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14. ABSTRACT

This project aims to further both our basic understanding of the effects of different oral statins, with and without steroid drugs, on hearing loss and to compare the ability to protect hearing with the ability to protect hair cells and synapses within the cochlea. Concomitant with the laboratory studies, we are undertaking a small, innovative clinical trial to determine if the prevailing treatment (steroids) of idiopathic sudden sensorineural hearing loss can be improved by adding a short course of statins. Despite equipment failure of our original equipment and delays in the supply line due to manufacturing and COVID, we acquired our new sound booth and stand, ABR/OAE setup, noise speaker, laptop and software to run the system, oscilloscope, noise generator, and amplifier. We created a new type of mouse cage in order to be able to expose control and experimental animals simultaneously. We have validated, by dose-response, the noise exposure on noise-induced hearing loss in mice on our new equipment in our old lab and in the new laboratory location. We tested oral lovastatin, atorvastatin, pravastatin and simvastatin for protective effects against high decibel noise induced hearing loss. Lovastatin gave the best protection. Lovastatin will be used in the clinical study. Preliminary data indicates that lovastatin does not protect inner hair cell synapses. clinical protocol has been approved by the local IRB and the Army and the study is listed it on ClinicalTrials.gov. After final approvals for slight modifications, we will begin accruing patients.

15. SUBJECT TERMS

Noise induced hearing loss, Statins, Sudden Sensorineural Hearing Loss, Clinical Trial

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1. **INTRODUCTION**

This project aims to further both our basic understanding of the effects of different oral statins, with and without steroid drugs, on hearing loss, and to compare the ability to protect hearing with the ability to protect hair cells and synapses within the cochlea. Concomitant with the laboratory studies, we are undertaking a small, innovative clinical trial to determine if the prevailing treatment (steroids) for idiopathic sudden sensorineural hearing loss can be improved by adding a short course of and oral statin.

2. KEYWORDS

Noise induced hearing loss, statins, Sudden sensorineural hearing loss, steroids, clinical trial

3. ACCOMPLISHMENTS

What were the major goals of the project?

	Percent Complete
Acquire IACUC approval and ACURO approval	100%
Validate new ABR measuring equipment	100%
Measure additional ABR of statins	60%
Statistics on ABR thresholds	Ongoing
Immunolabel all cochleas, count HC and synapses Statistics (above ABR study)	Ongoing
ABR study of Statin and Steroid and controls	x
Immunolabel all cochleas, count HC and synapses, statistics (statin+steroid ABR study)	X
Acquire Human Subjects Approval	100%
Acquire HRPO approval	100%
Hire and train clinical coordinator, put patient questionnaires on line, set up clinic for study	50%
Set up drugs at the pharmacy	50%
Initiate clinical trial, acquire data, calculate results	X
Begin writing paper on clinical Trial	X
Final calculations and final paper writing	x

What was accomplished under these goals?

Major Activities:

Northwestern Medical School decreed that our entire floor and all the investigators had to move to another floor. The move date was changed multiple times. This was finally accomplished in January of 2022. After the move, we then had to setup the general laboratory, but also, we had to reset up our ABR testing in a new room in the basement. We had to purchase some replacement

equipment, including a new microphone. In the basement, we were required, once again, to calibrate the equipment and validate our earlier results. We then carried out ABR recordings on noise exposed mice, plus and minus oral statin administration; histology - dissection, immunolabeling, confocal microscopy and counting of synaptic ribbons and synapses on inner hair cells, and numbers of outer hair cells. Our IRB protocol was reapproved in September by NU and by the Army in October, but is now under review once again for the small modification of specifically naming the statin for our clinical trial, and for a slight change in timeline to ensure that the study patients were treated on the same timeline as any non-study patients who had the same medical problem. We are creating a small group to evaluate adverse reactions. The listing on clinicaltrials.gov is up-to-date. A parttime, experienced clinical coordinator (MD) was hired to start the clinical study and ensure that all the legal and scientific paperwork was created and managed. An arrangement with the Investigational Research Pharmacy was made to ensure that the delivery of research drug and placebo could be expedited over their usual procedure.

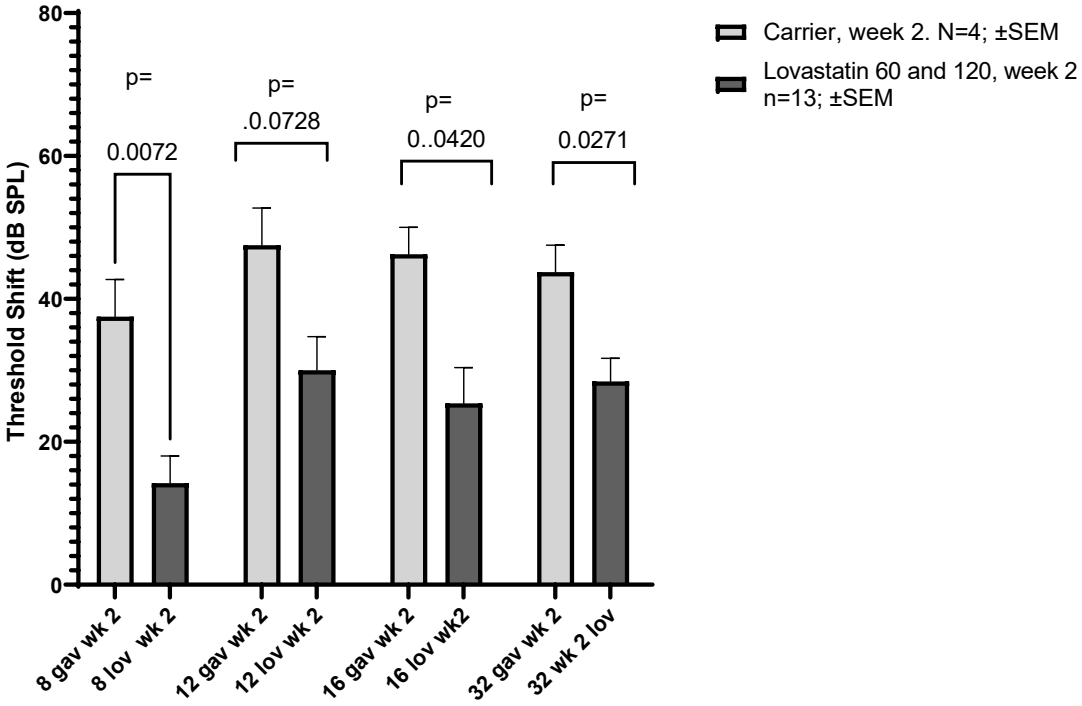
Specific objectives:

The main objectives were to get our physiology system back up and running after the move and to validate the results compared to prior results; to start our ABR studies of oral statin protection in noise exposed mice; to move the paperwork and plans forward and troubleshoot for our clinical trial.

