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Cochlear lateral wall changes secondary to hypercholesterolemia and noise exposure in the chinchilla model

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ABSTRACT

Objective: To investigate the effects of hypercholesterolemia on the cochlear lateral wall structures in chinchillas and its impact on the susceptibility of the inner ear structures to noise exposure.

Materials and Methods: Fifteen chinchilla temporal bones were selected from the Animal Temporal Bone Collection of the Paparella Otopathology and Pathogenesis Laboratory at the University of Minnesota. The experimental group was subjected to 3-month 1% cholesterol diet, while the control group maintained a standard diet. After 3 months, the experimental group's left ears exposed to noise trauma for 10 minutes while right ears did not. One month later the animals were euthanized, and the temporal bones harvested from the animals underwent histopathological examination with morphometric assessments of stria vascularis (SV) and spiral ligament (SL).

Results: Histopathological analysis revealed no significant differences ($p>0.05$) in total SL area across cochlear turns between the experimental and control groups. However, distinct variations were observed in SV area within the lower basal ($p<0.01$) and upper basal ($p = 0.01$) turns of the hypercholesterolemia and noise-exposed group compared to the control group.

Conclusion: Hypercholesterolemia is one of the conditions that may contribute to sensorineural hearing loss. This study highlights its importance in auditory health by revealing the relationship between hypercholesterolemia and cochlear lateral wall structures, and increased susceptibility to noise-induced damage.

Keywords: *Hypercholesterolemia, Noise exposure, Cochlear lateral wall, Chinchilla*

INTRODUCTION

In recent years, hypercholesterolemia has emerged as a significant research topic, necessitating detailed investigation into the condition to uncover its growing prevalence and complex interaction with various health parameters. More than a century ago, Virchow's revelation that atheroma contains a yellow fatty substance, later identified as cholesterol by Windaus, pointed the involvement of lipids in the development of atherosclerosis (Mayerl et al., 2006). The early recognition of cholesterol's pivotal role in arterial plaques laid the foundation for the cholesterol hypothesis in understanding the pathogenesis of atherosclerosis (Axelsson and Lindgren, 1985). Numerous researchers exploring the potential link between high serum cholesterol levels and hearing loss have observed a correlation. These researchers suggest that elevated blood lipids may impact hearing by triggering premature widespread atherosclerosis in major arteries and vessels that

supply blood to the auditory organ (Lee et al., 2023).

In the late 1970s and early 1980s, Morizono and colleagues conducted studies investigating the effects of a high-cholesterol diet on auditory function in rabbits and chinchillas (Morizono and Paparella, 1978; Morizono and Sikora, 1978; Morizono et al., 1985). These studies found that animals subjected to a cholesterol-rich diet exhibited a progressive decline in auditory evoked potentials, particularly affecting higher frequencies.

The cochlear lateral wall, including the spiral ligament (SL) and stria vascularis (SV), plays a key role in maintaining cochlear fluid balance. This process involves four distinct capillary networks within the SL and SV, crucial for modulating cochlear endolymph homeostasis (Hosoya et al., 2023). These capillaries are essential for regulating sensory hair cell transduction by controlling endocochlear potential, ion transport, and maintaining fluid balance in the endolymph (Wangemann, 2006; Peeleman et al., 2020). Importantly, the cochlear wall is sensitive to noise exposure, primarily due to potential disruptions in cochlear microcirculation following acoustic trauma (Hirose and Liberman, 2003; Yu et al., 2021). However, no study has quantitatively analyzed changes in the cochlear lateral wall (to the best of our knowledge), impeding a complete understanding of the specific mechanisms underlying alterations in the cochlea due to hypercholesterolemia and noise exposure.

The main goal of this study was to evaluate the influence of hypercholesterolemia on cochlear lateral wall through histopathological analysis of the temporal bones in the chinchilla model. Additionally, this research aimed to conduct further analysis on cochlear changes in the context of exposure to acoustic trauma in the presented hypercholesterolemia model.

MATERIALS and METHODS

Animal temporal bone specimens

A total of 15 temporal bones from 10 adult chinchillas (*Chinchilla lanigera*) were selected from the Animal Temporal Bone Collection of the Paparella Otopathology and Pathogenesis Laboratory at the University of Minnesota.

