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Modeling the Measurements of Cochlear Microcirculation and Hearing Function after Loud Noise

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Abstract

Objective. Recent findings support the crucial role of microcirculatory disturbance and ischemia for hearing impairment especially after noise-induced hearing loss (NIHL). The aim of this study was to establish an animal model for in vivo analysis of cochlear microcirculation and hearing function after a loud noise to allow precise measurements of both parameters in vivo.

Study Design. Randomized controlled trial.

Setting. Animal study.

Subjects and Methods. After assessment of normacusis (0 minutes) using evoked auditory brainstem responses (ABRs), noise (106-dB sound pressure level [SPL]) was applied to both ears in 6 guinea pigs for 30 minutes while unexposed animals served as controls. In vivo fluorescence microscopy of the stria vascularis capillaries was performed after surgical exposure of 1 cochlea. ABR measurements were derived from the contralateral ear.

Results. After noise exposure, red blood cell velocity was reduced significantly by 24.3% (120 minutes) and further decreased to 44.5% at the end of the observation (210 minutes) in contrast to stable control measurements. Vessel diameters were not affected in both groups. A gradual decrease of segmental blood flow became significant (38.1%) after 150 minutes compared with controls. Hearing thresholds shifted significantly from 20.0 ± 5.5 dB SPL (0 minutes) to 32.5 ± 4.2 dB SPL (60 minutes) only in animals exposed to loud noise.

Conclusion. With regard to novel treatments targeting the stria vascularis in NIHL, this standardized model allows us to analyze in detail cochlear microcirculation and hearing function in vivo.

Keywords

Cochlea, stria vascularis, microcirculation, ABR, in vivo fluorescence microscopy, animal model, noise-induced hearing loss, NIHL

Noise-induced hearing loss (NIHL) is a common cause of acquired sensorineural hearing loss. NIHL can be caused by a one-time exposure to an intense impulse sound or by a continuous exposure to loud sound over an extended period of time. Both temporary and permanent threshold shifts are observed in NIHL.

It has been demonstrated that cochlear microcirculation is altered by exposure to loud noise.[1-6] In animals, there was evidence that high-intensity noise of at least 85-dB sound pressure level (SPL) for 6 hours can increase hearing thresholds and decrease cochlear blood flow.[1] Most authors agree that there is a prompt decrease in cochlear blood flow soon after the onset of noise exposure, which becomes progressively worse later on. An increase of cochlear blood flow that subsequently presents several days after the exposure might reflect a recovery period for hearing function.[1] Since the cochlea demands high energy supply, the labyrinthine function is closely linked to proper homeostasis. Any reduction of cochlear blood flow may induce

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local ischemia that subsequently alters normal metabolic homeostasis and results in a reduction of auditory function.[7]

In the past years, numerous studies have focused on changes in cochlear blood flow and hearing threshold after high-intensity noise by usage of laser Doppler measurements in combination with hearing measurements of either auditory brainstem response (ABR) or otoacoustic emissions (OAE). ABR seems more sensitive to noise exposure duration than OAE does.[8] This method allows measurement of threshold shift after loud noise exposure precisely and with high reproducibility.

For in vivo studies of cochlear microcirculation, intravital fluorescence microscopy (IFM) is an optical imaging method that literally offers the most reliable outcome due to its ability to directly access blood flow within single cochlear vessels. IFM has been implemented to observe cochlear microcirculation since the 1950s.[9-11] However, this method became less popular than laser Doppler techniques due to the necessity of a wide surgical approach. The laser Doppler technique is certainly technically easier to perform, but it is susceptible to artifacts based on temperature changes or movements. Compared with IFM, the laser Doppler technique does not allow measurements at the single capillary level.

In the present study, we aimed to establish an animal model with acute NIHL by using IFM and ABR to achieve more precise and simultaneous data of cochlear microcirculation and hearing function. The establishment of such a model is crucial for further insights into the pathophysiological background of inner-ear disorders and therapeutic options of novel blood flow–promoting agents.

