

Main Articles

Efficacy of intratympanic methylprednisolone acetate in treatment of drill-induced sensorineural hearing loss in guinea pigs

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Abstract

Intratympanic steroids offer direct access to the inner ear with high concentration and without systemic effects. In this study, the efficacy of intratympanic methylprednisolone acetate (IT-MPA) was evaluated in a guinea-pig model of drill-induced inner ear trauma.

Twenty-five guinea pigs were divided into a control group to document the baseline distortion product otoacoustic emissions (DPOAEs) and the normal scanning electron microscopic (SEM) morphology of the inner ear. The animals in the study group were subdivided into a steroid-only group (S), a trauma-only group (T), a trauma-plus-time group (TT), and a trauma-plus-steroid (TS) group.

IT-MPA was found to have no damaging effect on the inner ear. Twelve days after trauma, there was spontaneous although incomplete recovery of the DPOAEs amplitudes and SEM morphology with scar tissue replacing lost outer hair cells. Statistically higher DPOAEs amplitudes ($p < 0.05$) were recorded in the TS group that had nearly normal SEM morphology compared to the TT group. The authors conclude that IT-MPA significantly improves drill-induced sensorineural hearing loss and inner ear morphological changes in guinea pigs.

Key words: Hearing Loss, Sensorineural; Hearing loss, Noise-induced; Guinea Pigs; Methylprednisolone; Microscopy, Electron, Scanning Transmission; Otoacoustic Emissions, Spontaneous

Introduction

Permanent sensorineural hearing loss following tympanoplasty is reported to be between 1.2 per cent¹ and 4.5 per cent.² Touching the intact ossicular chain with burrs produces pressure conducted towards the footplate of the stapes comparable to 130–150 dB sound pressure levels.^{3,4} Paparella demonstrated that drilling on the intact ossicular chain in experiments on cats produced changes in the organ of Corti located in the basal turn of Corti, with the most severe disruption of the cytoarchitecture and cellular degeneration occurring in the outer hair cells (OHCs).⁵

Experimental evidence showed the efficacy of corticosteroids in protecting the mouse from noise-induced hearing loss. Mice injected with methylprednisolone acetate (MPA) had less of a threshold shift on their brainstem auditory evoked

responses than did their non-injected littermates.⁶

Systemic corticosteroids are also known to improve hearing in autoimmune inner ear diseases⁷ and sudden sensorineural hearing loss.⁸ The intratympanic route provides direct access to the inner ear without systemic side-effects. It gives a much higher concentration of steroids into the cochlear fluids compared to systemic administration.^{9–11} Intratympanic MPA (IT-MPA) was found to have the best profile within the perilymph and endolymph.¹²

This experimental study was carried out to evaluate the efficacy of IT-MPA in preventing or reversing sensorineural hearing loss and cochlear morphological changes in a guinea-pig model of drill-induced trauma, using distortion product otoacoustic emissions (DPOAEs) and scanning electron microscopy (SEM).

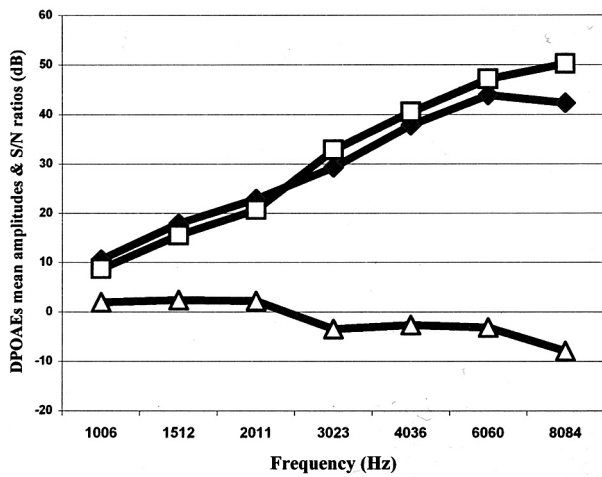


FIG. 1

DPOAEs mean amplitudes, S/N ratio in the control group. Notice the increasing amplitude and S/N ratio from the low to the higher frequencies. (◆) mean amplitude, (□) S/N ratio, (△) noise floor.