Key outcomes:

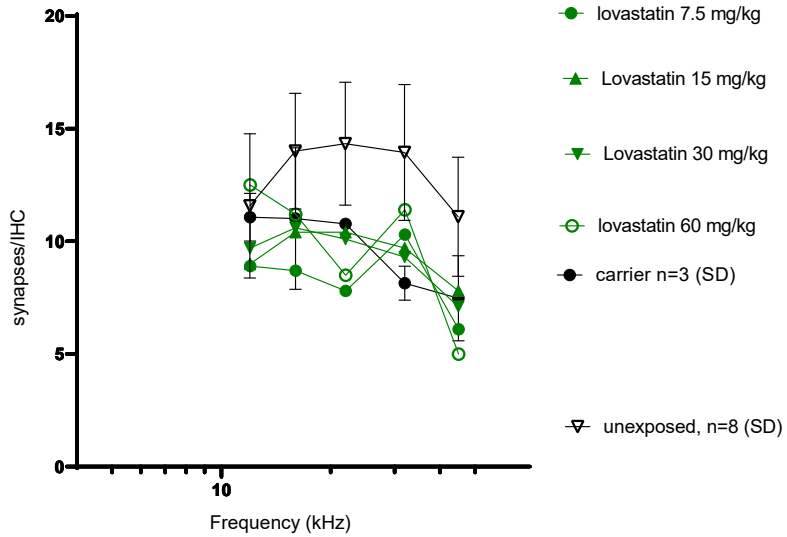
Due to the COVID delay as well as the delay caused by moving the laboratory, we truncated the initial studies of oral steroids in protection against noise induced hearing loss in mice. Since we had seen with prior work that ABR threshold elevations after 110dB SPL x 2 hour x 8-16 kHz stabilitized by 1 week after noise, we tested several doses of oral atorvastatin, lovastatin, simvastatin and pravastatin as protectants against ABR threshold elevation 1 week after noise exposure. Lovastatin (60 mg/kg) showed the best protection (Figure 1). We therefore moved forward by naming lovastatin as the drug we would test in the clinical trial. Counting of synapses and outer hair cells is ongoing. Preliminary results indicate that regardless of ABR threshold protection, lovastatin does not protect against loss of inner hair cell synapses (Figure 2). We also counted outer hair cells up to 45kHz. As seen by Fernandez et al.(Neuroscience, 2020), in the presence of high decibel noise, we maintain outer hair cells in mice regardless of drug at least to 32kHz (Figure 3). We do not have sufficient counts at 45kHz to determine if the lovastatin protects against OHC loss. We did not originally propose to use Distortion product otoacoustic emissions in these studies, but we recently worked up that methodology in our lab and can use DPOAE to gain some insight in the function of OHC after noise +/- statin in the next year. The clinical trial is in its final review by the IRB.

Figure 1. Effect of lovastatin on ABR threshold shift



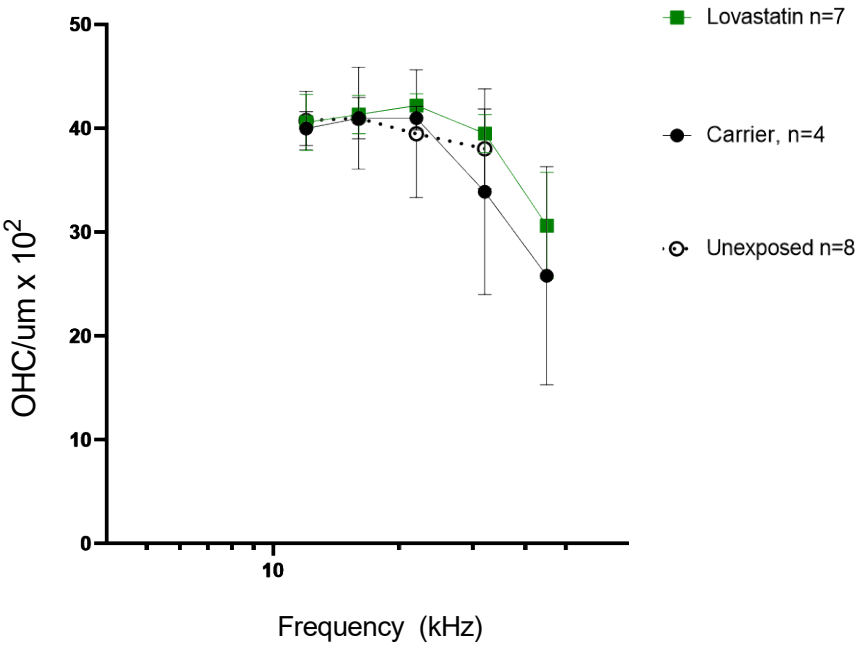
Oral Lovastatin (data from 60 mg/kg and 120 mg/kg combined) protects against high decibel noise (110 dB SPL x 2 hr x 8-16 kHz) exposure. Lovastatin given by gavage a few days before, the day of and one day after noise exposure. Threshold shifts at 2 weeks after noise are depicted. Unpaired t-test at each frequency.

Figure 2. Effect of oral lovastatin on Inner hair cell synapses



In this experiment, oral lovastatin at 60 mg/kg protected against ABR threshold shift. But none of the treatments protected against synapses loss.

Figure 3. Effect of oral lovastatin on outer hair cell numbers



Outer hair cell numbers are maintained to 32 kHz. There is no data for 45 kHz for the unexposed control. The number of outer hair cells in the lovastatin treated was similar to that in the carrier treated.

Other achievements:

Invited review: Whitlon, DS. (2022) Statins and Hearing. Hearing Research 425: 108453

What opportunities for training and professional development has the project provided?

This project was not intended to provide training and professional development opportunities. However, we do participate in weekly journal clubs with the auditory scientists across departments in the Medical School (Otolaryngology, Neurology, Anesthesiology). In addition, Dr. Depreux often works one-on-one with Dr. Richter in developing and carrying out physiological methods for our study.

How were the results disseminated to communities of interest?

Journal Club

What do you plan to do during the next reporting period to accomplish the goals?

We plan to begin the studies of steroids and statins after noise exposure, to label and count synapses and hair cells in the mice. And to add DPOAE to the study as we can. After final protocol approval by Northwestern and the Army, we will initiate the clinical trial. If possible, given the hiring problems across the country, we will hire an assistant to help with the mouse studies.

4. IMPACT

If one judges from the literature, the idea of statins and hearing is really in its infancy. From my research in writing the review article on statins and hearing, it is very clear that in humans, the

handful of studies of statins in hearing are so widely varied in methodology and results that no meaningful conclusions can be drawn. Most of the few clinical studies published are retrospective studies, so our clinical study will break new ground in being a fully prospective study with very carefully controlled inclusion and exclusion criteria. Outside the hearing field, the results may open up the awareness of possible effects of statin therapy on hearing.

Technology transfer: Nothing to report

5. CHANGES/PROBLEMS

We are still coming back from the setback of COVID and now of the move to our new laboratory. We have found hiring very difficult, but this is not unique to our University. We will now lower our requirements for hiring and add some assistants to help with physiology and with histology. There are now more COVID safeguards in place at the Hospital so we will be able to recruit patients more freely. Lately, JAX mice had trouble filling our CBA/CaJ mouse orders, but they are getting better. This mouse is widely used in auditory research so the problem is not limited to our laboratory.

The only delay now to our initiating the clinical trial is the final approval of the IRB and the ARMY to our modifications. Hiring is also taking a bit more time, as with most other laboratories.

