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Safety of Intracochlear Dexamethasone injection through Round Window Membrane in Guinea Pigs

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ABSTRACT
Background: Delivering drugs to the cochlea is challenging due to its complex anatomical structure, almost entirely encased in bone. Intratympanic steroid injections are commonly used for inner ear conditions like Meniere's disease and sudden sensory-neural hearing loss, but they have limitations, including rapid clearance via the Eustachian tube and the need for patients to remain in a supine position post-injection. Intracochlear drug delivery bypasses these limitations, offering potential for more effective treatment.
Objective: This study aims to assess the safety and histopathological effects of intracochlear dexamethasone administration via microneedle through the round window membrane (RWM) in guinea pigs.Methods: Using a Hamilton syringe, 5 µl of dexamethasone sodium phosphate was administered through the RWM into the cochlea of guinea pigs. Animals were observed for six weeks, then euthanized for histopathological analysis. The cochlear specimens were examined for inflammation, fibrosis, neo-ossification, hair cell counts, and spiral ganglion neuron density, comparing the treated ear to the untreated ear as a control.
Results: Histopathological analysis showed no signs of acute or chronic inflammation, fibrosis, or neo-ossification in the treated cochleae. There were no significant differences in hair cell or spiral ganglion neuron densities between treated and control cochleae.
Conclusion: Intracochlear dexamethasone injection via microneedle is a safe, minimally invasive approach for direct cochlear drug delivery, showing no long-term histopathological changes. Microneedles hold promise for targeted treatments in inner ear diseases with minimal anatomic or physiological disruption.

Keywords: Intracochlear drug delivery, dexamethasone, cochlea, microneedle, histopathology

INTRODUCTION

Drug delivery to the cochlea is a challenge in inner ear diseases due to the anatomical restrictive nature of the cochlea as it's almost surrounded by bone.(1)
The intratympanic injection of steroids have been used for long as a method of drug delivery to the cochlea beside the systemic route in treatment of diseases such as Meniere's disease and sudden sensory-neural hearing loss.(2)
Intratympanic injection has demonstrated a superior influence on therapeutic effect and gene expression compared to systemic administration. However, its efficacy is constrained by the requirement that therapeutic agents remain in the middle ear for sufficient time to enable diffusion across the round window membrane (RWM). Post-injection, patients must also stay still in a supine position for approximately 30 minutes to facilitate this diffusion into the inner ear. Additionally, a substantial portion of the administered agent may be cleared via the Eustachian tube.(3)
Intracochlear drug administration gained popularity over time specially in conjunct with CI. It has the advantage of bypassing inner ear barriers like the RW, the stapes, and the blood labyrinth barriers. In addition, it has been demonstrated that intracochlear application of only small amounts e.g. of dexamethasone phosphate by injection through the round window membrane leads to a lower variability of the intracochlear concentration, to an increased absolute perilymph concentration, and to a more uniform distribution of the substance in scala tympani. It also avoids the systemic side effect of the applied drugs. (4). It can be achieved by 3 means: direct injection by microneedles, micropumps and drug-eluting electrodes.(5)

This study aims to investigate the long-term effects of intracochlear dexamethasone injection using microneedle through the round window membrane on the cochlea of guinea pigs by histopathological evaluation.

MATERIALS AND METHODS

Suez Canal University Higher Committee for research Ethics has reviewed and approved all the study procedures and approved the use of 10 male Guinea pigs in this study. The housing and operative procedures on the animals were performed at the Animal House facility located in the faculty of Medicine, Suez Canal University. Intracochlear injection of 5 µl of Dexamethasone sodium phosphate (4mg/ml) through the round window (RW) was done using a Custom-made Hamilton syringe with capacity of 10 µl, needle length 30 mm, 30-gauge style 2 (Hamilton Company, Reno, NV). Access to the RW Gained through a postauricular skin incision and dorsal bulla opening under complete aseptic condition. Using zero-degree 2.7 mm endoscope (Karl Storz, Germany); the round window membrane is visualized, and injection is done (Figure 1). Anesthesia was done using a mixture of ketamine & xylazine. Animals were allowed to recover and followed up for 6 weeks then Euthanization was done using lethal dose of xylazine. Procedures performed on the right ear while the left side was used as a control.Specimens processed for histopathology, 3 sequential mid-modiolar sections of 5 µm thickness were obtained using microtome for each specimen and processed for H & E stain (Figure 2). (6) Using Leica DM 2000 Microscope with digital camera (Leica Microsystems GmbH, Wetzlar, Germany) sections were examined for signs of inflammatory reaction (lymphocytic & macrophage infiltrate), fibrosis, neo-ossification, Inner and Outer hair cells count at (basal, middle, and apical) cochlear turns (Figure 3). We obtained Spiral ganglion neuron (SGN) density in the Rosenthal's canal in cells per mm2 at the basal, middle and apical turns using ImageJ program (version 1.54; National Institutes of Health, Bethesda, MA, USA) on the obtained micrographs of the Rosenthal canal (Figure 4) at each transection. (7)

