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Research Paper

Noise-induced trauma produces a temporal pattern of change in blood levels of the outer hair cell biomarker prestin

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ABSTRACT

Biomarkers in easy-to-access body fluid compartments, such as blood, are commonly used to assess health of various organ systems in clinical medicine. At present, no such biomarkers are available to inform on the health of the inner ear. Previously, we proposed the outer-hair-cell-specific protein prestin, as a possible biomarker and provided proof of concept in noise- and cisplatin-induced hearing loss. Our ototoxicity data suggest that circulatory prestin changes after inner ear injury are not static and that there is a temporal pattern of change that needs to be further characterized before practical information can be extracted. To achieve this goal, we set out to 1) describe the time course of change in prestin after intense noise exposure, and 2) determine if the temporal patterns and prestin levels are sensitive to severity of injury. After assessing auditory brainstem thresholds and distortion product otoacoustic emission levels, rats were exposed to intense octave band noise for 2 h at either 110 or 120 dB SPL. Auditory function was re-assessed 1 and 14 days later. Blood samples were collected at baseline, 4, 24, 48, 72 h and 7 and 14 days post exposure and prestin concentrations were measured using enzyme-linked immunosorbent assay (ELISA). Functional measures showed temporary hearing loss 1 day after exposure in the 110 dB SPL group, but permanent loss through Day 14 in the 120 dB SPL group. Prestin levels temporarily increased 5% at 4 h after 120 dB SPL exposure, but not in the 110 dB SPL group. There was a gradual decline in prestin levels in both groups thereafter, with prestin being below baseline on Day 14 by 5% in the 110 dB group (NS) and more than 10% in the 120 dB SPL group ($p = 0.043$). These results suggest that there is a temporal pattern of change in serum prestin level after noise-induced hearing loss that is related to severity of hearing loss. Circulatory levels of prestin may be able to act as surrogate biomarker for hearing loss involving OHC loss.

1. Introduction

Biomarkers in circulation are powerful indicators of normal and pathological biological processes as well as response to pharmacological treatment. They are widely used for early detection of various diseases and conditions (e.g. myocardial infarction and osteoporosis). The greater the specificity of markers to specific organs, the greater the potential to inform on the state of that organ. The inner ear has been found to possess a number of unique proteins that serve its specialized functions. We have demonstrated that an inner ear protein, otolin-1, has increased levels in circulation in patients with benign paroxysmal positional vertigo, thus providing proof of concept for use of inner ear proteins as biomarkers in the clinical setting (Parham et al., 2014; Sacks and Parham, 2015). We have also proposed that these proteins can be used as biomarkers for hearing loss (Parham, 2015). One example, prestin, is localized to the lateral plasma membrane of outer hair cells (OHCs), where electromotility occurs (Zheng et al., 2000; Liberman et al., 2002; Surovtseva et al., 2012; He et al., 2014). Electromotility is believed to be the physical process that underlies the cochlear amplifier, which means prestin plays a central role in cochlear sensitivity and tuning (Brownell et al., 1985; Zenner et al., 1985). Damage to OHCs is one of the earliest events that lead to permanent hearing loss (Saunders et al., 1991; Rybak et al., 2007). Apoptosis of OHCs is followed by phagocytosis by supporting cells (Abrashkin et al., 2006; Bird et al., 2010) and release of their cellular

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contents, including structural proteins, into circulation. Therefore, prestin is uniquely suited to serve as a biomarker of inner ear damage, and possibly hearing loss.

We have demonstrated changes in blood prestin after inner ear injury leading to hearing loss. As an initial effort, we examined the relationship of functional, histological and serological measures in a rodent model of noise trauma comparing baseline to 2 weeks post trauma. We demonstrated that elevated ABR thresholds, decreased DPOAE levels and loss of OHCs were related to decreased prestin levels (Parham and Dyherfjeld-Johnsen, 2016). We subsequently investigated whether ototoxicity resulted in similar changes (Liba et al., 2017; Naples et al., 2017). In mouse and guinea models of cisplatin-induced hearing loss, we also found changes in prestin levels. However, we noted a temporal pattern of change such that prestin rose over 3–7 days (depending on the animal model's sensitivity to ototoxicity) before decreasing. The early rise in prestin occurred in advance of changes in ABR thresholds which suggested the potential for early detection of hearing loss. We now seek to establish the temporal pattern of change in blood prestin levels after noise trauma. Since permanent noise-induced hearing loss is also characterized by injury and loss of OHCs, based on our observations in cisplatin ototocixity, we hypothesize that blood prestin levels will rise before dropping below baseline.