Methods

Animals

Protocols are concordant with the policy on the use of animals according to international standards (EC directive 86/609/EEC). All procedures are further approved by the Institutional Animal Care and Use Committee. Twelve male albino Hartley guinea pigs (weight range, 250-400 g) were used in this study. The animals were randomly divided into 2 groups: a noise-exposed and a control (unexposed) group. Animals in both groups received identical surgical approaches (see below).

Surgical Preparation

All animals with a normal Preyer's reflex were anesthetized with a combination of ketamine 85 mg/kg (Ketavet; Parke-Davis, Berlin, Germany) and xylazine 8.5 mg/kg (Rompun; Bayer, Leverkusen, Germany) intraperitoneally initially, then repeated with the supplementary half-dose of ketamine and xylazine every 45 minutes. A polyethylene catheter (PE50; Portex Ltd, Hythe, Kent, United Kingdom) was inserted into the left femoral artery to monitor mean arterial blood pressure (MAP). For intravenous administration, the right external jugular vein was cannulated with another microcatheter (Portex Ltd). Heart rate and pulse oxygenation were monitored thoroughly, and animals breathed spontaneously during the experiments.

For microsurgery, a postauricular approach was performed on the right ear with fixation of the animal's head, and the right mastoid bulla was exposed and opened as described before.[12,13] The right pinna was retracted by a 3-0 silk suture in the anterolateral direction. Temporalis muscle was dissected aside, and the periosteum was then removed. To provide better access to the cochlea, the posterior annulus, the posterosuperior part of tympanic membrane, and the ossicles had to be removed. A small rectangular window (0.2 × 0.3 mm) was meticulously created over a convex part of the second turn of the cochlea using the tip of a small knife blade (blade #11) and microforceps, elevating the cochlear bony wall without traumatization to the underlying spiral ligament and stria vascularis.[14] Only a tiny and intact piece of bone was removed from the cochlea. Spiral ligament and strial vessels were then clearly exposed within the window.

In Vivo Fluorescence Microscopy and Analysis of Cochlear Microcirculation

We used a modified Zeiss microscope (Axiotech Vario; Zeiss, Goettingen, Germany) for IFM. Fluorescein isothiocyanate–labeled dextran (molecular weight 500,000; 0.05-0.1 mL of a 5% solution in 9% NaCl; Sigma, Deisenhofen, Germany) was injected intravenously as a plasma marker to allow fluorescence microscopy of cochlear microvessels in the stria vascularis as described before.[12,13] Blood flow was recorded immediately after the cochlear window had been opened. The initial recording of red blood cell velocity (v_{RBC}) and strial capillary diameters was performed at time point 60 minutes (30 minutes of noise exposure plus 30 minutes of operative procedure in noise-exposed animals, or 30 minutes without manipulations plus 30 minutes of operative procedure in the controls, respectively). Serial documentation was performed every 30 minutes up to 210 minutes.

Analysis of microcirculatory parameters was performed offline by an image analysis system (Cap Image; Zeintl, Heidelberg, Germany). This system was described in detail by Zeintl et al[15] and Klyscz et al.[16] It allows measurement of microcirculatory parameters such as functional vessel density, red blood cell velocity (v_{RBC}), and vessel diameters (d). Red blood cell velocities in selected vessels and vessel diameters were the primary values obtained from the analysis system. Blood flow in vessel segments (Q) was calculated according to the equation first described by Baker and Wayland[17]:

$$Q = \frac{v_{RBC}}{1.6} \cdot \left(\frac{d}{2}\right)^2 \cdot \pi$$

Measurement of Auditory Function

Auditory function was assessed by ABR recordings. Both ears had been examined and cleaned and baseline hearing thresholds were determined before loud noise or surgical procedures were performed (0 minutes). Afterward, only the threshold from the left ear was recorded every 30 minutes up to 180 minutes. Generation of acoustic stimuli and subsequent recordings of evoked potentials were performed using needle electrodes and a GSI Audera device (VIASYS HealthCare Inc, Madison, Wisconsin). The stimulus response thresholds