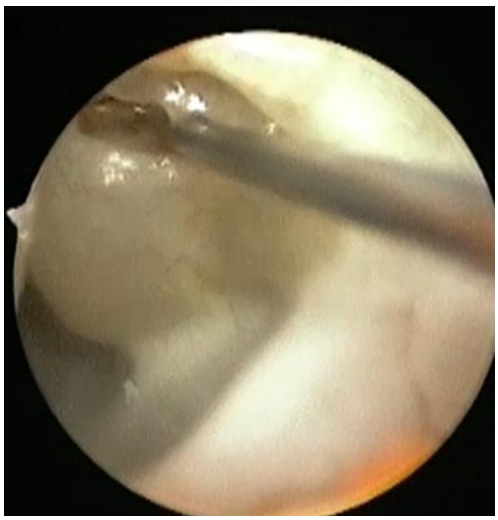

Figure 1: Injection of Dexamethasone through round window using Hamilton syringe.

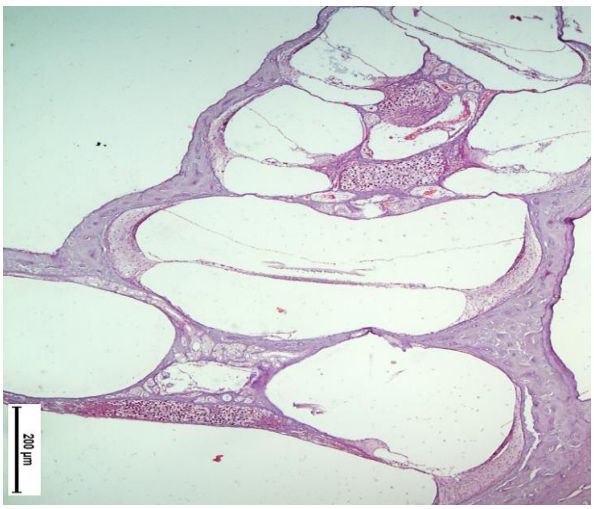

Figure 2: Mid-modiolar section of a specimen from study group shows normal cochlear architecture and no signs of chronic inflammatory reaction.

RESULTS

We found no significant histopathological findings in the study group suggesting acute or chronic inflammatory reaction inside the cochlea. The cochleae from the study group shows normal architecture and preserved microstructures.

The study founds no statistically significant difference between inner hair cell count or outer hair cell count in the study and control groups at basal, middle and apical turns of the cochlea (table 1). The same was found in Spiral Ganglion Neurons packing density /mm^3 at basal, middle and apical turns of the cochlea.

Table 1. Mean Inner and outer hair cell count at different cochlear turns.

	Group	**Basal Turn**	**Middle turn**	**Apical Turn**
Mean OHC	**Injection** (n=10)	2.6 (SD 0.5)	2.7 (SD 0.5)	2.7 (SD 0.5)
	Control (n=10)	2.8 (SD 0.8)	2.7 (SD 0.6)	2.8 (SD 0.6)
	***p* value**	0.68	0.91	0.79
Mean IHC	**Injection** (n=10)	1.0 (SD 0)	0.9 (SD 0.3)	0.8 (SD 0.4)
	Control (n=10)	1.0 (SD 0)	0.8 (SD 0.4)	0.8 (SD 0.4)
	***p* value**	1.0	0.73	1.0
• Using Independent sample Mann-Whiteny U test, there are no statistically significant difference between inner hair cells count or outer hair cells count. IHC= inner Hair cells, OHC= Outer hair cells				

Table 2: Mean Spiral Ganglion Neurons (SGN) packing density/mm^3 at different cochlear turns.

Group	**Basal Turn**	**Middle Turn**	**Apical Turn**
Study (n=10)	1012 (SD 107)	1092 (SD 140)	1017 (SD 130)
Control (n=10)	1106 (SD 159)	1072 (SD 155)	1046 (SD 142)
***p* value**	0.123	0.481	0.436
* Using Independent sample Mann-Whiteny U test, there are no statistically significant difference between SGN packing densities at basal, middle and apical cochlear turns.			

