach to high-performance liquid chromatography-fluorescence detection for aminoglycoside antibiotics based on a ligand displacement reaction. *J Chromatogr* 2001; 939: 59-67.
13. Jezowska-Bojczuk M, Karaczyn A, Kozlowski H. Copper (II) binding to tobramycin: potentiometric and spectroscopic studies. *Carbohydr Res* 1998; 313: 265-269.
14. Priuska E, Schacht J. Formation of free radicals by gentamicin and iron: evidence for an iron/gentamicin complex. *Biochem Pharmacol* 1995; 50: 1749-1752.
15. Song BB, Schacht J. Variable efficacy of radical scavengers and iron chelators to attenuate gentamicin ototoxicity in guinea pig in vivo. *Hear Res* 1996; 94: 87-93.
16. Lopez-Gonzalez MA, Delgado F, Lucas M. Aminoglycosides activate oxygen metabolites production in the cochlea of mature and developing rats. *Hear Res* 1999; 136: 165-168.
17. Sha SH, Zajic G, Epstein CI, Schacht, J. Overexpression of copper/zinc-superoxide dismutase protects from kanamycin-induced hearing loss. *Audiol Neurootol* 2001; 6: 117-123.

18. Pirvola IJ, Xing-Oun L, Virkkala J, *et al*. Rescue of hearing, auditory hair cells, and neurons by CEP-1347/KT7515, an inhibitor of c-Jun N-terminal kinase activation. *J Neurosci* 2000; 20: 43-50.
19. Eshraghi AA, Wang J, Adii E. Blocking c-Jun N-terminal kinase signaling can prevent hearing loss induced by both electrode insertion trauma and neomycin ototoxicity. *Hear Res* 2007; 226: 168-177.
20. Lesniak W, Pecoraro VL, Schacht J. Ternary complexes of gentamicin with iron and lipid catalyze formation of reactive oxygen species. *Chem Res Toxicol* 2005; 19: 357-364.
21. Kennaway DJ, Wright H. Melatonin and circadian rhythms. *Curr Top Med Chem* 2002; 2: 199-209.
22. Reiter RJ. The melatonin rhythm: both a clock and a calendar. *Experientia* 1993; 49: 654-664.
23. Reiter RJ. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. *Endocr Rev* 1991; 12: 151-180.
24. Hardeland R. The presence and function of melatonin and structurally related indoleamines in a dinoflagellate, and a hypothesis on the evolutionary significance of these tryptophan metabolites in unicellulars. *Experientia* 1993; 49: 614-622.
25. Dubbles R, Reiter RJ, Klemke E, *et al*. Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. *J Pineal Res* 1995; 18: 28-31.
26. Hattori A, Migitaka H, Iigo M, *et al*. Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptor in vertebrates. *Biochem Mol Biol Int* 1995; 35: 627-635.
27. Paredes SD, Korkmaz A, Manchester LC, Tan DX, Reiter RJ. Phytomelatonin: a review. *J Exp Bot* 2009; 60: 57-69.
28. Tan DX, Hardeland R, Manchester LC, *et al*. The changing biological roles of melatonin during evolution: from an antioxidant to signals of darkness, sexual selection and fitness. *Biol Rev* 2010; 85: 607-623.
29. Lopez-Gonzalez MA, Guerrero JM, Delgado F. Presence of the pineal hormone melatonin in the rat cochlea: its variation with lighting conditions. *Neurosci Lett* 1997; 238: 81-83.
30. Tan DX, Chen LD, Poeggeler B, Manchester LC, Reiter RJ. Melatonin: a potent, endogenous hydroxyl radical scavenger. *Endocrine J* 1993; 1: 57-60.
31. Rodriguez C, Mayo JC, Sainz RM, *et al*. Regulation of antioxidant enzymes: a significant role for melatonin. *J Pineal Res* 2004; 36: 1-9.
32. Tan DX, Manchester LC, Reiter RJ, *et al*. A novel melatonin metabolite, cyclic 3-hydroxymelatonin: a biomarker of in vivo hydroxyl radical generation. *Biochem Biophys Res Commun* 1998; 253: 614-620.