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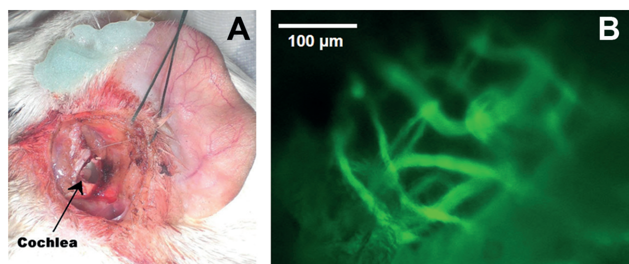

Figure 1. In vivo fluorescence microscopy of the stria vascularis. (A) Surgical access to the guinea pig cochlea before cochleostomy. (B) After intravenous injection of fluorescent dyes, strial microcirculation is visualized.

were detected with tone bursts at the frequency of 8 kHz. The acoustic stimuli were delivered monaurally through a tubal insert phone foam tip fitted well to the animal's ear canal. The supraliminal potentials were recorded in 10-dB steps, whereas the near-threshold potentials were recorded in 5-dB steps. Thresholds at each evaluation were verified at least twice.

Noise Exposure

For animals in the noise-exposed group, noise was generated by a sound stimulator (GSI Audera; VIASYS HealthCare Inc, Madison, WI), amplified and delivered through the ear phones. The 4-kHz octave band noise of 106-dB SPL was used as an exposure stimulus and delivered continuously for 30 minutes over the right and left ears, respectively.

Statistical Analysis

Results are presented as mean ± SD (standard deviation). Sample sizes were estimated prior to the experiments using SigmaStat (Jandel Scientific, San Rafael, California) software. Values of independent groups were compared with the Kruskal-Wallis and the Mann-Whitney *U* test. The Spearman coefficient was calculated to analyze correlation. *P* values smaller than 5% were considered to be significant.

Results

In Vivo Fluorescence Microscopy of Cochlear Microcirculation

After surgical exposure of the cochlea, a bony window to the cochlear capillary bed of the stria vascularis was prepared, allowing in vivo fluorescence microscopy and offline quantitative analysis after intravenous injection of fluorescent dyes (**Figure 1**). Standard parameters such as MAP, heart rate, and pulse oxygenation were stable throughout the experiments. The average MAP was 50.5 ± 13.8 mm Hg, and the heart rate was 197 ± 7.9 per minute. The pulse oxygenation varied from 96% to 100% (98.7% ± 1.4 %).

Cochlear Microcirculation

Diameters of capillaries in the stria vascularis (mean, 9.1 ± 0.8 µm) appeared to be constant throughout the observation period and showed no significant differences between groups (**Figure 2A**). The vessel diameters in the noise-exposed group slightly declined over time but did not change significantly.