In the presented study, the experimental group of animals had *ad-libitum* access to a diet comprising 1% cholesterol, which was incorporated into the standard Chinchow and custom-blended by the manufacturer (Ralston-Purina). This dietary regimen was maintained for a duration of 3 months, while the control group, consisting of 5 animals, was kept on a standard diet. After dietary intervention, the animals in the experimental group (n=5) were divided into two subgroups.

The right ears of these chinchillas constituted the first subgroup to explore the changes in hypercholesterolemia condition while the second group, including the left ears, were additionally exposed to noise.

For chinchillas included in the noise exposure subgroup, the animals were anesthetized with ketamine hydrochloride (20 mg/kg) and sodium pentobarbitol (30 mg/kg). Only the left ears of the animals in the experimental group underwent a 12-kHz pure-tone stimulation at 95 dB SPL for 10 minutes. One month later, serum samples were collected from all animals, and they were euthanized using a high dose of ketamin-HCl injection. Detailed information regarding our experimental methodology has been documented in a previous study conducted by our laboratory (Morizono and Sikora, 1982).

Following euthanasia, temporal bones underwent further processing, including perfusion, fixation, decalcification, embedding, and horizontal sectioning at a thickness of 20 μm. Every 10th section was stained with hematoxylin-eosin to enable histological analysis for comparisons among the high-cholesterol-fed group (Group 1), the high-cholesterol-fed and noise-exposed group (Group 2), and the control group (Group 3).

Analysis of the cochlear lateral wall

At the mid-modiolar level of all cochlear turns and the two neighboring sections, morphometric assessments of the SV and SL were conducted. Images were captured at either ×40 or ×100 magnification using a digital camera, and image analysis software (SPOT Advanced, SPOT Imaging Solutions, MI, USA) was employed on a computer to quantify the cut surface areas of the SV and SL.

Additionally, the average loss of fibrocytes in the SL on sections at the mid-modiolar level was assessed, following the techniques outlined by Hequembourg and Liberman (2001). A classification scale was utilized to evaluate fibrocyte loss in the SL, ranging from 0 for normal conditions (missing less than 1/3 of fibrocytes) to 3 for severe or complete loss of fibrocytes on mid-

modiolar-level sections. The calibrated image was captured at an original magnification of ×40.

Statistical analysis

The Kruskal-Wallis test was employed to assess group differences. Post-hoc pairwise comparisons using the Dunn-Bonferroni test were conducted to identify specific group differences in the significant results. All statistical analyses were carried out using SPSS 23.0 software for Windows (SPSS Inc., Chicago, IL), with statistical significance established at a P-value less than 0.05.

Ethical approval

Studies utilizing our archival collection are University of Minnesota Institutional Review Board and IACUC-exempt, as they originate from previously approved protocols (Decision ID: 00003249).

RESULTS

The serum cholesterol levels in the included animals exhibited a nearly 3.5-fold increase ($p<0.01$) within the high-cholesterol group compared to the control group after a 3-month dietary intervention. This finding suggests clinical hypercholesterolemia in the experimental group, consistent with a prior study conducted by our laboratory (Morizono and Sikora, 1982). Additionally, despite considerable variability in the obtained values, there were noteworthy elevations in glucose, alanine transaminase (ALT) and aspartate transaminase (AST)levels in the animal group fed with a cholesterol diet.

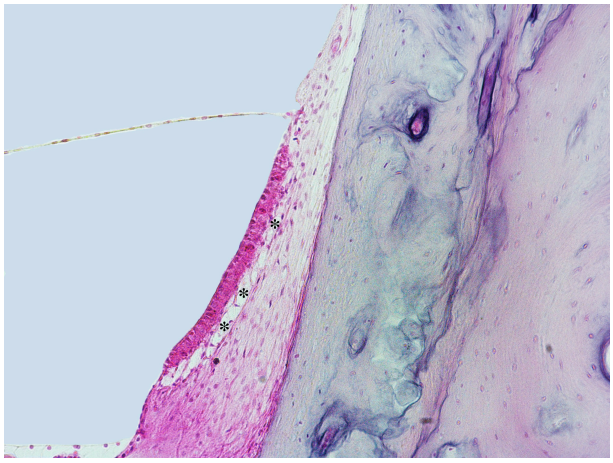

Figure 1. The separation of the SV and fibrous stroma (x20). *: shows the large empty-looking areas. Staining with hematoxylin-eosin (H&E)

Table 1. Median, minimum (min), and maximum (max) values of the total areas (μm²) for the Spiral ligament and Stria vascularis within each respective group. The rows in bold indicate the statistical significance ($p<0.05$) when compared to third group.