Changes that had a significant impact on expenditures:

The main impacts had to do with delayed start of the clinical trial, the slow hiring, and the forced move of the laboratory to a new floor. The clinical trial awaits final approval. The hiring will now be done at a lower qualification level for assistants that will be overseen by the experienced Ph.Ds in my lab; The move has been sorted out.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Nothing to report.

6. PRODUCTS

Invited review: Whitlon, DS. (2022) Statins and Hearing. Hearing Research 425: 108453. Federal Support? Yes.

7. PARTICIPANTS

	Project Role	Identifier	Person-months	Contribution
Donna Whitlon, Ph.D.	PI		4.8	Experimental design, writing, calculating, troubleshooting
Claus- Peter Richter, MD, PH.D.	Co-Investigator		1.2	Auditory physiology and equipment advice and troubleshooting; software development
Sumit Dhar, Ph.D.	Co-Investigator		0.6	Audiology consult
Alan Micco, MD	Physician, Co-Investigator		0.1	Clinical Trial planning
Fred Depreux, Ph.D.	Senior Research Associated		6	ABR research
Yingjie Zhou, Ph.D.	Technician		0.5	Cochlea dissection
Aditi Argawal MD	Clinical Coordinator		0.6	Clinical coordinator setting up documents
Lyubov Czech, Ph.D.	Research Tech II		6	Histology – immunolabeling, confocal, counting of synapses and hair cells.

Has there been a change in the active other support of the PD/PI or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report

APPENDEX

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Statins and hearing

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ABSTRACT

Statins are a class of drugs that are widely used for the treatment of hyperlipidemia and the prevention of heart attack and stroke. These drugs inhibit the rate-limiting step in the synthesis of cholesterol, HMG-CoA reductase. In addition, statins have effects unrelated to cholesterol, stemming from the non-cholesterol synthesizing arms of the HMG-CoA reductase pathway and the regulation of gene expression. In multiple studies, depending on the cell type and type of statin, statins have been shown to increase antioxidant activity, decrease inflammatory mediators and alleviate damage to blood vessels. When the cochlea experiences stresses that can lead to damage and to hearing loss, increases in reactive oxygen species, inflammatory mediators and blood vessel damage are generally observed. This review summarizes the published *in vitro*, animal, and clinical studies that examine effects of statins on damaged cochlear structures and on hearing loss. Preclinical results show largely protective effects of statins and raise the possibility that statins will find a place in interventions for hearing loss. Thus far, however, clinical studies and trials are rare and inconsistent. Given the volumes of information on pharmacology and toxicology that is known about statins from their more than 30 years in medicine, relative safety of the drugs and their broad accessibility, the time is ripe for more well-controlled, prospective, and focused clinical trials to determine whether statins might be repurposed for the protection or repair of hearing.

1. Introduction

For decades, medical interventions have been sought to prevent or alleviate hearing loss. Although various chemical compounds, drugs and approaches have been studied *in vitro*, in animals and in clinical trials (Chester et al., 2021; Le Prell, 2021; Yu et al., 2020) and some have seemed promising, to date, not one drug has been approved for hearing loss by the Food and Drug Administration (FDA) of the USA.

Hearing loss can be congenital or acquired (onset is after birth). Acquired hearing loss occurs as a result of a variety of causes and insults – for examples: genes, noise exposure, drug and chemical ototoxicity, aging. The different types of cochlear insults causing hearing loss often induce similar characteristic biochemical and cellular responses in the cochlea (Prasad and Bondy, 2020), such as the generation of excessive damaging reactive oxygen species (Yu et al., 2020), an excessive inflammation and its molecular mediators (Hough et al., 2021; Yu et al., 2020; Zhang et al., 2020), and decreases in vascularity or increases in vascular pathology (Clinkard et al., 2013; Nakashima et al., 2003; Ohinata et al., 1997; Shin et al., 2019).

In the cochlea, biochemical changes induced by insults to the cochlea can lead to injury in the most often documented cell types and structures: hair cells and synapses, neurons, and cells of the stria vascularis (Eckert et al., 2021; Keithley, 2020; Mehraei et al., 2016; Waqas et al., 2018). Damage or degeneration of these cochlear cells changes the way that sound is detected and transduced in the cochlea, leading to altered signaling from the cochlea to the brain through the spiral ganglion, and causing hearing impairment/deafness. To protect hearing, medicine cannot rely on endogenous hair cell and neuron regeneration because it does not occur spontaneously in mammals.

Taking a broader perspective, the damaging cellular and biochemical responses of the cochlea to insults and stresses, given as examples above, are not unique to the cochlea, but are akin to damage and degeneration responses in other tissues and other diseases (Caso et al., 2021; Dammak et al., 2021; Ganguly et al., 2021; Park and Yang, 2021; Rodrigues et al., 2021; Ruan et al., 2021), raising the possibility that drug interventions that are effective in other regions will have positive effects in the auditory system.

The similarity in biochemical and cellular responses caused by stresses to the cochlea such as noise, aging and chemical or drug toxicity, suggests the possibility that one drug may have activity

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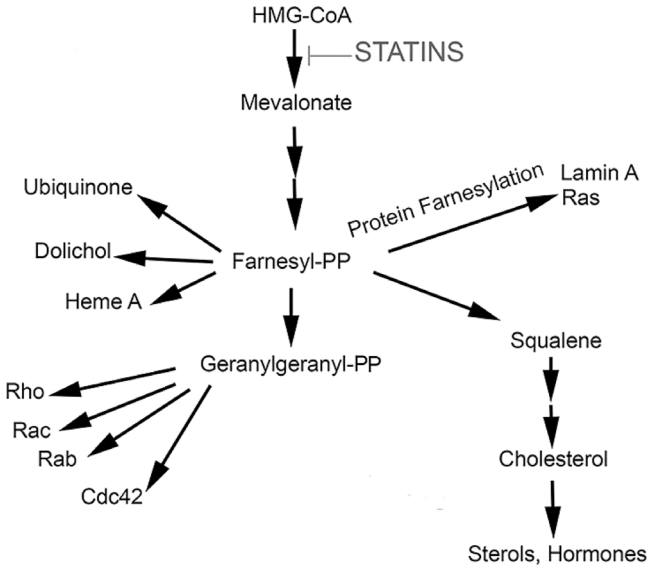

Fig. 1. The HMG-CoA reductase pathway. HMG-CoA is the starting material for a variety of biological compounds. The first step in the pathway, HMG-CoA reductase is inhibited by statins. Downstream of mevalonate is a 15-carbon isoprenoid pyrophosphate, farnesyl-pyrophosphate (farnesyl-PP). Farnesyl-PP is used not only to synthesize cholesterol but also to synthesize compounds such as ubiquinone, dolichol, heme A, squalene and geranylgeranyl-pp. It is also used in a post translational modification, farnesylation, of a variety of proteins including Lamin A and Ras. Geranylgeranyl-PP in mammalian systems is known to be the source of a 20-carbon isoprenoid modification (geranylgeranyl group) of a number of proteins including Rho, Rac, Rab and Cdc42. Modified and reprinted with permission from Whitlon et al. (2015) Novel high content screen detects compounds that promote neurite regeneration from cochlear spiral ganglion neurons. Scientific Reports 5: 15960, https://doi.org/10.1038/srep15960.

against deafness induced in different ways. However, because the responses are not identical and because thus far, no single drug has been successful in protecting or curing all of the ensuing hearing loss, it is likely that a drug or group of drugs that target different arms of the response will be most successful in protecting hearing. It also must be considered that the biochemical and pathological responses that occur in the central auditory pathways are not yet well-documented. It is possible that new drugs may also have to target impaired neuronal pathways and associated cells in the brain as well as in the cochlea.