Materials and methods

Twenty-five healthy pigmented guinea pigs of either sex, of the same age, and average weight of 400 g were used. They had normal otoscopic findings and a preserved Preyer reflex threshold.¹³ The animals were divided into a control group of five animals and a study group of 20 guinea pigs. The control group was used to document baseline DPOAEs and the normal SEM morphology of the inner ear. The animals in the study group were divided into the following:

- (1) Steroid-only group (S): five animals were used to evaluate the safety of IT-MPA on the inner ear, 12 days after injection.
- (2) Trauma group: fifteen animals were subjected to a standardized drill-induced trauma as carried out by Schneider *et al.*¹⁴ They were then divided further into:
 - (a) Trauma-only group (T): five animals were used to document inner ear SEM morphological changes immediately following trauma.

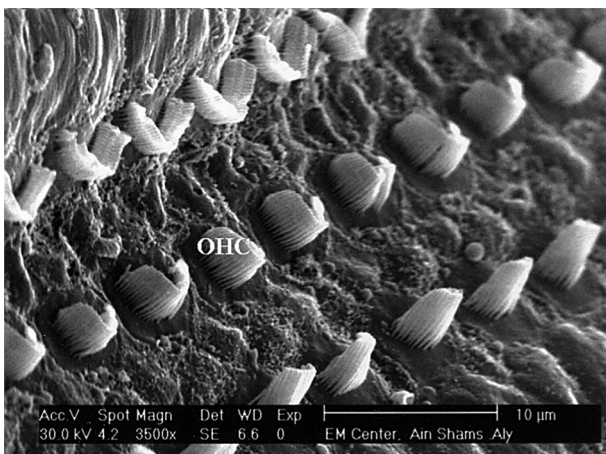


FIG. 2

Normal SEM of the outer hair cells (OHCs). Stereocilia are arranged in a V pattern.

- (b) Trauma-plus-time group (TT): five animals were allowed to recover from anaesthesia in order to look for any spontaneous recovery of hearing and inner ear morphology, 12 days after trauma.
- (c) Trauma-plus-steroid group (TS): five animals were injected with IT-MPA immediately after trauma and allowed to recover from general anaesthesia. They were used to assess the effect of IT-MPA on hearing and inner ear morphology 12 days after trauma.

The protocol was approved by the Animal Care and Use Committee in the Faculty of Medicine- Suez Canal University.

Methods

All steps were performed under general anaesthesia using intramuscular injections of ketamine (45 mg/kg) and xylazine 8 mg/kg. DPOAEs were recorded using the following protocol: (1) two pure tone stimuli F1 and F2 with an F2/F1 ratio of 1.2 were used. The intensity levels of the two tones were equal to approximately 65 dB SPL and measurements were done for the frequency range 1 to 8 kHz. Higher levels (≥ 70 dB) were not used because they are accompanied by more noise rejection, and recording below 1 kHz was found difficult due to the noisy respiration of the anaesthetized animal. The DPOAEs amplitude was measured at 2F1–F2 and plotted against the geometric mean of F1 and F2 (i.e. DP-gram). The signal to noise (S/N) ratio was calculated by subtracting the noise floor from the corresponding amplitude. Baseline recording was done for all groups and repeated after 12 days for groups S, TT, and TS).

The tympanic membrane of the right ear was visualized under the operating microscope and MPA solution (40 mg/ml, Depo Medrol, Upjohn) was injected through the upper rear quadrant of the tympanic membrane using a 22-gauge spinal needle. Approximately 0.11 ml (total dose of 4.4 mg) was injected, this being generally the volume required to completely fill the middle ear.¹² The head was positioned to pool the solution over the round window niche for 30 minutes.

Surgical steps for drill-induced trauma in groups (T, TT, TS). After recording the baseline DPOAEs, a post-auricular incision was made and the soft tissue was retracted from the field by a self-retaining mastoid retractor. A small opening in the bulla was made with a needle and curette exposing the ossicular chain. A 1.4 mm diamond burr with a rotating speed of 20 000 rpm was applied against the body of the incus for 10 seconds.¹⁴ The wound was then sutured and a pressure dressing was applied to the wound for 24 hours. Amoxicillin 40 mg/kg/d was given i.m. to prevent secondary infection.