Cochlear turns	Groups	Spiral ligament			Stria vascularis		
		Median	min	max	Median	min	max
Lower basal	1	74152.89	68787.71	78639.86	4677.7	4150.25	5596.34
	2	74919.87	67693.44	76247.89	**4130.14**	**4061.07**	**4247.3**
	3	68694.6	56067.31	77573.12	5497.08	4350.72	6652.5
Upper basal	1	72717.63	60069.1	76886.04	4651.84	4042.18	5599.5
	2	72357.41	63218.35	75864.53	**4159.88**	**3564.33**	**5444.82**
	3	62589.19	51404.16	68905.71	5310.86	5039.23	5913.15
Lower middle	1	59295.31	49582.31	63436.09	4887.06	4289.66	5396.17
	2	56631.62	49099.46	63417.08	4044.67	3143.09	5557.34
	3	49761.68	45721.79	55008.83	5405.3	5405.3	6135.12
Upper middle	1	46668.39	41847.65	58347.41	3396.32	2736.86	4130.49
	2	48332.04	41882.49	56880.32	3385.65	2568.22	4531.77
	3	39097.88	33344.15	49961.94	4499.2	3685.54	4690.01
Apical	1	33291.01	25937.7	37786.27	2707.26	2499.89	3887.57
	2	25991.44	23259.39	40028.47	3649.16	2527.8	4330.89
	3	25417.55	23849.5	32271.02	3513.89	3396.63	4425.27

In our histopathological analysis, no significant differences ($p>0.05$) were identified between the groups regarding the total SL area or total fibrocyte loss across any cochlear turn (Table 1). In the first and second groups, large empty-looking areas surrounding Type I and type II fibrocytes were observed, leading to a reduction in intercellular contacts and the separation of the SV and fibrous stroma. This finding was evident in the lower basal turn, observed in 3 out of 5 samples in the first group and 4 out of 5 samples in the second group (Figure 1). Similarly, in the upper basal turn, the pathology was observed in 2 out of 5 samples in the first group, while it was evident in 4 out of 5 samples in the second group. Notably, no observable pathology was detected in any cochlear turns within the third group.

Furthermore, the mean area of SV was measured, and statistical analysis were performed among all three groups (Table 1). Specifically, we found significant differences in the lower basal ($p<0.01$) and upper basal ($p=0.01$) turns of the SV area within the second and third groups (Figure 2). No differences were observed in the remaining cochlear turns between these two groups. Additionally, the second group showed a significant decrease in the mean area of SV compared to those in the first group only in the lower basal ($p=0.03$) turn. Although a slight decline in the SV area was seen in the first group, no significant differences were found between the first and third groups.