Besides specificity to the inner ear, prestin as a useful biomarker blood would have to demonstrate sensitivity to the severity of damage. For example, the dose regimen of cisplatin delivered dictates the severity of OHC disruption and hearing loss. Unfortunately, the higher the dose of cisplatin, the greater the morbidity and mortality is, which hampers the ability to carry out a systematic investigation in experimental models. On the other hand, magnitude of noise trauma can readily be varied without mortality or impact on the experimental models' overall well-being. Therefore, we hypothesize that blood prestin levels are not only expected to show a temporal pattern of change after noise trauma, we also believe that the amount of change in blood prestin will be related to severity of noise trauma. Here, we test these hypotheses in our established rat model of noise-induced hearing loss (Parham and Dyherfjeld-Johnsen, 2016).

2. Methods

2.1. Subjects

A total of 46 male Wistar rats were used in this study. 20 rats were assigned to noise trauma groups. Blood samples from additional 26 naïve rats were included for establishment of a larger pool of data for baseline prestin quantification. They were 6–9 weeks of age at sampling. Rats were provided ad lib access to food and water and housed in 12 h light-dark cycle. This research was approved by the Ethical Committee for Animal Research no 22 and the French Ministry for Higher Education and Research.

2.2. Noise exposure

Noise trauma was induced in awake, animals with exposure to intense octave band of noise (8–16 kHz) for 2 h. Noise-exposed rats were divided into two groups according to the intensity of the noise: 110 dB SPL (n = 11) and 120 dB SPL (n = 9). This octave band corresponds to a distance of 35–50% distance from the apex (Viberg and Canlon, 2004).

2.3. Functional measurements

Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) were recorded at baseline, and after noise trauma on Days 1 and 14. Animals were deeply anaesthetized using 80 mg/kg ketamine, 8 mg/kg xylazine and 1 mg/kg acepromazine and placed on a 35 °C recirculating heating pad inside a IAC Acoustics (North Aurora, IL) sound-proof audiology chamber throughout the recording session.

ABRs were recorded using a custom setup with AM Systems 1700 multichannel differential amplifiers and a CED Power 1401–3 interface with CED Signal Software (Cambridge Electronic Design, Cambridge, England). Three stainless steel needle electrodes were placed subdermally over the vertex, the left mastoid, and right hind leg of each animal. Tone-pips (2 ms duration, 300 repetitions at a rate of 20 stimuli/s) at 8, 16, and 24 kHz generated in MATLAB (MathWorks, Natick, MA) were delivered to the left ear through a Tucker-Davis Technologies SA-1 Stereo Amplifier using calibrated MF-1 speakers (Tucker-Davis Technologies, Alachua, FL) in closed-field configuration at attenuating intensity. Responses were band-pass filtered from 100 Hz to 5 kHz. The threshold was defined as the lowest level at which a response peak (waves I through V) was distinctly and reproducibly present. For each frequency, the sound pressure level was decreased from 90 dB SPL to 10 dB in steps of 5 dB. For ABR thresholds exceeding 90 dB SPL, a maximal value of 95 dB SPL was assigned.

DPOAEs were recorded using a Tucker-Davis Technologies RZ6 Auditory Workstation with an ER10B + Low Noise DPOAE microphone (Etymotic Research, Inc., Elk Grove Village, IL) and acoustic stimuli delivered by two calibrated MF-1 speakers in closed-field configuration. DPOAEs were recorded at fixed stimulus levels (L1 = 80 dB SPL, L2 = 70 dB SPL), with an f2/f1 ratio of 1.2. The 2f1-f2 DPOAE responses were recorded at f1 and f2 frequencies with geometric means of 4, 8, 16, 24, and 32 kHz.