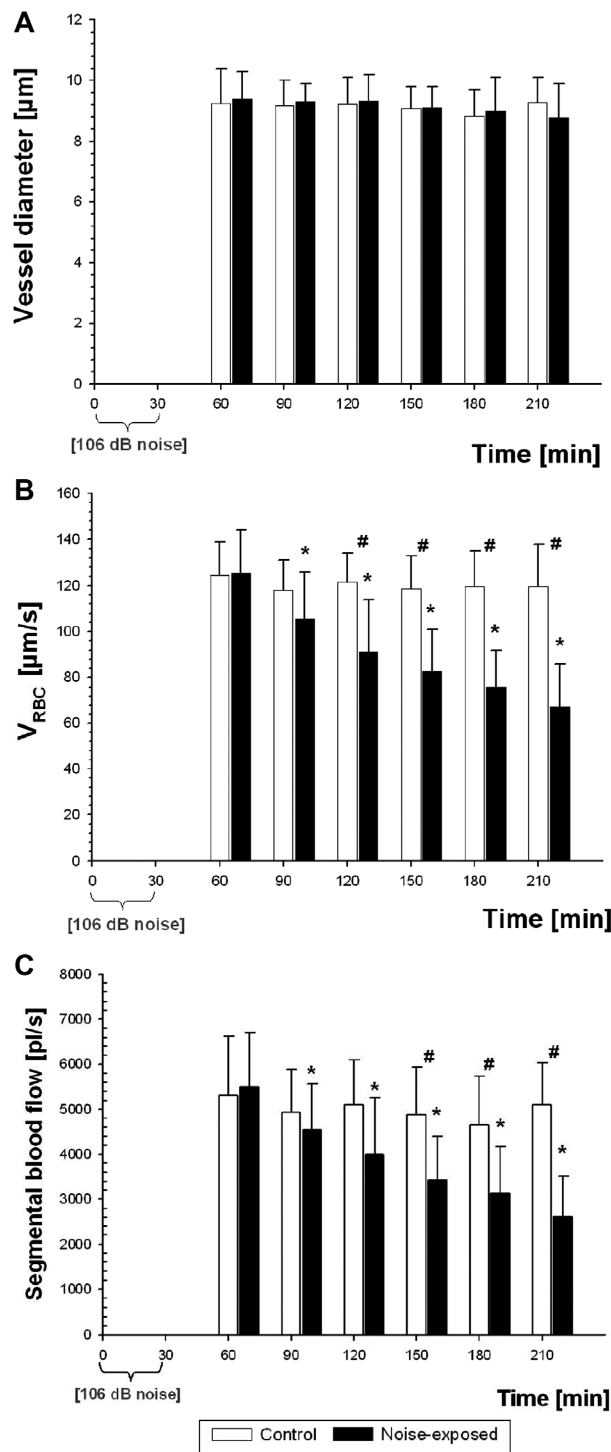

Figure 2. Analysis of strial microcirculation. (A) Vessel diameters of strial capillaries were not affected by loud noise. (B) Significant reduction of v_{RBC} in strial capillaries after loud noise exposure in contrast to control measurements. (C) Significant decrease in segmental blood flow of strial capillaries after noise exposure in contrast to control measurements. $^{*}P < .05$ versus measurements at 60 minutes. $^{\#}P < .05$ versus controls.

Initial v_{RBC} of strial capillaries from all animals, recorded at 60 minutes, was 125.3 ± 13.3 µm/s in the noise-exposed

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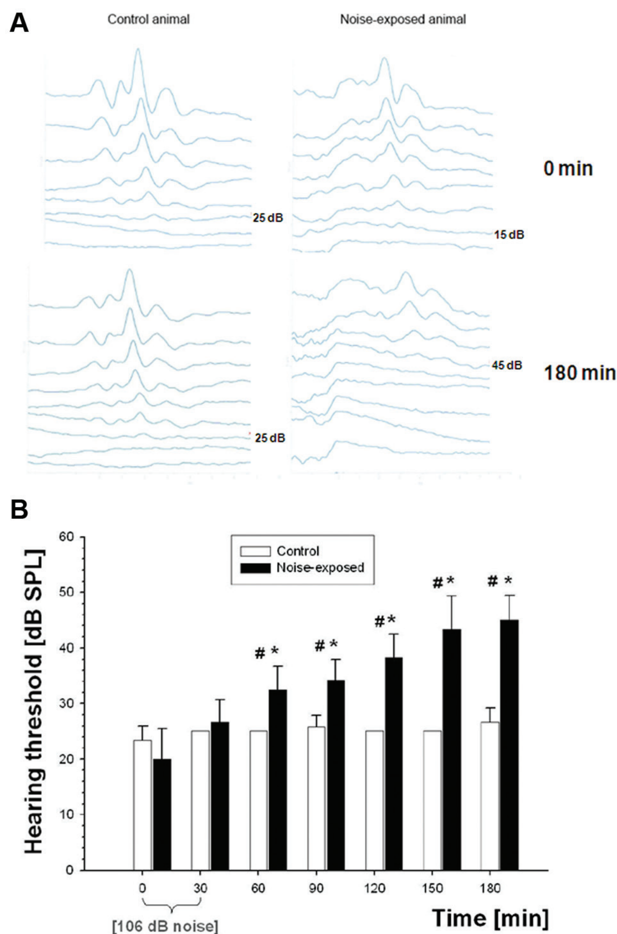

Figure 3. Measurements of hearing thresholds. (A) Representative auditory brainstem response (ABR) recordings of left ears comparing a noise-exposed animal with a control. Hearing thresholds are given in dB sound pressure level (SPL). (B) Hearing thresholds shifted significantly only in animals exposed to loud noise in contrast to controls. $^*P < .05$ versus measurements at 0 minutes. $^{\#}P < .05$ versus controls.