33. Tan DX, Manchester LC, Burkhardt, *et al*. N1-acetyl-N2-formyl-5-methoxykynuramine, a biogenic amine and melatonin metabolite, functions as a potent antioxidant. *FASEB J* 2001; 15: 2294-2296.
34. Ressmeyer AR, Mayo JC, Zelosko V, *et al*. Antioxidant properties of the melatonin metabolite, N1-acetyl-5-methoxykynuramine (AMK): scavenging of free radicals and prevention of protein destruction. *Redox Rep* 2003; 8: 205-213.
35. Hardeland R, Tan DX, Reiter RJ. Kynuramines, metabolites of melatonin and other indoles: the resurrection of an almost forgotten class of biogenic amines. *J Pineal Res* 2009; 47: 108-126.
36. Reiter RJ. Melatonin: lowering the high price of free radicals. *News Physiol Sci* 2000; 15: 246-250.
37. Tan DX, Reiter RJ, Manchester LC, *et al*. Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger. *Curr Top Med Chem* 2002; 2: 181-188.
38. Hardeland R. Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. *Endocrine* 2005; 271: 119-130.
39. Konturek PC, Konturek SJ, Celinski K, *et al*. Role of melatonin in mucosal gastroprotection against aspirin-induced gastric lesions in humans. *J Pineal Res* 2010; 48: 318-323.
40. Barlow-Waldon LR, Reiter RJ, Abe M, *et al*. Melatonin stimulates brain glutathione peroxidase activity. *Neurochem Int* 1995; 26: 497-502.
41. Pablos MI, Agapito MT, Gutierrez R, *et al*. Melatonin stimulates the activity of the detoxifying enzyme glutathione peroxidase in several tissues of chicks. *J Pineal Res* 1995; 19: 111-115.
42. Limson J, Nyokong T, Daya S. The interaction of melatonin and its precursors with aluminum, cadmium, copper, iron, lead and zinc: an absorptive voltammetry study. *J Pineal Res* 1998; 24: 15-21.
43. Leon J, Sainz RM, Mayo JC, Tan DX, Reiter RJ. Melatonin and mitochondrial function. *Life Sci* 2004; 75: 765-790.
44. Lopez A, Garcia JA, Escames G, *et al*. Melatonin protects the mitochondria from oxidative damage reducing oxygen consumption, membrane potential, and superoxide anion production. *J Pineal Res* 2009; 46: 188-198.
45. Paradies G, Petrosillo G, Paradies V, Reiter RJ, Ruggiero FM. Melatonin, cardiolipin and mitochondrial bioenergetics in health and disease. *J Pineal Res* 2010; 48: 297-310.
46. Urata Y, Homna S, Goto S, *et al*. Melatonin induces gamma-glutamylcysteine synthetase mediated by activator protein-1 in human vascular endothelial cells. *Free Rad Biol Med* 1999; 27: 838-847.
47. Leon J, Escames G, Rodriguez MI, *et al*. Inhibition of neuronal nitric oxide synthase activity by N1-acetyl-5-methoxykynuramine, a brain metabolite of melatonin. *J Neurochem* 2006; 98: 2023-2033.
48. Reiter RJ, Paredes SD, Manchester LC, Tan DX. Reducing oxidative/nitrosative stress: a newly discovered genre for melatonin. *Crit Rev Biochem Mol Biol* 2009; 44: 175-200.
49. Jou MJ, Peng TI, Yu PZ, *et al*. Melatonin protects against common deletion of mitochondrial DNA-augmented mitochondrial oxidative stress and apoptosis. *J Pineal Res* 2007; 43: 389-403.
50. Jou MJ, Peng TI, Hsu LF, *et al*. Visualization of melatonin's multiple mitochondrial levels of protection against mitochondrial Ca^{2+}-mediated permeability transition and beyond in rat brain astrocytes. *J Pineal Res* 2010; 48: 20-38.
51. Milczarek R, Hallmann A, Sokolowska K, Kaletha K, Klimek J. Melatonin enhances antioxidant action of α-tocopherol and ascorbate against NADPH- and iron-dependent lipid peroxidation in human placental mitochondria. *J Pineal Res* 2010; 49: 149-155.