2.4. Histological measurements

After audiometry and phlebotomy, on day 14, animals were deeply anaesthetized with an intraperitoneal injection of pentobarbital (100 mg/kg). The cochleae were removed, fixed with 4% paraformaldehyde, pH 7.4, for 1 h, rinsed in phosphate buffered saline (PBS) and then stored in PBS with 0.1% sodium azide. Cochleae were decalcified with 10% EDTA, pH 7.4 at room temperature for 3 days and then stained with rabbit antimyosin VIIa (1:1000; Proteus BioSciences, Ramona, CA) for 48 h at 48 °C then donkey antirabbit IgG conjugated with Alexa Fluor 594 (1:700; ThermoFisher Scientific, Waltham, MA) overnight at 48 °C. Whole cochleae were subsequently mounted on 8-well slides (#P36930; Dutscher, Brumath, France) and image stacks obtained using a Zeiss Axioimager Z2 apotome (X10; mosaic; stacks of 4 mm). Using Imaris analysis software (Bitplane, Zürich, Switzerland), markers for hair cell position were created and superimposed on the original apotome image mosaic in the Fiji image processing software, and a cochlear hair cell counting algorithm was then applied to count OHCs (Saleur et al., 2016). The region containing OHCs was manually delineated from the apex to the base and subdivided into segments of 200 mm by the algorithm. After manual correction, the number of OHCs per segment was determined and the mean number per segment of 10% distance from the apex was calculated.

2.5. Enzyme-linked immunosorbent assay (ELISA)

Half a milliter of blood was obtained from the sublingual vein in heparinated tubes from each rat under deep anesthesia at baseline. After noise exposure, additional samples were obtained at the following time intervals after trauma: 4, 24 and 72 h and 7 and 14 days. After centrifugation (2000 g) for 10 min and the resulting supernatant (plasma) was collected, frozen and stored at −80 °C. Prestin concentration in the supernatant was measured using a rat

SLC26A5 ELISA kit (MyBioSource.com) as described in the manufacturer's instruction manual. The optical density in the wells of the ELISA micro-plate was measured at 450 nm using a Biotek ELx808 plate reader and data were compiled using the KCJunior software package (BIO-TEK INSTRUMENTS, INC., Winooski, VT).

2.6. Statistical analyses

ABR threshold change was determined by subtracting ABR pre-exposure threshold from threshold on Days 1 and 14 for each subject. DPOAE level change was similarly calculated by subtracting DPOAE level on Days 1 and 14 from pre-exposure level for each subject. Descriptive statistics, including mean and standard error of the mean (SEM) were calculated. Statistical significance of the differences in the dependent variables were compared using repeated measures ANOVA. Significant interactions were followed up with one-way ANOVAs and post-hoc pairwise comparisons were carried out with least significant difference (LSD) tests. Prestin concentration change at each time point was determined by subtracting prestin level for each subject from its own pre-exposure level. Pair-wise statistical comparisons were carried out using t-tests. Small group comparisons were carried out using nonparametric Mann-Whitney *U* test. Statistical significance was set at $p = 0.05$.

3. Results

3.1. Histological findings

Noise trauma resulted in loss of hair cells in the cochleae of both noise-exposure groups. Fig. 1 shows cochleograms for 110 dB SPL (Panel A) and 120 dB SPL (Panel B) exposure levels, 14 days after trauma. Mean hair cell counts decreased strongly in the basal region above 25 kHz, more steeply so in the 120 dB SPL group. The 120 dB SPL noise-exposed group also showed smaller decreases of mean hair cell counts in the mid portion of the cochlea, between 10 and 25 kHz, as well as in the apical portion of the cochlea (particularly for the region corresponding to less than 7 kHz) compared to the 110 dB SPL group.