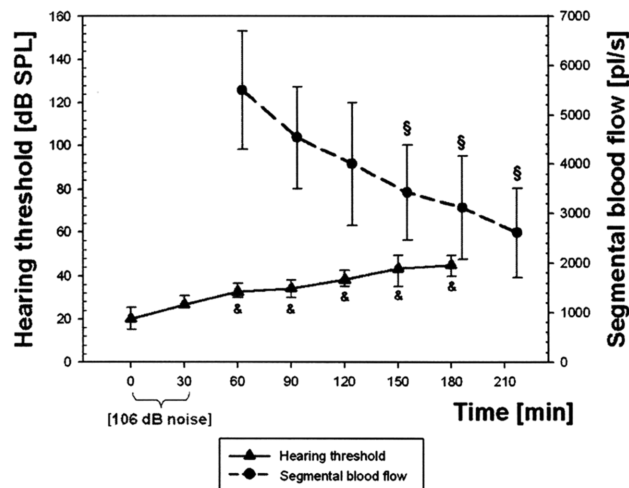

Figure 4. Synopsis of segmental blood flow of strial capillaries and hearing thresholds. Significant impairment of hearing function was detected 60 minutes after loud noise and further slowly increased up to approximately 45-dB sound pressure level (SPL), accompanied by a gradual decrease of strial capillary blood flow. The change in segmental blood flow was significant beyond 150 minutes. $^{§}P < .05$ versus segmental blood flow measurements at 60 minutes. $^{\&}P < .05$ versus hearing threshold at 0 minutes.

group and 124.5 ± 12.5 µm/s in the control group (**Figure 2B**). The differences at this early time point were not significant (P = .91). In noise-exposed animals, the v_{RBC} further declined from 91.3 ± 16.4 µm/s at 120 minutes to 82.7 ± 13.0 µm/s at 150 minutes, and 76.0 ± 10.1 µm/s at 180 minutes. v_{RBC} was only 66.8 ± 11.2 µm/s at the end of the observation (210 minutes). With regard to the velocities, there were statistically significant differences between noise-exposed and control animals after time point 120 minutes (P = .004). Gradual decreases in v_{RBC} of the strial capillaries were observed in the noise-exposed group. In contrast, within the control group, no significant changes of v_{RBC} were observed over time.

Comparably, segmental blood flow of the strial capillaries, which is dependent on v_{RBC} and vessel diameters, decreased continuously after noise exposure (**Figure 2C**). A gradual decline of segmental blood flow in noise-exposed animals was observed at 90 and 120 minutes compared with baseline values at 60 minutes. Comparing the 2 groups, significant differences were measured after 150 minutes (P = .041). The maximum reduction in blood flow was –53.5%. ± 6.7% at 210 minutes (P = .004).

Hearing Thresholds

ABR recordings were performed simultaneously as in vivo fluorescence microscopic measurements after noise exposure (**Figure 3A**). At the beginning of the experiment, all animals demonstrated equal hearing thresholds on both ears (**Figure 3B**). There was no difference between hearing levels of noise-exposed animals and the control group up to time point 30 minutes. However, animals exposed to loud noise showed an average hearing threshold shift from 20 ± 5.5 dB SPL to a maximum of 45 ± 4.5 dB SPL (P = .002) at the end of the observation (180 minutes).

In fact, the hearing thresholds began to raise immediately after cessation of a 30-minute loud noise; however, this threshold shift at 30 minutes did not differ significantly from baseline and control values. The threshold shifts between time point 60 minutes and time point 180 minutes increased subsequently in noise-exposed animals and were statistically significant (P = .002). In contrast, no increase of hearing thresholds was observed in the control group.

Synopsis of values derived only from the noise-exposed animals revealed a decrease of cochlear blood flow accompanied by a simultaneous increase of hearing thresholds (**Figure 4**).