52. Brownell WE. Outer hair cell electromotility and otoacoustic emissions. *Ear Hear* 1990; 11: 82-92.
53. Kemp DT, Ryan S, Bray PA. A guide to the effective use of otoacoustic emissions. *Ear Hear* 1990; 11: 93-105.
54. Henley CM, Owings MH, Stagner BB, Martin GK, Longsbury-Martin BL. Postnatal development of $2f_1$-f_2 otoacoustic emissions in pigmented rat. *Hear Res* 1989; 43: 141-148.
55. Biesalski HK, Welker HA, Thalmann R, Vollrath L. Melatonin and other serotonin derivatives in the guinea pig membraneous cochlea. *Neurosci Lett* 1988; 91: 41-46.
56. Lopez-Gonzalez MA, Guerrero JM, Rojas F, Osuna C, Delgado F. Melatonin and other antioxidants prolong postmortem activity of the outer hair cells of the organ of Corti: its relation to the type of death. *J Pineal Res* 1999; 27: 73-77.

57. Ariano RE, Zelenitsky SA, Kassum DA. Aminoglycoside-induced vestibular injury: maintaining a sense of balance. *Ann Pharmacother* 2008; 42: 1282-1289.
58. Forge A, Schacht J. Aminoglycoside antibiotics. *Audiol Neurootol* 2000; 5: 3-22.
59. Forge A, Li L. Apoptotic death of hair cells in mammalian vestibular sensory epithelia. *Hear Res* 2000; 39: 97-115.
60. Kahlmeter G, Dahlager JI. Aminoglycoside toxicity: a review of clinical studies published between 1975-1982. *J Antimicrob Chemother* 1984; 13(Suppl A): 9-22.
61. Black FO, Peterka RJ, Elardo SM. Vestibular reflex changes following aminoglycoside induced ototoxicity. *Laryngoscope* 1987; 97: 582-586.
62. Song BB, Sha SH, Schacht J. Iron chelators protect from aminoglycoside-induced cochleo- and vestibule-toxicity. *Free Rad Biol Med* 1998; 25: 189-195.
63. Proctor L, Petty B, Lietman P, Glackin R, Shimizu H. A study of potential vestibulotoxic effects of once daily versus thrice daily administration of tobramycin. *Laryngoscope* 1987; 97: 1443-1449.
64. Tran Ba Huy P, Bernard P, Schacht J. Kinetics of gentamicin uptake and release in the rat. Comparison of inner ear tissues and fluids with other organs. *J Clin Invest* 1999; 77: 1492-1500.
65. deVries PJ, Verbooyen RP, Leguit P, Verbrugh HA. Prospective randomized study of once-daily versus thrice-daily netilmicin regimens in patients with intraabdominal infections. *Eur J Clin Microbiol Infect Dis* 1990; 9: 161-168.
66. Kim JB, Jung JY, Ahn JC, Rhee CK, Hwang HJ. Antioxidant and anti-apoptotic effect of melatonin on the vestibular hair cells of rat utricles. *Clin Exp Otorhinolaryngol* 2009; 2: 6-12.
67. Ravi R, Somani SM, Rybak LD. Mechanism of cisplatin ototoxicity: antioxidant system. *Pharmacol Toxicol* 1995; 76: 386-394.
68. Kopke RD, Liu W, Gabaiyadeh R, *et al*. Use of organotypic cultures of Corti's organ to study the protective effects of antioxidant molecules on cisplatin-induced damage of auditory hair cells. *Am J Otol* 1997; 18: 559-571.
69. Lopez-Gonzalez MA, Guerrero JM, Rojas F, Delgado F. Ototoxicity caused by cisplatin is ameliorated by melatonin and other antioxidants. *J Pineal Res* 2000; 28: 73-80.
70. Reiter RJ, Tan DX, Sainz RM, Mayo JC, Lopez-Burillo S. Melatonin: reducing the toxicity and increasing the efficacy of drugs. *J Pharm Pharmacol* 2002; 54: 1299-1321.
71. Phillips JS, McFerran D. Tinnitus Retraining Therapy (TRT) for tinnitus. *Cochrane Database Syst Rev* 2010; 17: 3:CD007330.
72. Gard AP, Klopper HB, Thorell WE. Successful endovascular treatment of pulsatile tinnitus caused by a sigmoid sinus aneurysm: a case report and review of the literature. *Intern Neuroradiol* 2009; 15: 425-428.