These results confirm that the more intense noise in the 120 dB SPL group, as expected, produced significantly greater hair cell loss than in the 110 dB SPL group ($p = 0.007$). It is also noteworthy that along with increased magnitude, there was increased variability in the magnitude of hair cell losses in the 120 dB group.

3.2. Functional findings

As predicted by the hair cell loss, noise trauma induced changes in DPOAE levels and ABR thresholds that corresponded to the level of exposure. DPOAE levels were examined from 4 to 32 kHz, a three-octave range covering approximately 60% of the cochlear length. Fig. 2 shows mean change in DPOAE levels 1 and 14 days after noise exposure. The 'DPOAE-gram' showed that on the Day 1, DPOAE levels decreased 25 dB or more, with slightly greater decreases for the 120 dB SPL group. Repeated measures ANOVAs confirmed statistically significant decreases in DPOAE level on Day 1 (Table 1). DPOAE levels were significantly below baseline on Day 1 but recovered on Day 14 to near base line for the 110 dB SPL group. No significant differences were found in DPOAE levels at baseline and Day 14 after 110 dB exposure (Table 1), suggesting many of the level decreases seen on Day 1 were temporary. Much smaller recovery was seen for the 120 SPL dB exposure group, with DPOAE levels remaining decreased relative to baseline by 15–20 dB at 8 kHz and above. Statistically significant changes in DPOAE level were present across frequencies, from Baseline to Day 1 and Day 14, but no significant difference was found between Day 1 and Day 14 levels (Table 1). These latter changes may represent permanent OHC system functional impairment or loss, and suggest little recovery after severe noise exposure.

Fig. 3 shows ABR threshold change after noise trauma. ABR thresholds rose substantially one day after trauma. There were differences between the two intensity levels in the amount of ABR threshold shift on Day 1 and the amount of ABR threshold recovery by Day 14. On Day 1, ABR thresholds increased up to 40 dB in the 110 dB SPL exposure group but the threshold shifts approached 60 dB in the 120 dB SPL exposure group. These changes were both statistically significant (Table 2). On Day 14, most of the temporary threshold shifts had resolved in the 110 dB SPL exposure group leaving behind ABR threshold elevations of less than 10 dB across frequencies. These changes were not statistically significant. In contrast, in the 120 dB SPL exposure group, only a small amount of recovery was noted, such that ABR thresholds remained elevated by about 40 dB. There was no statistically significant differences between ABR thresholds on Day 1 and Day 14. ABR thresholds remained significantly higher than baseline on Day 14 (Table 2).

3.3. Serological findings

Prestin levels in naïve, unexposed rats ranged from 125 to 245.7 pg/mL ($n = 46$; 177.9 ± 4.3, mean ± SEM). Complete time

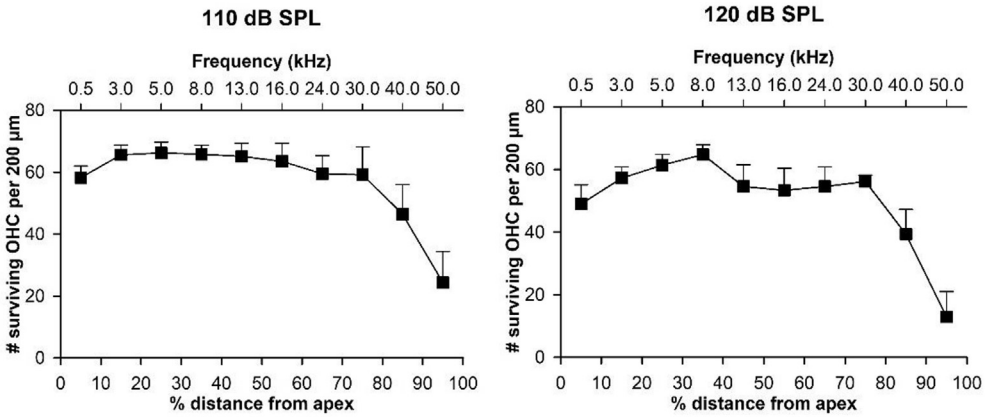

Fig. 1. Cochleograms showing mean hair cell counts for 110 (left panel) and 120 (right panel) dB SPL exposure levels as a function of distance from cochlear apex. The corresponding cochlear frequencies are shown on the upper x-axis of each plot. Error bars represent SEMs.