Discussion

The present study for the first time shows a hearing threshold shift and impairment of cochlear microcirculation after acute loud noise by using simultaneous measurements of IFM in combination with ABR in guinea pigs.

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To establish this model, we followed the steps of cochlear preparation described by Nuttall in 1987.[18] Using IFM, we were able to directly visualize capillary networks within the cochlear lateral wall. Diameters and velocity of a single vessel in at least 6 vessels per region of interest were investigated. The average diameter of all observed vessels in our study was 9.1 ± 0.8 μm, which is comparable to the value of 9.3 μm and 12.2 μm shown by Nuttall.[14] Even though Nuttall categorized these vessels into strial capillaries (larger in size and slower in velocity) and spiral ligament capillaries, there was no clear cut between the 2 types of vessels in our experiments.

Comparably, average red blood cell velocity values observed in our control animals appeared to be in accordance with the mean strial vessels' velocity of 112 μm/s measured by Perlman and Kimura[19] and within the range of mean red cell velocity in the spiral ligament vessels (120 ± 60 μm/s) measured by Nuttall.[14] Although a removal of surrounding structures is necessary for the surgical procedure, IFM is an excellent method to evaluate cochlear microcirculation in animals. The advantages of IFM compared with other methods such as laser Doppler flowmetry, reactive or labeled microsphere injection techniques, oxygen microelectrodes, and magnetic resonance imaging are (1) to measure the velocity of cells, (2) to study the dynamics of microcirculatory changes, (3) to define the exact region of interest, and (4) to investigate morphological and physiological changes within single visualized vessels.[12]

To examine acute effects of loud noise on cochlear microcirculation and hearing function, we used an exposure to 106-dB SPL noise with a duration of 30 minutes as a noxa. This SPL seemed to be sufficient to provoke cochlear blood flow impairment as well as the decrease of intracochlear oxygen tension.[6] Studies from Thorne and Nuttall[2] and Scheibe et al[4] have suggested that high-intensity noise above 105-dB SPL is capable of reducing cochlear blood flow. Even though Lamm and Arnold[6] also used 106-dB noise, they measured the cochlear blood flow with the laser Doppler method, which is less precise than the IFM. They were able to report only a relative change from baseline with a total cochlear blood flow reduction of –31.6% ± 12% at time point 210 minutes, while in the experiments shown here, all relevant microcirculatory parameters were measured in absolute values (diameter, red blood cell velocity, and segmental blood flow). The maximum reduction in blood flow at the 210-minute time point in our study was –53.5% ± 6.7%, and a significant decline in v_{RBC} was noticed even before at the 120-minute time point after noise exposure. According to Lamm and Arnold,[6] a significant reduction of cochlear blood flow is observed not before 90 minutes after exposure. Therefore, even if IFM recordings of cochlear capillaries had been feasible approximately 60 minutes after noise exposure, we did not expect to miss any important changes at this early time point.

It is in line with these considerations that initial segmental blood flow recorded at 60 minutes did not differ significantly between the 2 groups. It has been speculated that there is a correlation of certain areas of perfusion with specific frequencies of hearing in the cochlea. For example, high frequent noise greater than 10 kHz given with high intensity corresponds to a decrease in blood flow only in basal turns of the cochlea.[4,20] However, the exact correlation between a specific frequency and a specific turn of the guinea pig cochlea has not been identified yet. In our study, we demonstrated an alteration of segmental blood flow in strial capillaries located at the second turn of cochlea after exposure to 106-dB SPL centered at 4-kHz noise.

A dip or notch at 4 kHz, or at 6 kHz, is a typical feature of NIHL in humans. However, this is different in guinea pigs. According to evidence from Thorne and Nuttall,[2] the maximum threshold shift in guinea pigs following the 4-kHz noise exposures occurred at 4, 6, and 8 kHz using ABR testing. In preliminary experiments (data not shown), we evaluated the guinea pigs' hearing thresholds at different frequencies, both before and after noise exposure. The experiments revealed that tone bursts at 8 kHz resulted in the best ABR waveform responses. In addition, the greatest increase of ABR threshold after loud noise was observed at 8 and 6 kHz, respectively.