2. Statins

Statins are a class of drugs that are widely used for the treatment of hyperlipidemia and the prevention of heart attack and stroke. The prime target of statins, and the rationale for developing these drugs for lowering serum cholesterol, is the enzyme HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase). HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, and this conversion is the rate-limiting step in the biochemical synthesis of cholesterol (Fig. 1).

Seven statins are currently used clinically: atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin and simvastatin. All of the statins inhibit the HMG-CoA reductase, but to varying degrees. The drugs have similar, but different, molecular structures, and show a range of lipophilicity or hydrophilicity (pravastatin and rosuvastatin are more hydrophilic). The chemical structures contribute to (for examples) the binding strength of the statin to the enzyme, the drug potency, the tissue selectivity, bioavailability, degradation mechanisms, uptake mechanisms and elimination half-life (McKenney, 2003)

By inhibiting HMG-CoA reductase, statins, based on their structure, inhibit cholesterol synthesis and lower serum cholesterol, by varying degrees. However, not all of the effects of the HMG-CoA reductase pathway and its inhibition by statins are confined to control of cholesterol (Fig. 1). Cholesterol is only one of multiple intermediates, multiple end products, and multiple arms of the HMG-CoA reductase pathway. The key precursor to cholesterol synthesis, before the multiple pathways diverge, is a 15-carbon isoprenoid pyrophosphate, farnesyl-pyrophosphate (FPP). FPP is a substrate for farnesyl transferase (FT), an enzyme that post-translationally adds the 15-carbon isoprenoid group to various proteins. Farther down the HMG-CoA reductase pathway and out of the direct flow to cholesterol, is the 20-carbon pyrophosphate, geranylgeranyl pyrophosphate (GGPP). GGPP is used by geranylgeranyl transferases (GGT) to post-translationally add the 20-carbon isoprenoid group to various proteins. The process of adding farnesyl or geranylgeranyl groups to proteins is termed “prenylation” and the added hydrophobicity of this modification to the protein aids in trafficking the protein to various membrane sites within the cells, which affects the protein activity and function (Wang and Casey, 2016). The PRENbase database [https://mendel.imp.ac.at/PrePS/PRENbase/] estimates known and predicted prenylated proteins in both eukaryotes and non-eukaryotes.

Examples of proteins and molecules that are prenylated: By FT: the mitochondrial redox protein Coenzyme Q (CoQ, Stefely and Pagliarini, 2017); the polyisoprenoid chain of dolichols (involved in N-glycosylation of proteins, Welti, 2013); of Heme A (part of the enzyme cytochrome oxidase, Rivett et al., 2021); of the nuclear intermediate filament protein lamin A (Murray-Nerger and Cristea, 2021); and small GTPases such as, Ras (Reddy et al., 2020; involved in signal transduction, activates enzymatic cascade, and involved in the growth, proliferation and migration of cells). By GGTs: various signaling molecules, such as the Rho family of GTPases including Rac1, RhoA, and Cdc42 (Reddy et al., 2020). The depletion of the farnesyl-PP or the geranylgeranyl-PP intermediates in the HMG-CoA reductase pathway by statins (Fig. 1), has the potential not only to affect cholesterol (through FPP) but also to diminish the prenylation of non-cholesterol related proteins. These effects of statins are called “pleiotropic” and may be the basis for some of statins’ non-cholesterol related protective as well as adverse side effects, such as diabetes, rhabdomyolysis, liver damage and myopathy.

In addition to the effects on the cholesterol levels and the activities of prenylated proteins, some statins have been shown to alter gene expression. The effects on gene expression and the mechanisms by which they occur are varied and seems to depend not only on the statin investigated, but also on the tissue or cell type under study. Additional statin-induced biochemical changes, depending on the model, are increases or decreases in neurotrophins in the rat brain (Moradi et al., 2019; Roy et al., 2015; Wang et al., 2015).

Overall, statins have been shown not only to regulate cholesterol synthesis, but also to increase antioxidant activity and decrease inflammatory mediators and in this way alleviate damage to blood vessels by multiple mechanisms. Statins can alter signaling pathways in cells and therefore have the potential to alter the signaling pathways in the cochlea and brain related to damaged auditory system and to protect or improve hearing.

3. Statins and hearing

In a medical hypothesis paper, Borghi et al. (2002) proposed that statins might be useful in treating sudden sensorineural hearing loss (SSNHL). The hypothesis was formulated with the idea that SSNHL might be caused by an impairment in the vessels serving the cochlea, limiting the oxygen flow to the organ.

Since 2002, however, there have been relatively few studies on statins and the auditory system. The existing information is var-

ied, and the results are inconsistent. This is not surprising since none of the reported methods or analyses are identical to any others. What the results have emphasized, however, is that the statins have potential to interfere with damaging biochemical mechanisms involved in propagating damage to the ear, either through cholesterol or non-cholesterol mechanisms, and perhaps to protect or repair hearing loss.

3.1. In vitro

Several studies have evaluated statins in cochlear tissue *in vitro*. Some have been carried out in primary cochlear cells in organotypic or dissociated culture and two studies are reported that used immortalized cell lines derived from cochlear tissue (STable I).

Brand et al. (2011) demonstrated by PCR that HMG-CoA reductase mRNA exists in the organ of Corti, spiral ganglion and stria vascularis in the cochlea of 5-day old rats. For an organ of Corti explant culture, a transgenic mouse expressing GFP driven by a hair cell specific Brn-3.1 promoter (on a C57BL/6J background, AF Ryan, Personal communication) was used. Brand et al. (2011) demonstrated that simvastatin, first chemically converted to its active form, could at least partially protect hair cells from apoptotic cell death in these cultures (in 10% serum) caused by the antibiotic gentamicin. The protective effects were prevented by mevalonate, the product of the simvastatin-blocked HMG-CoA reductase enzymatic reaction (Fig. 1), indicating that the formation of an intermediate in the HMG-CoA reductase pathway was necessary for gentamicin-mediated cell death. Alone, simvastatin had no toxic effect on the hair cells, even at 100 μM. When organs of Corti from 5-day old Wistar rats were exposed to 10 μM simvastatin for 1 hour, a large increase in phosphorylated AKT (p-AKT) could be detected by Western Blot. A prior study from this group, also using P5-rat basal turn cochlear sensory epithelium, indicated that the general G-protein inhibitor GDP-βS protected auditory hair cells from gentamicin, as did the FT inhibitors B581 and FTI-277, indicating that a prenylated G-protein which, they identified as H-Ras p21 protein, was involved in the gentamicin toxicity (Battaglia et al., 2003). These authors have also reported that C. difficile toxin B (an inhibitor of small GTPases Rho/Rac/Cdc42) protects outer hair cells from gentamicin in rat basal turn explants (Bodmer et al., 2002). In work by others, inhibitors of JNK signaling were shown to prevent gentamicin induced hair cell death in rat cochleas *in vitro* and in guinea pigs *in vivo* (Pirvola et al., 2000). Taken together, Bodmer et al. (2002) hypothesized that Ras-Rac/Cdc42-JNK signaling may be important in aminoglycoside toxicity signaling. It is relevant that the activities of Ras, Rac and Cdc42 are regulated by the HMG-CoA reductase prenylation system and can be inhibited by statins (Fig. 1).