Inner ear preparation and SEM. Animals were sacrificed either immediately (group T) or two weeks

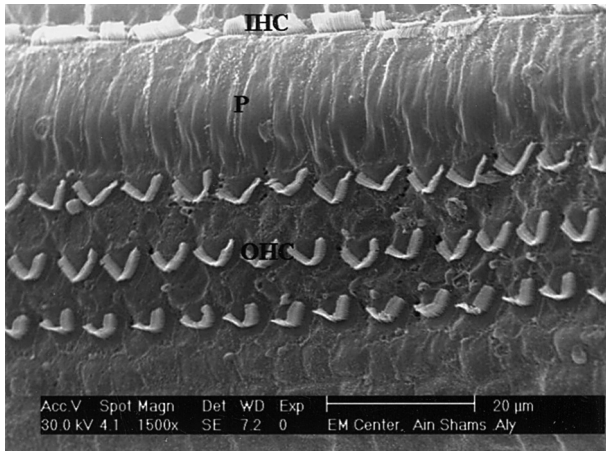


FIG. 3

Steroid-only group (S). SEM of the organ of Corti. No evidence of stereociliary abnormalities 12 days after IT-MPA. IHC = inner hair cells; OHC = outer hair cells, P = pillar cells.

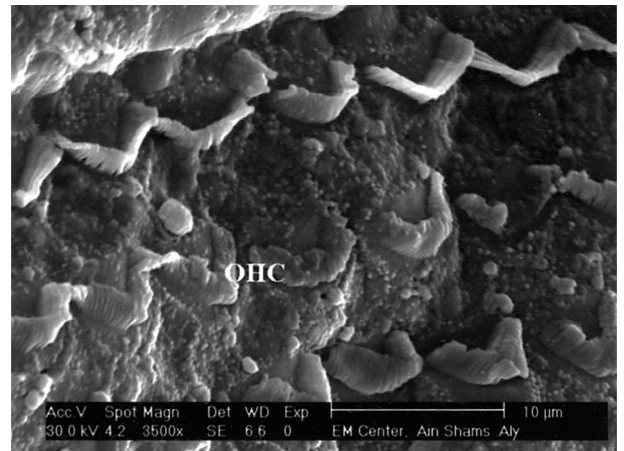


FIG. 4

Trauma-only group. SEM of organ of Corti showing marked stereociliary changes in the OHCs, especially the outer row.

later (group S, TT and TS) using an intraperitoneal injection of 100 mg of sodium pentobarbital. The tympanic bulla was harvested. It was opened and excess bone was removed. The cochlea was perfused using glutaraldehyde 2.5 per cent in phosphate-buffered saline (pH 7.2) through the round window after removing the stapes. The cochlea was fixed in the same solution for 24 to 72 hours. Specimens were then transferred to saline.

The bony wall of the cochlea was thinned with a high-speed surgical drill. Only the lateral aspect of the cochlea was widely exposed and the medial wall was left for support. For dehydration, the specimen was placed in 30 per cent acetone for 24 hours, then in 70 per cent acetone for another 24 hours.

Any remaining bone fragments were removed. The stria vascularis was stripped away, and Reissner's membrane was removed. After dissection, the specimen was placed in 90 per cent acetone and then in three changes of 100 per cent acetone at intervals, each of at least 12 hours for complete dehydration.

The next process was critical point drying. This involved placing the tissue in a pressure container and replacing the acetone with liquid carbon dioxide. The container was warmed to the critical point, which is the combination of pressure and temperature at which the carbon dioxide is transformed from a liquid to gas without a change in volume. The gas was gradually released, leaving the dry specimen without distortion.

The specimen was then placed in a vacuum chamber and coated with a 200-Å layer of gold.

A Hitachi-50 scanning electron microscope was used to examine the specimen. Low magnification was used for orientation purposes. The basilar membrane was examined at a higher magnification ($\times 500$ to $\times 2000$).

Results

Control group

The mean amplitudes and S/N ratios of DPOAEs

showed a generally rising slope from low to high frequencies. The mean amplitude was lowest at 1006 Hz (10.6 dB) and highest at 6006 Hz (43.9 dB) (Figure 1). The S/N ratio similarly was lowest at 1006 Hz (8.7 dB) and highest at 8086 Hz (50.2 dB). The normal SEM view of organ of Corti is shown in Figure 2.