After loud noise exposure, the maximum increase in hearing thresholds was 45 ± 4.5 dB SPL. This value is in accordance with the measurements by Thorne and Nuttall 1 hour after exposure to 103-dB SPL noise.[2] Taken together, loud noise shifted hearing levels in our experimental animals from normal hearing to a moderate degree of hearing loss within 3 hours after exposure.

An acute impairment of hearing function was detected within 1 hour after loud noise: hearing thresholds shifted significantly 30 minutes after exposure—at timepoint 60 minutes, respectively—and tended not to increase further beyond 150 minutes. In contrast, segmental blood flow of cochlear capillaries continuously decreased up to 210 minutes (end of observation). The limited change in hearing threshold shift might probably be because the decrease of blood flow is followed by an injury to the outer hair cells that has reached a steady state already. Another reason might be that ischemia of strial cells leads to a decrease of the endocochlear potential to such an extent that it may have an impact only on the outer hair cells.

There is still a long-standing controversy regarding how microcirculatory disturbances are linked to NIHL. Many authors hypothesize that NIHL is caused by mechanical destruction of hair cells and supporting structures of the organ of Corti. Recently, there is growing evidence that metabolic exhaustion combined with free radical formation plays an important role in NIHL. High-intensity noise can create intense metabolic activity, which increases mitochondrial free radical formation in the inner ear.[21,22] Therefore, noise-induced free radical formation is thought to be a significant factor in cochlear blood flow reduction.[5,23] Le Prell et al[24] revealed a linkage between reactive oxygen species (ROS) production and reduced cochlear blood flow: ROS peroxidizes lipids that exert formation of a potent vasoconstrictor, 8-isoprostane-F2α (8-iso-PGF2α).[25] In addition to vasoconstriction, sludging of blood cells in the cochlear lateral wall and spiral lamina vessels are recognized in the noise-damaged cochleae as well.[26] However, since there was no change in diameter of the strial capillaries after loud noise in our study, we assumed that vasoconstriction may occur

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elsewhere in the feeder vessels such as arterioles from spiral modiolar artery (SMA). Apart from ROS and isoprostane formation following loud noise, spingosine-1-phosphate (S1P) might be another potent vasoconstrictor that contributes to cochlear blood flow reduction because it controls SMA smooth muscle contraction.[27] As demonstrated by Fujioka et al,[28] tumor necrosis factor–α (TNF-α) is the earliest cytokine that increases within a few hours after the onset of noise exposure. We were able to show most recently that TNF-α indeed appears to play a crucial role inducing such S1P-mediated ischemic conditions in the inner ear.[13]

In NIHL, adequate supplies of oxygen and nutrients are interrupted and/or the elimination of waste products fails. So, vascular permeability is affected, resulting in local ischemia.[5,7] In the stria vascularis, capillaries are located closely to the strial cells, which contain active potassium ion transport channels. When these cells function properly, a high concentration of potassium ions is maintained in the endolymph.[29] The gradient of potassium ion levels leads to changes of the endocochlear potential, which is a sufficient driving force for cochlear amplification and hair cell transduction. Impairment of cochlear microcirculation can thus lead to significant hearing loss.

Conclusion

A new standardized animal model allowing measurements of cochlear microcirculatory parameters in the stria vascularis and hearing threshold shift after NIHL was established using in vivo fluorescence microscopy and ABR. This methodology allows one to analyze in detail effects and kinetics of treatments targeted at cochlear microcirculation.

Author Contributions

Warangkana Arpornchayanon, acquisition of data, analysis and interpretation of data, drafting the article, final approval; **Martin Canis**, analysis and interpretation of data, conceptual design, drafting the article, final approval; **Markus Suckfuell**, interpretation of data, revising the article, final approval; **Fritz Ihler**, interpretation of data, revising the article, final approval; **Bernhard Olzowy**, interpretation of data, revising the article, final approval; **Sebastian Strieth**, corresponding author, conceptual design, analysis and interpretation of data, revising the article, final approval.

Disclosures

Competing interests: None.

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