In dissociated newborn mouse spiral ganglion cultures, which are mixed cultures containing spiral ganglion neurons, fibroblasts, and glia at minimum, in medium containing 10% FBS, statins promote neurite elongation from spiral ganglion neurons (Whitlon, 2017; Whitlon et al., 2015). In this assay system, originally designed as a drug screen for promoting neurite elongation, all of the hydrophobic statins but not the hydrophilic statins were active, but at different optimal concentrations. Fluvastatin and the discontinued drug cerivastatin were most sensitive at the lowest concentration tested, 1 μM. The elongation activity could be blocked by the addition of geranylgeraniol, a compound that is known to be converted to geranylgeranyl-PP *in vivo* (Crick et al., 1997). These data indicate that a prenylated protein(s) is involved in slowing neurite elongation and that by depleting this pool of geranylgeranyl-PP with statins, the regrowth is stimulated. The most likely candidate affecting neurite elongation in this model is Rho GTPase, which can be geranylgeranylated for activity. Rho has effects on the actin cytoskeleton and likely works, at least in part, through its downstream enzyme Rho Kinase (ROCK), whose chemical inhibition with H1152, also promotes neurite elongation, but is not blocked by geranylgeraniol (Lie et al., 2010; Whitlon et al., 2015; Whitlon, 2017). Inhibition of ROCK has also been shown to increase neurite growth in other *in vitro* and *in vivo* systems in mammalian CNS tissue (Lingor et al., 2007) and *in vitro* in dorsal root ganglion neurons (Fuentes et al., 2008).

Two other studies of spiral ganglion neurite growth, carried out *in vitro* under different conditions, appear to show opposite effects from those of Whitlon et al. (2015).

In the first, Leitmeyer et al. (2016) used organotypic cultures of the rat spiral ganglion from 5-day old rats and found that simvastatin (chemically converted to its active form) caused neuronal cell death. The explants contain spiral ganglion neurons, fibroblasts, and glial cells at a minimum, and were cultured in serum-free medium. Simvastatin caused a dose dependent (1–100 μM) decrease in spiral ganglion neurite number in the outgrowth zone of the explants, a dose dependent decrease in neurite length, as well as in the measured area of supporting cells surrounding the explant and in spiral ganglion neuronal survival. Addition of mevalonate did not affect either spiral ganglion neurite number, neurite length nor the area of non-neuronal cells around the explants but did partly improve neuronal survival in the presence of simvastatin. The report concluded that simvastatin is toxic for spiral ganglion neurons *in vitro* and suggested caution was warranted in using simvastatin as a potential otoprotective drug.

One image in another study shows primary organ of Corti basal turn explants from postnatal day 2 rats maintained for 3 days (Park et al., 2009). The images document a complete loss of neurons and fibers in the cultures maintained in 10 μM simvastatin, but that coculture with mevalonate blocked this loss of neurons. Although the paper presents limited experimental evidence, this study is consistent with the Leitmeyer study that simvastatin is toxic to spiral ganglion neurons.

One way to reconcile this data with the Whitlon et al. (2015) experiments, which did not see statin induced neuronal cell death, (besides differences in species and culture methods), is that the medium used for maintaining the growth in the Whitlon et al. (2015) study had about 10 times the fetal bovine serum (FBS) concentration as in the Leitmeyer et al. (2016) (serum free) and Park et al. (2009) (1.5% FBS) cultures. *In vivo*, over 95% of simvastatin that gets into the blood is carried by serum proteins (Corsini et al., 1999; Vickers et al., 1990) leaving only a small concentration of active drug. Thus, the active concentration of simvastatin, whose lowest effective dose was reported to be 5 μM in the Whitlon et al. (2015) study, may have been significantly lower in the medium than the total added dose. The 10 μM concentration reported as toxic in the serum free cultures by Leitmeyer et al. (2016), or the 10 μM concentration reported as toxic in the cultures by Park et al. (2009) with only 1.5% serum may have had a much higher active concentration of simvastatin than in the Whitlon et al. (2015) study. The differences in effects at different active drug concentrations would be consistent with the literature that indicates biphasic effects of statins in some studies – a protective effect at lower doses, which is lost at higher concentrations (Hu and Wan, 2019; Katsumoto et al., 2005; Weis et al., 2002).

In cell lines, the relevance to hearing of statin exposure is more difficult to assess. The VOT-33 mouse cochlear neuroblast cell line was created from the Immortomouse™, which contains a temperature sensitive mutant of the SV40 large T antigen gene (Jat et al., 1991; Nicholl et al., 2005). At the permissive temperature, the cells continually divide. At the nonpermissive temperature, they stop dividing. Park et al. (2009), used this line, cultured in 1.5% FBS, at the permissive temperature to examine the effects of simvastatin on cell viability. They found a decrease in cell viability of about

20% to 60% between the concentrations of 2.5 μM and 10 μM, which was reversed with mevalonate, indicating that depletion of an HMG-CoA reductase pathway metabolite was responsible for cell death, which they report as apoptotic. This, too, is consistent with other results in the literature, where statins, depending on the cell line, the statin and dose, can show pro- or anti-survival effects in dividing cells – especially cancer cells (Cerezo-Guisado et al., 2005; Chen et al., 2020; Wang et al., 2021) . In fact, statins have been proposed as single or adjunct treatments for cancer chemotherapy in certain cell types (Duarte et al., 2021; Tilija Pun and Jeong, 2021).

Another, *in vitro* model system, also derived from the Immortomouse™, is the HEI-OC1 cell line, developed from the auditory organ of this transgenic mouse. These cells divide at the permissive temperature, and at non-permissive temperature stop dividing and eventually die. Because in some reports, hyperlipidemia is correlated with risk factors for sensorineural hearing loss, Lee et al. (2020) used these cells under nonpermissive conditions (cultured with 10% FBS) and treated with palmitic acid (PA) in an attempt to model effects of diet-induced obesity. The presence of PA in the cultures caused reduced cell viability, increased number of apoptotic cells, increased reactive oxygen species, and decreased ATP. These effects of PA were partially or completely blocked by atorvastatin (0.25 μM in 10% serum,-note the low concentration). In addition, in the presence of PA, atorvastatin upregulates the mRNA expression of the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2), superoxide dismutase, catalase, thioredoxin, all involved in antioxidant activity. Atorvastatin increases the level of phosphorylated-AKT (pAKT, protein kinase B) as also seen in the Brand et al. (2011) study of simvastatin protection on the cultured organ of Corti (above). The authors hypothesize that atorvastatin promoted the viability of the PA treated cells by inhibiting mitochondrial dysfunction and excessive ROS production via upregulation of PI3K-pAKT-Nrf2 pathway. The transcription factor, Nrf2, is known to regulate gene expression of hundreds of genes, many of which are involved in redox homeostasis and inflammation (Saha et al., 2020).