73. Ralli M, Lobarinac E, Fetoni AR, Stolzberg D, Paludetti G, Salvi R. Comparison of salicylate- and quinine-induced tinnitus in rats: development, time course and evaluation of audiologic correlates. *Otol Neurotol* 2010; 31: 823-831.
74. Rosenberg SI, Silverstein H, Rowan PT, Olds MJ. Effect of melatonin on tinnitus. *Laryngoscope* 1998; 108: 305-310.
75. Lopez-Gonzalez MA, Santiago AM, Estaban-Ortego F. Sulpiride and melatonin decrease tinnitus perception modulating the autolimbic dopaminergic pathway. *J Otolaryngol* 2007; 36: 213-219.
76. Tenn CC, Niles LP. Central-type benzodiazepine receptors mediate the antidopaminergic effect of clonazepam and melatonin in 6-hydroxdopamine lesioned rats: involvement of a GABAergic mechanism. *J Pharmacol Exp Ther* 1995; 274: 84-89.
77. Neri G, De Stefano A, Boffa, *et al*. Treatment of central sensorineural stimulus with orally administered melatonin and sulodexide: personal experience from a randomized controlled study. *Acta Otorhinolaryngol Ital* 2009; 29: 86-91.
78. Megwalu VC, Fennell JE, Piccivillo JF. The effects of melatonin on tinnitus and sleep. *Otolaryngol Head Neck Surg* 2006; 134: 210-213.
79. Pirodda A, Raimondi MC, Ferri GG. Exploring the reasons why melatonin can improve tinnitus. *Med Hypotheses* 2010; 79: 190-191.
80. Simko F, Paulis L. Melatonin as a potential antihypertensive treatment. *J Pineal Res* 2007; 42: 319-322.
81. Aoki K, Zhao K, Yamazaki F, *et al*. Exogenous melatonin administration modifies cutaneous vasoconstriction response to whole body skin cooling in humans. *J Pineal Res* 2008; 44: 141-148.
82. Robeva R, Kirilov G, Tomova A, Kumanov PH. Melatonin-insulin interactions in patients with metabolic syndrome. *J Pineal Res* 2008; 44: 52-56.
83. Wasdell MB, Jan JE, Bomben MM, *et al*. A randomized, placebo-controlled study of controlled release melatonin treatment for delayed sleep phase syndrome and impaired sleep maintenance in children with neurodevelopmental disabilities. *J Pineal Res* 2008; 44: 57-64.
84. Gitto E, Pellegrino S, Gitto P, Barberi I, Reiter RJ. Oxidative stress of the newborn in the pre- and postnatal period and the clinical utility of melatonin. *J Pineal Res* 2009; 46: 128-139.
85. Korkmaz A, Reiter RJ, Topal T, Manchester LC, Oter S, Tan DX. Melatonin: an established antioxidant worthy of use in clinical trials. *Mol Med* 2009; 15: 43-50.
86. Chahbouni M, Escames G, Venegas C, *et al*. Melatonin treatment normalizes plasma pro-inflammatory cytokines and nitrosative/oxidative stress in patients suffering from Duchenne muscular dystrophy. *J Pineal Res* 2010; 48: 282-289.
87. de Castro-Silva C, de Bruin VM, Cunha GM, Nunes DM, Medeiros CA, de Bruin PF. Melatonin improves sleep and reduces nitrite in the exhaled breath condensate in optic fibrosis - a randomized, double-blind, placebo-controlled study. *J Pineal Res* 2010; 48: 65-71.
88. Nordlund JJ, Lerner AB. The effects of oral melatonin on skin color and on release of pituitary hormones. *J Clin Endocrinol Metab* 1977; 45: 768-774.
89. Sanchez-Barcelo A, Mediavilla MD, Tan DX, Reiter RJ. Clinical uses of melatonin: evaluation of human trials. *Curr Med Chem* 2010; 17: 2070-2095.

Received: January 12, 2011
Accepted: March 30, 2011

Author's address: Prof. Russel J. Reiter, Department of Cellular and Structural Biology, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, Texas, USA 78229; Fax: 210-576-3863; E-mail: reiter@uthscsa.edu