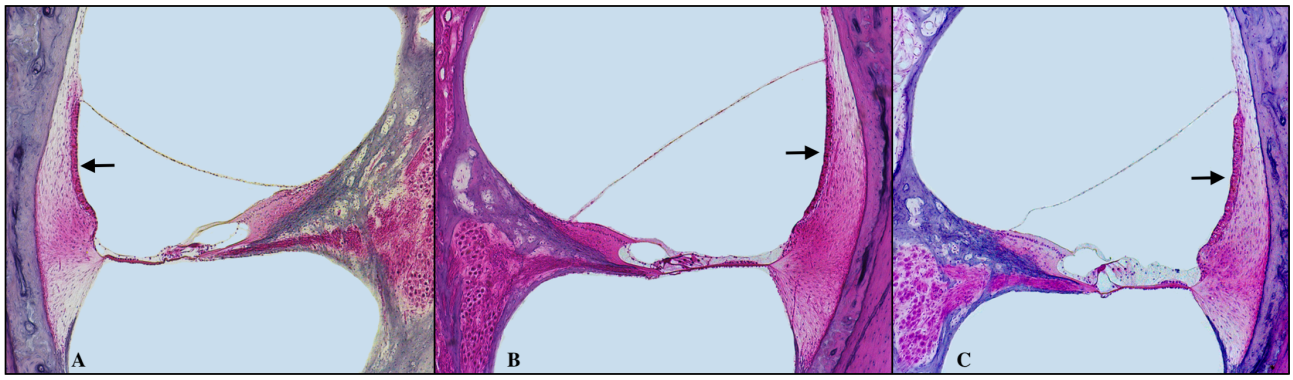

Figure 2. Photomicrographs showing the decrease in the mean area of SV within the representative basal turn of chinchilla temporal bones (x20). A: A section from hypercholesterolemia group. B: Section showing the decreased area in the hypercholesterolemia and noise exposed group. C: Section from non-diseased control group. The arrows specifically point to the SV. Staining with hematoxylin-eosin (H&E)

DISCUSSION

Hypercholesterolemia, characterized by elevated levels of cholesterol in the blood, can contribute to sensorineural hearing loss. The potential impact on auditory function involves multifaceted mechanisms, suggesting a complex interplay between elevated cholesterol levels and the development or exacerbation of sensorineural hearing impairment (Evans et al., 2006). Although prior research indicates that hyperlipidemia affects cochlear function and increases susceptibility to noise exposure, the exact mechanism explaining this correlation remains unclear (Morizono and Paparella, 1978; Sikora et al., 1986).

In this study, the chinchilla animal model was employed to induce a hypercholesterolemic condition. Hypercholesterolemia, a significant contributor to cardiovascular disease, not only affects the cardiovascular system but also has broader systemic impacts due to abnormal lipid levels (Kathak et al., 2022). In the presented study, in addition the hypercholesterolemia, increases in liver enzymes levels were observed. Serum ALT is a specific marker for hepatic damage primarily situated in the liver (Kathak et al., 2022), and AST, present in the cytoplasm of hepatocytes (Aulbach and Amuzie, 2017). The increases in these biomarkers indicate hepatic dysfunction due to dyslipidemia. Considering their role in energy metabolism, the close connection between glucose and lipid metabolism, and the elevated glucose level in the experimental group in the presented study, are not surprising. High glucose levels in hypercholesterolemia can result from insulin resistance, inflammation, beta cell dysfunction, oxidative stress, and fatty tissue dysfunction, collectively leading to increased blood sugar levels (Parhofer, 2015). These increases observed in serum analysis underline the complex relationship

between dyslipidemia and other systems in the presented model, providing valuable information about the broader effects of abnormal lipid levels.

In a previous study conducted in our laboratory investigating the cochlear effects of hypercholesterolemia utilizing the chinchilla model (Morizono et al., 1985), a noteworthy extension of P1 latency in the Auditory-Evoked Brainstem Response test was noted at various high frequencies in animals subjected to a cholesterol-rich diet, even without exposure to noise. Moreover, these cholesterol-fed animals exhibited increased vulnerability to noise-induced damage, particularly at extremely high frequencies, as evaluated through auditory perception. Our present research expands upon these earlier discoveries by investigating further into the impact on high cholesterol diet-fed animals. Notably, our investigation revealed specific changes in the cochlear lateral wall, particularly within the group exposed to noise.