Overall, the *in vitro* reports show more protective than negative effects of statins. The protective effects *in vitro* are all associated with higher serum percentages in the medium which suggests that the cell death effects might be due to elevated concentrations of free statins.

3.2. Animal models

Experimental procedures in animal models are also variable. Studies use different animal models (mouse, rat, guinea pig), different hearing loss generation (noise at different levels and energy, aging, cisplatin, hyperlipidemia) and study different statins. All, however, have shown protective effects of statins (STable II).

Syka et al. (2007) examined the effects of atorvastatin on presbycusis in the C57BL/6J mouse, that due to a mutation in the cadherin 23 gene, is a mouse model for age-related hearing loss. Animals treated with atorvastatin (10 mg/day in chow) for 2 months had higher amplitude distortion product otoacoustic emissions (DPOAE) at all frequencies than the non-treated control, indicating better outer hair cell function at this age. Atorvastatin did not affect the cholesterol levels in either the C57BL/6J treated or controls over the span of this study. Syka et al.(2007) observed decreased expression of intercellular and vascular adhesion molecules in the aortic wall of the atorvastatin treated mice, indicating reduced endothelial inflammatory activity in this region. The authors hypothesized that in addition to the heart, the drug may also improve the blood supply to the inner ear and could therefore slow down the progression of presbycusis by a cholesterol independent mechanism. This would be consistent with a previous study in gerbils indicating vascular involvement in age-related hearing loss (Gratton and Schulte, 1995).

Cai et al. (2009) examined the effect of simvastatin on hearing loss in a an ApoE knockout (KO) model (on the C57BL/6J background). These mice spontaneously develop hypercholesterolemia, endothelial dysfunction and atherosclerosis that is associated with structural and functional changes in the inner ear and hearing loss (Guo et al., 2005). Animals were fed experimental diet for 14 days from postnatal day 10. Control mice were fed regular chow. Experimental ApoE-KO mice were either fed a high lipid atherosclerotic diet (KO-A) or the same diet also containing simvastatin (KO-AS). KO-A mice demonstrated elevated click stimulated ABR thresholds (about 25 dB SPL), hair cell and spiral ganglion cell loss, elevated cholesterol, and severe atherosclerotic lesions in the aorta. The KO-AS group showed no elevation in ABR thresholds, no hair cell loss or obvious loss of neurons, less elevation in cholesterol and fewer atherosclerotic lesions. The authors conclude that simvastatin might be used to treat the sensorineural hearing loss associated with hyperlipidemia. Interestingly, in the aging study of Syka above (Syka et al., 2007) in which atorvastatin appeared to slow down the deterioration of hearing with age in the C57BL/6J mice, there was no associated reduction in hearing loss with atorvastatin in similarly aged ApoE KO mice, but the Syka et al. (2007) mice were treated with normal chow, not a high lipid atherosclerotic diet.

Lee et al. (2020) studied the effects of atorvastatin on hearing impairment in another model of hyperlipidemia in mice, using a C57BL/6J wild type mouse with diet induced obesity. The diet induced obesity mice had a significant hearing impairment (by ABR) and showed increased levels of reactive oxygen species in the cochlea as well as hair cell death. This was associated with reduced levels of activated pAKT and superoxide dismutase (SOD2). These changes were partially prevented by treatment with atorvastatin (20 mg/kg, IP, every other day).

Three studies of statins in noise-induced hearing loss (NIHL) models have been published, one in mice (Park et al., 2012), one in guinea pigs (Richter et al., 2018) and one in rats (Jahani et al., 2016).

Using 7–8-week-old Balb/c mice, Park et al. (2012) examined the effect of pravastatin (gavage 25 mg/kg daily for 5 days before noise exposure) on hearing 1 day and 14 days after noise exposure (112-dB, 1–20 kHz for 3 hours). Serum total cholesterol levels were reported to be reduced after the pravastatin treatment, but both treated and control levels were in the reference range reported by Morris et al. (1982) for their Balb/c control mice. Park et al. (2012) report about a 20 dB ABR threshold shift decrease in animals exposed to pravastatin compared to non-treated controls at 16 kHz and at 32 kHz at both 1 hour and 14 days after noise. Hair cells were counted at the 32 kHz place and the numbers were decreased in the animals exposed only to noise but maintained to near control levels in the noise+pravastatin treated animals. The authors report that the levels of 4-hydroxynonenal (4HNE) adducts (a measure of oxidative stress) increase after noise, but that the levels approached those in the unexposed mice in noise+pravastatin treated animals, suggesting that pravastatin induced protection against oxidative stress. In addition, activated Rac1 (which, among other activities, is involved in the generation of reactive oxygen species via NADPH oxidase, Hordijk, 2006), was increased immediately after exposure to noise, but was less activated in the pravastatin treatment group. The authors suggest that pravastatin protects the hair cells from oxidative stress at least in part by blocking the activation of the prenylated protein, Rac1.

In guinea pigs, which unlike the mouse strains, are outbred animals, the responses to noise exposure are more variable (Luebke and Foster, 2002). Nonetheless, Richter et al. (2018) were able to demonstrate that delivery of fluvastatin directly to one ear

via osmotic pump through a cannula in a cochleostomy, could protect the contralateral side from ABR threshold shifts caused by exposure to high level noise (120 dB SPL x 4–8 kHz x 4 hours). The protection of the contralateral side was unexpected, and the mechanism remains unexplained, however it was fortuitous. The effects of fluvastatin therefore could be assessed in a cochlea that had not been subjected to any surgical manipulations. Protection, although variable among animals, occurred in a subset of guinea pigs at each frequency measured (2,4,8, 16 and 32 kHz). The noise exposure caused a reduction in hair cells above the 1 kHz place. Hair cells were not reduced in the animals treated with fluvastatin. In addition, in a preliminary report of a mouse study by this group, contralateral hearing was protected by similarly delivered fluvastatin in the CBA/CaJ strain in animals exposed to high level noise (110 dB SPL x 2 hours x 8–16 kHz) (Whitlon et al., 2019).

It is difficult to compare the Jahani et al. (2016) study to the other NIHL reports because the paper does not report detailed methodology and it uses nonstandard interpretations of temporary (TTS) and permanent (PTS) threshold shifts for animals. In this report TTS and PTS are defined as 24 hours and 72 hours respectively after noise exposure. Rats are divided into groups which are pretreated for 2 weeks by gavage either with 3 different atorvastatin concentrations or with carrier, or not at all. Then the study exposes animals to a broadband noise (125–20,000 Hz at 110 dB SPL for 2 hours) in a glass cage and assesses hearing solely by DPOAE, using both ears of 40 animals. The authors report that atorvastatin only at 5 mg/kg, not at higher doses, prevents permanent hearing loss.