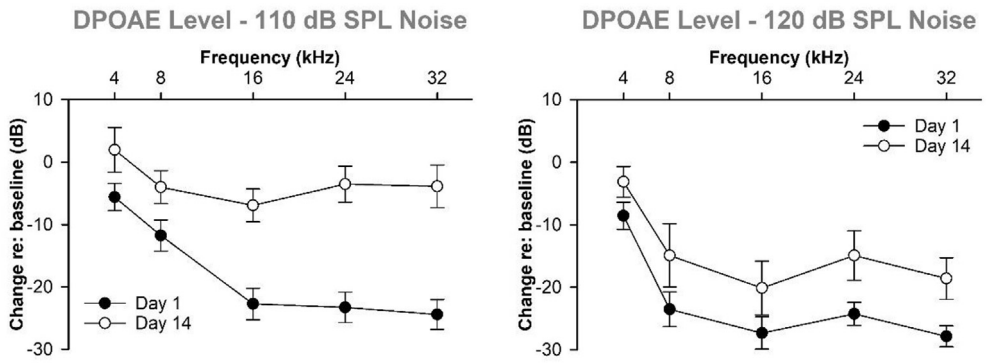

Fig. 2. Mean change in DPOAE levels relative to baseline as a function of stimulus frequency 1 and 14 days after acoustic trauma for 110 (left panel) and 120 (right panel) dB SPL exposure levels. Error bars represent standard error of the means (SEMs).

Table 1
Statistically significant results of repeated measures ANOVAs and post-hoc LSD tests examining the effects of Test Day (Baseline, and Day 1 and 14 post acoustic trauma) and Stimulus Frequency (4, 8, 16, 24 and 32 kHz) on DPOAE levels.

110 dB SPL DPOAE	p	Observed power	120 dB SPL DPOAE	p	Observed power
Day	<0.01	1.00	Day	<0.01	1.00
Stimulus Frequency	<0.01	1.00	Stimulus Frequency	0.042	0.68
Day			**Day**		
Baseline vs. Day1	<0.01		Baseline vs. Day1	<0.01	
Day 1 vs. Day 14	<0.01		Baseline vs. Day 14	<0.01	
			Day 1 vs. Day 14	<0.01	
Stimulus Frequency					
4 vs 8 kHz	<0.01		**Stimulus Frequency**		
4 vs. 16 kHz	<0.01		4 vs 8 kHz	<0.01	
4 vs. 24 kHz	<0.01		4 vs. 16 kHz	<0.01	
4 vs. 32 kHz	<0.01		4 vs. 24 kHz	<0.01	
8 vs. 24 kHz	<0.01		4 vs. 32 kHz	<0.01	
8 vs. 32 kHz	0.016				
16 vs. 24 kHz	0.029				

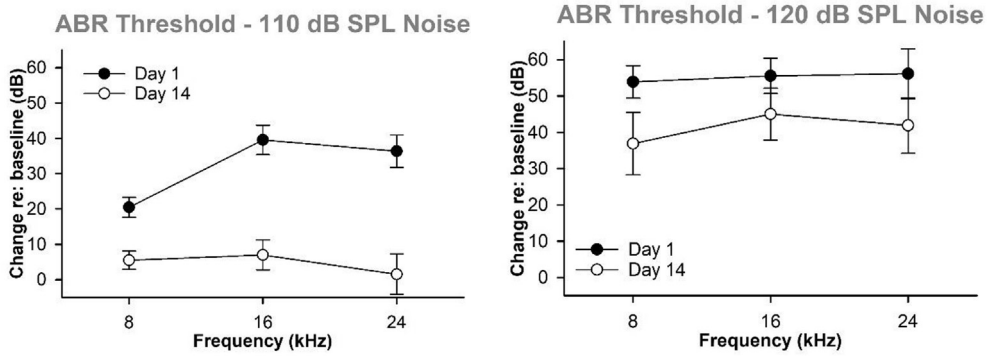

Fig. 3. Mean change in ABR thresholds relative to baseline as a function of stimulus frequency 1 and 14 days after acoustic trauma for 110 (left panel) and 120 (right panel) dB SPL exposure levels. Error bars represent standard error of the means (SEMs).

series data were obtained from 10 to 9 rats for the 110 and 120 dB SPL exposure groups, respectively. Fig. 4 shows mean change of prestin concentrations in the blood of the two groups as a function of time after noise exposure.