Lateral wall structures take part in acoustic transduction in the mammalian cochlea. The cochlear lateral wall encompasses the SL and SV. The SL, comprising the fibrocytes, serves primarily as structural support for the cochlea (Peeleman et al., 2020) while SV plays an important role in preserving potassium homeostasis within the scala media in collaboration with SL (Locher et al., 2015). The diverse fibrocyte subtypes in the SL actively express various ion transporters and enzymes, contributing to K+ absorption and participating in an intricate gap junction network essential for the crucial K+ recycling process in endocochlear potential generation (Spicer and Schulte, 1996). In the presented study, while there was not a significant change in the mean area of SL between the groups, there was a significant pathology separating type I and type II fibrocytes and SV in the first and second groups compared to those in the third group. This anomaly presents itself as a pathological observation, with potential roots traced to the loss of basal cells that form the interface between the SV and SL, SV detachment, type I and type II fibrocyte loss in the SL, or the presence of edema. Nevertheless, across all scenarios, a diminished K+ supply to the SV emerges due to tight junctions hindering K+ movement from perilymph. This could pose a potential risk of compromising the K+ recycling pathway by disrupting the gap junction network that connects fibrocytes and the SV (Liang et al., 2005). Temporal bone studies, exemplified by Kusunoki et al. (2004), revealed a significant relationship between aging and fibrocyte loss in the basal turn in human temporal bones, potentially leading to hearing loss in the elderly. Furthermore, in a murine model, Schmutzhard et al. (2012) demonstrated that the impairment of type I fibrocytes in the SL may disrupt the blood labyrinth barrier, leading to the breakdown of the endocochlear potential and contributing to significant hearing loss. Moreover, exposure to loud noise then leads to an increase in the production of pro-inflammatory cytokines in type I and type II fibrocytes, suggesting that these cells play a vital role in regulating immune responses following exposure to loud sounds (Zhang et al., 2019). However, further investigation is needed to determine the contributing factors to this cochlear pathology in the context of elevated cholesterol diets and acoustic trauma.

The SV, a constituent of the lateral wall, accommodates crucial marginal, intermediate, and basal cells (Locher et al., 2015). The relationship between the SL and the SV is critical for establishing optimal conditions essential for cochlear hearing function (Peeleman et al., 2009). In the presented study, a notable reduction in the mean area of the SV was observed in the second group compared to that in the third group in the basal turn. In animal models, the isopotential curve of cochlear microphonics, action potential thresholds, and endocochlear potential showed no distinctions between the hypercholesterolemia and control groups. However, when subjected to moderately intense sound, the hypercholesterolemia group exhibited a significantly greater reduction in the thresholds (Morizono and Paparella, 1978; Morizono and Sikora, 1982). In a later study, consistent adherence to a high-cholesterol diet was reported to be associated with the likelihood of high-frequency hearing loss, potentially attributed to vascular pathology arising from a hyperlipidemic condition, indicating that hypercholesterolemia may play a role as a contributing factor in differential susceptibility to noise (Sikora et al., 1986). The strial degeneration observed in the basal turn in the presented study might help explain the threshold changes and high-frequency hearing loss identified in previous animal studies.

The development of hypercholesterolemia is linked to endothelial cell dysfunction, reduced vascular nitric oxide availability, increased oxidant stress, and the establishment of a highly pro-

inflammatory state (Zhang et al., 2019). In the case of noise exposure, cochlear microcirculation and lateral wall pathologies are influenced in varying ways depending upon the severity of the exposure, revealing associations with alterations in vasoactive factors and inflammatory responses within the cochlea (Shin et al., 2019). In the presented study, two conditions influencing cochlear microcirculation were assessed. However, it is crucial to note in the clinical context that various factors, including cardiovascular diseases and genetic disorders, may also impact the circulatory system.

This study is not without limitations. Firstly, the nature of the study (archival temporal bone samples) prevents the conduct of more comprehensive research. In addition, limited detailed procedural information regarding animal experiments in the archival files is restricting access to detailed data. Lastly, the unavailability of hearing analysis causes lack of detailed information to assess auditory function in the animals.

The data obtained by histopathological quantitative analyzes in this study has important outcomes, highlighting that noise exposure in the context of hypercholesterolemia induces changes in the lateral wall structure. The integrity of the lateral wall is critical for maintaining normal cochlear physiology and sensitivity. The histopathological alterations caused by noise in the lateral wall might play a crucial role in noise-related hearing loss. Attempting to understand the mechanisms that undergird damage to these lateral wall structures—a significant contributor to noise-induced hearing loss-may lead to innovative intervention approaches that extend beyond the preservation of hair cell integrity (Hirose and Liberman, 2003). This understanding has the potential to reshape our understanding of auditory vulnerabilities and present opportunities for preventive strategies against hearing loss stemming from excessive acoustic stimulation.

CONCLUSION

This study reveals remarkable histopathological changes in the cochlear lateral wall structures, highlighting the significant impact of hypercholesterolemia and its accompanying noise exposure. Understanding these changes may lead to new interventions targeting these structures and provide opportunities for innovative strategies to prevent noise-induced damage in the cochlea.

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