Finally, in a model of cisplatin toxicity, Fernandez et al. (2020) examined the effect of lovastatin in 12-week-old CBA/CaJ mice of both sexes. Lovastatin was begun by gavage (40 or 60 mg/kg) and carried through the entire study. After 3 days, there was a 4-day cycle of cisplatin given IP, then a 10-day recovery, followed by two more cisplatin and recovery periods, then by auditory testing. The ABR data indicated that lovastatin reduced the cisplatin hearing loss in mice by 10–22 dB SPL, and the protection varied with dose and sex of the animal. Lovastatin also reduced cisplatin-induced cochlear outer hair cell loss. The expression of mRNA for two heat shock proteins HSPD1 (HSP60) and Hmox1 (HO-1; HSP32) were significantly upregulated in the cochlea after 4 days of lovastatin treatment. Hmox1 is inducible by stress conditions caused by various stimuli, and, among other functions, is involved in regulating oxidative stress by its downstream effectors (Consoli et al., 2021).

All the mammalian models indicated above showed positive effects of statins. On the other hand, in a non-mammal model, the zebrafish lateral line, simvastatin caused hair cell death after only 1 hour of treatment (Coffin et al., 2010), albeit at a relative high concentration (100 μM).

3.3. Clinical studies

Clinical studies of statins and hearing have not provided consistent answers (STable III). Given the multiple ways that statins can function in different tissues, this should not be surprising. Complicating the picture in addition, are the many polymorphisms and alternative splicings in the human genome relating to statins, that can cause variations in statin pharmacokinetics and effectiveness, such as those in, for example, HMG-CoA reductase, in statin transporters, metabolizing enzymes, and in drug targets and pathways (Ahangari et al., 2020; Kitzmiller et al., 2016; Mangravite and Krauss, 2007; Sirtori et al., 2012).

In clinical studies of statins and hearing, drawing any conclusions about statins is not presently possible given the major proportion of retrospective studies, which, as with all retrospective studies, cannot determine cause and effect. Key data are not always available. The inclusion and exclusion criteria are nonuniform. Outcome measures, study lengths, controls for morbidities or confounding factors all differ. The retrospective studies, nonetheless, do help to raise critical questions that must be addressed with well-designed, prospective clinical trials.

The Korean National Health Insurance Service Screening Cohort was used to investigate (retrospectively) the association of previous statin use with hearing impairment in an adult population ≥40 years of age (Kim et al., 2021a). This study had the benefit of a large cohort, and the hearing-impaired persons were paired 1:4 with controls for age, sex, income and region of residence. This analysis included 4397 hearing impaired participants matched with 17,548 controls from medical claim codes between 2002 and 2015. Multiple statins were involved and the number of days of statin prescription was assessed for 2 years before the onset of hearing impairment. Within the two years of statin use assessment, the durations were not significantly different between the hearing impairment and control groups. The distributions of obesity, smoking status, systolic or diastolic blood pressure, fasting blood glucose, hemoglobin, and dyslipidemia were not different between the hearing impairment and control groups. The hearing impairment group had lower frequencies of alcohol usage and lower total cholesterol. The older subgroup (≥70 years old) had a lower rate of hearing impairment associated with long durations of statin use, and overall, the male subgroups showed a lower rate of hearing impairment related to long durations of statin use. Interestingly, in the ≥70-year-old group, it was the hydrophilic statins that were associated with the lower rate of hearing impairment. The authors report the limitations of this study included the lack of knowledge of the types of hearing impairment, that previous statin use was assessed based on prescription data – compliance could be a factor. Other confounders, such as noise exposure and stress levels could not be assessed. The doses of the statins were also not determined.

Hameed et al. (2014) report a prospective study of atorvastatin in the management of tinnitus with hyperlipidemias. Patients (n=98) with persistent tinnitus and sensorineural hearing loss of at least one year duration and elevated cholesterol were included. All patients were evaluated with the Tinnitus Handicap Questionnaire, given 40 mg atorvastatin once daily, and asked to follow a low cholesterol diet. After 8 months, the patients were divided into “responsive” (reduced cholesterol) and “unresponsive” (no reduction in cholesterol) groups depending on their serum lipids. Tinnitus score fell by 10 or more points in 70.5% of the responsive group, but only in 4.2% of the unresponsive group. The authors conclude that lowering of serum cholesterol levels in patients with tinnitus helps alleviate the symptoms of tinnitus. There were no controls on atorvastatin without the cholesterol lowering diet, nor on the diet without atorvastatin. Thus, it is not possible to determine if the lowering of the cholesterol was a cause or a concomitant change with that alleviating tinnitus.

A study of hearing and subjective tinnitus in hyperlipidemic patients evaluated the effects of statins in a prospective analysis (Yucel et al., 2019). Patients (n=84) aged 18–84 with hyperlipidemia receiving a statin between 2012 and 2013 were included. The groups were heterogeneous with 48 of the total having hypertension, 40 having coronary artery disease and 39 taking aspirin. Patients were evaluated before starting the drug and after 6 months of treatment. Several statins were represented with a total of 5 doses. No significant difference was found in the pure tone averages of the patients before and after statin use. The authors do report a significant decrease in hearing thresholds at 6 kHz. In addition, in patients using rosuvastatin, they report an increase in the speech discrimination percentages and a decrease in the tinnitus frequency, duration, severity and degree of annoyance.

The anti-tumor drug cisplatin is highly toxic to cochleas and hearing (for example, see Moke et al., 2021). Addressing this toxi-

city, a recent study evaluated statin use on cisplatin induced hearing loss in retrospective data from the Walter Reed National Military Medical Center (n=34) and the University of Rochester Medical Center (n=215), combined with prospective data from 28 patients at NIH/Johns Hopkins University (Fernandez et al., 2021). Patients were ≥18 years of age with no bilateral profound hearing loss ≤ 90 days before the start of the cisplatin treatment. All patients were newly diagnosed with head and neck squamous cell carcinomas and treated with cisplatin-based chemoradiation therapy. A follow-up audiogram was carried out ≤ 90 days from the end of the cisplatin treatment. For most of the subjects, both ears were included in the analysis, and the ears were treated independently in the data analysis due to ear specific differences in baseline hearing sensitivities and differences in radiation doses to the cochlea. Multiple statins were represented in the studied population, although atorvastatin (n=50) was most highly represented. On average, cisplatin therapy resulted in a 13.7 dB ±18.6 high frequency threshold shift (pure tone average of 6, 8 and 12.5 kHz). Threshold shifts at frequencies equal to or greater than 4 kHz were significantly reduced among cisplatin subjects taking any statin relative to non-statin users. Threshold shifts in the subset of atorvastatin users were even more reduced in a dose independent manner than in the non-statin users. Statin use did not alter the three-year overall and disease-free survival. After controlling for cumulative cisplatin dose and baseline hearing status, atorvastatin users were calculated to be 53% less likely to acquire a cisplatin induced hearing loss compared with a non-user of statin.

The Blue Mountains Hearing Study (Australia) was a population-based survey of age-related hearing loss from 1997–2004. Surviving and new members were used in this follow-up study evaluating effects of dietary intake of cholesterol and cholesterol lowering medication on hearing (Gopinath et al., 2011). After adjusting for age and sex, participants in the highest quartile of dietary cholesterol intake were calculated to have a 33% higher likelihood of having prevalent hearing loss. Although serum total cholesterol, HDL and TG were not associated with hearing loss, participants self-reporting statin use were 48% less likely to have hearing loss than non-users of statin.