Little change in prestin concentrations were seen 4 h after trauma in the 110 dB SPL exposure group, while a small decrease in prestin was seen through Day 14 reaching about 10 pg/mL. In contrast, prestin concentrations increased by 15 pg/mL at 4 h after trauma in the 120 dB SPL group, but then gradually decreased producing reductions of 30 pg/mL on Day 14. In other words, the overall decrease in prestin concentration was less than 5% in the 110 dB SPL group versus more than 10% in the 120 dB SPL group. The difference between the Day 14 prestin concentrations and baseline was not statistically significant for the 110 dB SPL exposure group, but statistically significant after 120 dB SPL exposure ($p = 0.043$).

The increased prestin level at 4 h in the 120 dB SPL group was not statistically significant compared to baseline. A closer examination suggested that the majority of subjects had increased prestin levels (22.8 ± 9 pg/mL, $n = 6$) and a minority had decreased prestin levels (-17.3 ± 7 pg/mL, $n = 4$). The difference in prestin levels of these two groups was statistically significant ($p = 0.01$). The rise in prestin in the subset whose prestin levels increased relative to

Table 2
Statistically significant results of repeated measures ANOVAs and post-hoc LSD tests examining the effects of Test Day (Baseline, and Day 1 and 14 post acoustic trauma) and Stimulus Frequency (8, 16 and 24 kHz) on ABR thresholds.

110 dB SPL ABR	p	Observed power	120 dB SPL ABR	p	Observed power
Day	<0.01	1.00	Day	<0.01	1.00
Stimulus Frequency	<0.01	1.00	Stimulus Frequency	0.022	0.77
Day * Stim Freq	0.025	0.78			
			Day		
8 kHz			Baseline vs Day 1	<0.01	
Day	<0.01	1.00	Baseline vs Day 14	<0.01	
Baseline vs Day 1	<0.01				
Day 1 vs Day 14	<0.01		**Stimulus Frequency**		
16 kHz			8 vs 16 kHz	<0.01	
Day	<0.01	1.00	16 vs 24 kHz	<0.01	
Baseline vs Day 1	<0.01				
Day 1 vs Day 14	<0.01				
24 kHz					
Day	<0.01	1.00			
Baseline vs Day 1	<0.01				
Day 1 vs. Day 14	<0.01				

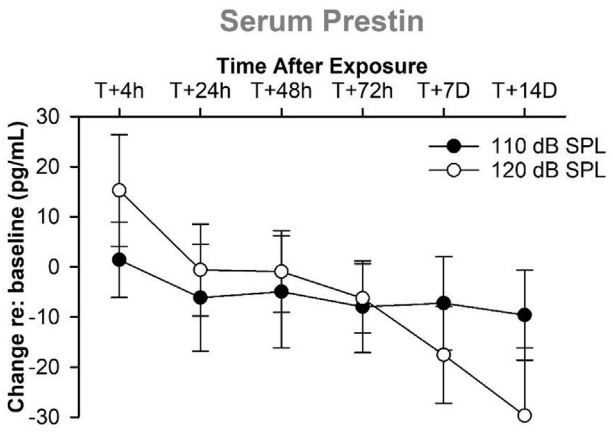

Fig. 4. Mean change in blood prestin concentrations of rats as a function of time after noise trauma at 110 and 120 dB SPL. Error bars represent SEMs.

baseline at 4 h was statistically significant ($p = 0.03$). The decrease in DPOAE levels of this group tended to be greater on Day 1 (-25.6 ± 1.4 dB) and Day 14 (-16.4 ± 3.3 dB) than those with decreased prestin levels (-17.2 ± 1.8 and -10.3 ± 5.2, respectively). The difference between the two groups was statistically significant on Day 1 ($p = 0.01$), but not on Day 14 due to small number of subjects and increased variability.