DISCUSSION

This study demonstrates that intracochlear injection of small amount of dexamethasone has no chronic inflammatory effects on the guinea pig's cochlea. No inflammatory cellular infiltration or fibrosis were found in the examined specimens from the study group. Also, hair cell counts and spiral ganglion neurons packing density shows no difference compared to the control group. In order to deliver dexamethasone through RWM intra-cochlear, we used a 10 µl Hamilton syringe with 30-gauge (Hamilton Company, Reno, NV). This microneedle has a diameter of 300 µm with an ultra-sharp beveled tip. The microneedle is advanced through RWM and DEX is injected slowly into scala tympani.

Leong S and colleagues used a custom-made microneedle with a diameter of 100 µm to perforate round window membrane of Guinea Pigs. The round window membrane perforation began to heal in 24 hours and completely healed by 1 week with complete closure after 48 hours. There were no measurable audiologic consequences by DPOAE. **(8)**

Yu et al in a similar study found complete healing of round window membrane perforations after 1 week in the histopathological sections with no granulation tissue formation. A temporary high frequency hearing loss occurred which resolves within 24 hours without producing permanent major hearing loss .**(9)**

Another study by Leong S. and his colleagues used microneedle to inject multiple volumes of artificial perilymph (1.0 - 2.5 – 5.0 – 10.0 µl) through round window membrane of Guinea Pigs, they found it a safe method to deliver small volumes into scala tympani, only high-frequency HL occurred when injecting large volumes and resolved after 48 hours.**(10)**

Reviewed studies prove that microneedles create a temporary micro-perforation in the RWM without causing significant anatomic or physiologic dysfunction. This perforation heals in a period of 7 days. Our study shows that the injection process has no long-term inflammatory effects on the microstructures of the cochlea.

Microneedles have the potential to mediate safe and effective intracochlear access that has many applications in the diagnosis and treatment of inner ear diseases.These applications include micro-aspiration of the intracochlear fluids that help in diagnosis of various cochlear disorders, and microneedle injection of treating agents and gene therapy.(11)

REFERENCES

1. Neuhauser HK. The epidemiology of dizziness and vertigo. Handbook of clinical neurology. 2016;137:67-82.

2. Salt AN, Plontke SK. Pharmacokinetic principles in the inner ear: Influence of drug properties on intratympanic applications. Hear Res. 2018;368:28-40.
3. Feng SJ, Leong S, Aksit A, Hebert D, Olson ES, Kysar JW, et al. Physiologic Effects of Microneedle-Mediated Intracochlear Dexamethasone Injection in the Guinea Pig. Laryngoscope. 2024;134(1):388-92.
4. Lyu AR, Kim DH, Lee SH, Shin DS, Shin SA, Park YH. Effects of dexamethasone on intracochlear inflammation and residual hearing after cochleostomy: A comparison of administration routes. PLoS One. 2018;13(3):e0195230.
5. Parys QA, Van Bulck P, Loos E, Verhaert N. Inner Ear Pharmacotherapy for Residual Hearing Preservation in Cochlear Implant Surgery: A Systematic Review. Biomolecules. 2022;12(4).
6. Kuthubutheen J, Coates H, Rowsell C, Nedzelski J, Chen JM, Lin V. The role of extended preoperative steroids in hearing preservation cochlear implantation. Hearing Research. 2015;327:257-64.
7. Ramekers D, Benav H, Klis SFL, Versnel H. Changes in the Electrically Evoked Compound Action Potential over time After Implantation and Subsequent Deafening in Guinea Pigs. J Assoc Res Otolaryngol. 2022;23(6):721-38.
8. Leong S, Aksit A, Feng SJ, Kysar JW, Lalwani AK. Inner Ear Diagnostics and Drug Delivery via Microneedles. Journal of Clinical Medicine. 2022;11(18).
9. Yu M, Arteaga DN, Aksit A, Chiang H, Olson ES, Kysar JW, et al. Anatomical and Functional Consequences of Microneedle Perforation of Round Window Membrane. Otol Neurotol. 2020;41(2):e280-e7.
10. Leong S, Feng SJ, Aksit A, Olson ES, Kysar JW, Lalwani AK. Microneedles Facilitate Small-Volume Intracochlear Delivery Without Physiologic Injury in Guinea Pigs %J Otol. Neurotol. 2023;44(5):513.
11. Leong S, Aksit A, Feng SJ, Kysar JW, Lalwani AK. Inner Ear Diagnostics and Drug Delivery via Microneedles. J Clin Med. 2022;11(18).