Another retrospective study (Han et al, 2021) investigated the association between statin use and the risk of sensorineural hearing loss (SNHL) or tinnitus in patients with Type 2 diabetes. Patients were chosen from the records of one hospital between 2015 and 2019. The study group contained 1379 Type 2 diabetes patients with SNHL or tinnitus matched with age, sex and index year of Type 2 diabetes patients without any type of hearing loss, n=5512. Patients were considered exposed to statin if they had ever been prescribed statins within 1 year before the index date. Use of most statins, except fluvastatin and pravastatin, were associated with reduced risk of SNHL or tinnitus. The reduction in risk of SNHL or tinnitus ranged from 24.2% to 36.8% in patients using atorvastatin, pravastatin, rosuvastatin or simvastatin. When the analysis was restricted to Type 2 diabetes patients with SNHL only (n=1061, matched controls, n=4241), a 29.4% reduction in risk of SNHL was calculated in the statin group. Overall, the protective effects of statins against SNHL and tinnitus were found regardless of age and sex. The authors indicate that limitations of the study include factors that were not assessed such as smoking habits, alcohol consumption, physical activity, noise exposure, specific diabetes treatment or a comprehensive assessment of administered drugs. The severity of SNHL or tinnitus was not assessed.

Several studies have returned no effect of statins on hearing. 1) Another analysis of the Korean National Health Insurance Service-Health Screening Cohort of adults ≥40 years of age (Kim et al., 2021b) indicated that previous statin use was not associated with sensorineural hearing loss. 2) Olzowy et al. (2007) in a prospective, randomized double blind clinical trial, studied patients 60–75 years old with presbycusis. Patients were treated with atorvastatin 40 mg or placebo and hearing was measured with pure tone audiometry. Tinnitus was evaluated subjectively with a standardized questionnaire. The study reports that development of hearing thresholds after 7 and 13 months showed no significant differences between the groups, but that there was a trend toward improvement of tinnitus scores (p=0.08). 3) A retrospective study by Canis et al. (2011) was carried out to determine if the reduction of serum cholesterol by simvastatin could relive subacute tinnitus. Remission rates were studied in 58 patients after 4 months of treatment with 40 mg simvastatin and compared to that with *Ginkgo Biloba* (previously shown to have no more effect on tinnitus than placebo) as a control. The study showed no significant efficacy in treatment of subacute tinnitus.

On the negative side, a study from Taiwan evaluating the association between statin use and sudden sensorineural hearing loss (SSNHL) (Chung et al., 2015) drew its study sample from the Taiwan Longitudinal Health Insurance Database. This study had 1263 subjects aged ≥40 years of age, and was matched with sex, age, hypertension, and coronary heart disease controls. This study reported a significant difference in the prevalence of statin use between SSNHL cases and controls (27% *vs* 21.3%). After adjusting for gender, age, hypertension, coronary heart disease, diabetes, renal disease and hyperlipidemia, the study found that SSNHL was significantly associated with previous statin use, regardless of whether it was regular or irregular use. The group of statins and their doses were used as a whole and were not individually identified. The authors report several limitations of the study. The study data did not give information on the severity of the SSNHL or contain the results of audiometric examinations. Further there was no information on body mass, race, smoking, alcohol, physical activity, other medication, or noise exposure.

One case report indicated irreversible atorvastatin associated hearing loss in a 32-year-old man with Crohn's disease (Liu et al., 2012). This case must be considered with caution. Hearing loss is a rare but known side effect of IBS and Crohn's (Oudman et al., 2021). Further, the patient was on multiple drugs – oral budesonide, oral cetirizine-Pseudoephedrine, diphenoxylate/atropine, oral famotidine, oral hyoscyamine, oral prednisone, oral moxifloxacin, oral metronidazole, oral valsartan, with unknown interactive effects on hearing and Crohn's Disease.

As part of the Beaver Dam Offspring Study of aging, hearing impairment and word recognition scores were measured (Nash et al., 2011). Participants ranged in age from 21 to 84 years. After considering multiple variables, participants taking statin drugs had lower word recognition in competing message (WRCM scores) than those not taking statins. Although serum cholesterol levels were not associated with WRCM scores, the authors hypothesize that individuals taking statins may represent the ones with the worst cholesterol profiles. They suggest that the WRCM task may be capturing age related changes in the central auditory cortex.

Overall, in the clinical studies, the results run from strongly positive to no effects to strongly negative effects on hearing depending on the study. Some of the studies hint at an effect of statins on tinnitus. In sum, there was one case report and 11 clinical studies, out of which 3 were prospective clinical trials.

4. Considerations and conclusions

Several of the above studies implicate the HMG-CoA reductase pathway in mechanisms that can slow spiral ganglion neurite growth, aid mechanisms that cause hair cell death, or synthesize deleterious amount of cholesterol that may impact auditory function. The importance of the regulation of the HMG-CoA reductase pathway in hearing is additionally emphasized in a recent paper by Seist et al. (2020). This work demonstrated that a bisphosphonate,

zoledronate, an inhibitor of farnesyl pyrophosphate synthase (end product farnesyl pyrophosphate, FPP), improved hearing after noise exposure and protected inner hair cell synapses. Another recent study implicating the HMG-CoA reductase pathway in deleterious mechanisms, documented the cochlear transcriptional response to acoustic trauma (Milon et al., 2021). This study identified HMG-CoA reductase as upregulated in Schwann cells and outer hair cells and placed statins on a list of drugs that potentially could be used to oppose the noise-induced gene expression changes.

The most direct way that statins might influence hearing is to inhibit excess cholesterol synthesis for serum lipids, which some studies have correlated with hearing impairment (Hong et al., 2015; Quaranta et al., 2015; Shao et al., 2021; Shargorodsky et al., 2010; Wang et al., 2020). Other protective mechanisms could utilize the non-cholesterol related arms of the HMG-CoA reductase pathway. These include the synthesis of prenylated small GTPases that are known to be involved, by way of downstream effector pathways, in neurodegenerative diseases (Arrazola Sastre et al., 2020). In addition, the regulation of signaling pathways and the regulation of gene expression in the cochlea by various mechanisms, for example lovastatin upregulation of two heat shock proteins (Fernandez et al., 2020) and the atorvastatin upregulation by of the transcription factor Nrf2, and several downstream antioxidant enzymes (Lee et al., 2020) are possible targets for statin therapy. Nrf2 has been suggested as a target for drugs for noise induced hearing loss (Honkura et al., 2016).

Statins have been in medical use since the first statin, lovastatin, was approved by the FDA in 1987. A great deal is known about their pharmacology, pharmacokinetics, and toxicity. The statins are considered safe drugs for the majority of the population, and they are widely accessible, with many of them currently available in generic form. As we move into more specifically defined clinical trials it is important to remember that the research is at such an early stage that the relative merits of different statins for different types of hearing loss has not been investigated, and there is no way of knowing whether a short course, an intermittent course or a chronic course of statins are required to protect or repair hearing. Some of the statins, particularly the lipophilic statins, can cross the blood brain barrier, potentially exerting positive or adverse effects on the brain. Whether or not statins can and must cross the blood-labyrinth barrier to protect hearing, is important to determine. Finally, because statins can initiate a variety of biochemical responses within cells, their actions may uncover new drug targets that might be further addressed with more specifically targeted interventions.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.heares.2022.108453.

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