4. Discussion

Our histological findings suggest that 110 dB SPL exposure level was associated with substantially less hair cell loss than the 120 dB SPL level. At the 120 dB SPL exposure level, hair cell losses were greatest in the basal region of the cochlea, followed by cochlear apex, but losses were also present all along the cochlear length. Our functional findings suggest that although significantly decreased DPOAE levels and increased ABR thresholds were seen at the 110 dB SPL exposure level one day after acoustic trauma, much of these changes resolved by 14 days after trauma. In contrast, at the 120 dB SPL exposure level, significantly decreased DPOAE levels and increased ABR thresholds showed little recovery by day 14 after trauma. Thus, there was agreement between our functional and histological findings to the extent that significantly greater loss of OHCs at 120 dB SPL was associated with a greater extent of functional changes after 120 dB SPL noise exposure than after 110 dB SPL. The DPOAE level and ABR threshold changes at 120 dB SPL at 14 days after trauma, however, appeared to be greater than that expected from the observed OHCs losses. Similar disproportionate changes between functional and histological measures, after noise trauma, have been previously reported (Harding et al., 2002; Chen and Fechter, 2003) and support the need for additional tools to measure of the effects of noise on the inner ear.

Consistent with the differences in OHC loss and ABR threshold and DPOAE level changes, temporal changes in the blood prestin concentration showed two different patterns. After the 110 dB SPL exposure, a small, but not statistically significant, decrease in mean prestin levels was found. In contrast, after 120 dB SPL exposure, there was a small rise in prestin levels, followed by a gradual decrease, which reached statistical significance 14 days after trauma. While the temporal pattern of the change in prestin appeared to be sensitive to the severity of damage, our data suggest that the best windows for assessing severity of injury is either early (a few hrs) or 2 weeks after exposure. In between these time points, there was no significant difference between the prestin levels after 110 or 120 dB SPL exposures. We previously postulated that the temporal pattern of change in prestin levels after exposure reflects the time course of injury (Liba et al., 2017). Specifically, early on after intense exposure, OHC injury is synchronous with a large population undergoing apoptosis and therefore, release of prestin simultaneously. With time, as the OHC die, there is less release of prestin into circulation and prestin levels are lower than baseline 14 days after exposure, presumably reflecting a dynamic equilibrium established with the lower number of surviving OHCs. Studies of prestin expression in the cochlea confirm a temporal pattern of change (Chen, 2006; Mazurek et al., 2007; Xia et al., 2013; Song et al., 2015). These studies also suggest that another factor that influences local prestin levels beyond the first few days is increased prestin expression in the remaining OHCs. Noise-induced hearing loss is associated with elevated prestin gene expression in the remaining OHCs, peaking at the 3rd post-exposure day and return to baseline 4 weeks later (Chen, 2006). Furthermore, there appears to be a base-to-apex gradient in prestin mRNA expression (Mazurek et al., 2007). Similarly, after noise-induced hearing loss resulting in OHC loss in the basal turn of the cochlea, prestin mRNA expression in residual, apical OHCs increases 7 days to 1 month later (Xia et al., 2013). We did not explore time points beyond 2 weeks after trauma, but the prestin changes had not plateaued in the 120 dB SPL exposure group at 14 days after trauma. Additional studies are needed to document the temporal pattern of change in blood prestin concentrations up to a month or more after exposure, as

further decreases in prestin level may occur as increased expression in the residual hair cells resolves. Further studies correlating changes in local and circulatory prestin levels could be helpful because they may have not only have implications for hearing loss, but also tinnitus in the post-exposure period.

It is, generally accepted that excess accumulation of reactive oxygen species and the accompanying degeneration and inflammation in the cochlea are the basis of many forms of sensorineural hearing loss. When comparing the temporal time course of changes in blood prestin concentrations in this study to that of our previous investigation of cisplatin ototoxicity, interesting differences are also noted. Specifically, a small rise in prestin levels occurred within hours in noise-exposed animals. In contrast, the rise in prestin levels continued over 3–7 days in cisplatin-exposed animals (Liba et al., 2017; Naples et al., 2017). This implies a differing time course of OHC damage onset between two different insults which may be related to the fact that early noise-induced injury combines an immediate mechanical event with metabolic stress (Yamashita et al., 2004), or could be related to lasting retention of cisplatin in the cochlea (Breglio et al., 2017) while acoustic trauma is a temporally limited event.

Changes in ABR thresholds and DPOAE levels shortly after exposure cannot reliably predict permanent or temporary hearing loss. In contrast, a rise in prestin early after intense trauma appears to be predictive of worse outcomes and permanent hearing loss. This predictive potential of prestin may have clinical implications. For example, the ability to distinguish temporary from permanent injury could identify patients who may require aggressive treatment. Although approved treatment options are not currently available, there is active development of otoprotectant treatments with clinical trials being in progress or planned. We have also reasoned that these early time frames, characterized by a rise in prestin, may in fact offer a “therapeutic window” during which intervention may still improve outcomes (Naples et al., 2017). Additional work is needed to investigate this potential.

Exactly how prestin ends up in circulation also need further investigation. Cellular breakdown products after damage are typically released into the blood for disposal and/or recycling. In fact, our exploration of inner ear biomarkers was inspired by disposal of collagen breakdown products from bone resorption into circulation, where they are used as biomarkers of osteoporosis (Parham et al., 2014). In the cochlea, dead OHCs from acoustic or ototoxic injury are phagocytosed by supporting cells in the organ of Corti (Abrashkin et al., 2006). This process is initiated within minutes of hair cell apoptosis and concurrent with loss of hair cell integrity (Bird et al., 2010). With degeneration of OHCs, prestin can be found in phagosomes in the supporting cells (Abrashkin et al., 2006). Our results support a view that at least some of contents of these phagosomes will end up in circulation (Parham, 2015). Release of membrane proteins into circulation after apoptosis where they may act as biomarkers have been demonstrated in other organ systems (Wells et al., 1997; Lavallard et al., 2011).

The current results add further promise to the potential of blood prestin as a biomarker target for therapeutic interventions. While our work has been primarily focused on assessing change in blood prestin concentration after ablation of OHCs, we foresee another potential application. Much effort is directed at regeneration of hair cells after injury, including in mammalian species. Examples of successful efforts include growing the cochlear progenitor cells into differentiated hair cells (Shi et al., 2013; McLean et al., 2017). Given our ablative findings, we expect that blood prestin concentrations would rise as new functional OHCs are generated and their numbers increase. If this is the case, quantitative assays of blood prestin could be a very useful adjunct to investigation of hair cell regeneration, because it may serve as a surrogate biomarker that replaces costly and time consuming microscopic quantification of hair cells following termination of the animal subject. If this relationship is confirmed preclinically, prestin blood levels could subsequently serve as a non-invasive measure of regenerative success in the clinical realm. This development could substantially improve research throughput, both in the preclinical and the translational/clinical investigation levels.

5. Conclusions

Outer-hair-cell-specific protein, prestin, in circulation may act as a biomarker for noise-induced damage. Prestin levels in circulation change after noise exposure and the pattern of change is dependent on the severity of injury. An early rise in prestin after noise exposure may be predictive of permanent hearing loss. An OHC-specific circulating biomarker may have both research and clinical applications, such as development of therapeutics and early diagnosis, respectively.

Author roles and disclosures

KP and JDJ designed the experiments and wrote the manuscript. MS, MP, CR, AB and CTVB carried out the experiments. KP, MS, MP, CR, AB, CTVB and JDJ analyzed the data. MP, CR, AB, CTVB, JDJ and the spouse of JDJ are employees of Sensorion and have stock options in the company.

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Appendix A. Supplementary data

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