Steroid-only (S) group

Twelve days after IT-MPA, there was no statistically significant difference in the DPOAEs' mean amplitude and S/N ratio between the (S) group and control group ($p > 0.05$). Again there was no change in the cytoarchitecture of the organ of Corti two weeks after IT-MPA compared to the baseline (Figure 3).

Trauma-only (T) group

The outer hair cells (OHCs) immediately underwent

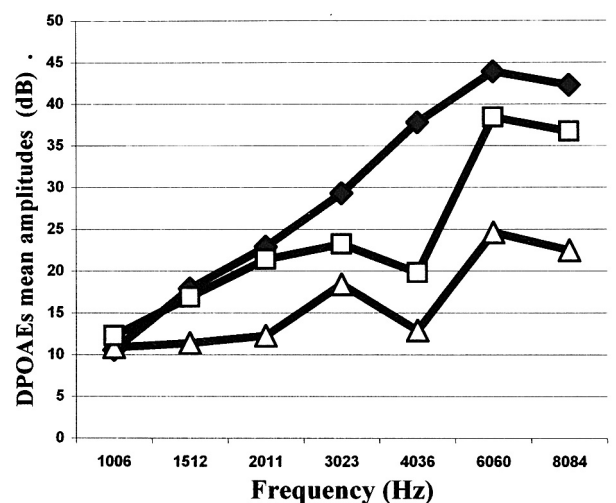


FIG. 5

DPOAEs mean amplitudes 12 days after trauma in the trauma plus time group (TT) and trauma plus MPA (TS) compared to the control group. (◆) control group, (□) trauma plus MPA, (Δ) trauma plus time groups.

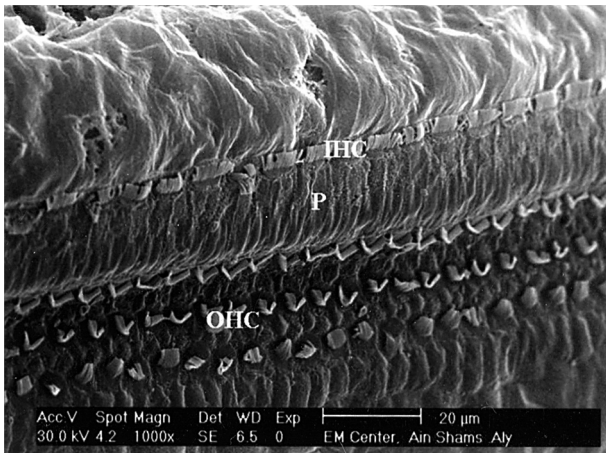


FIG. 6

Trauma plus time group. SEM showing apparently normal IHCs. The innermost and second rows of OHCs look normal, while the outermost row shows some stereociliary abnormalities with some cells replaced by scar tissue. P = pillar cells

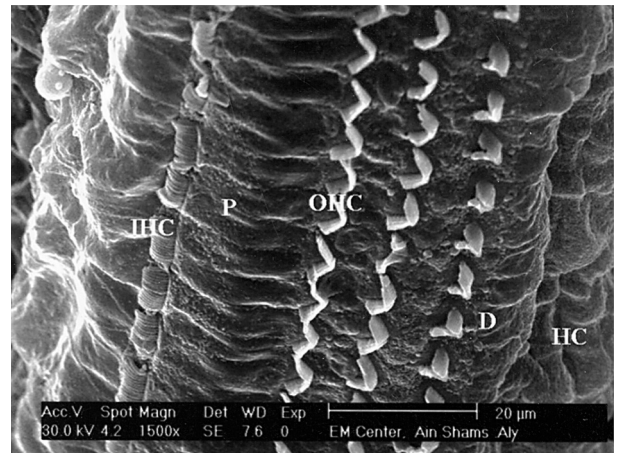


FIG. 7

Trauma plus steroid group SEM showing normal IHCs, normal OHCs except for some minimal stereociliary disarray in the outermost row. P = pillar cells

stereociliary changes in the form of disarray, collapse and fracture at the cuticular plate. Fused and dissolved stereocilia were also seen and some OHCs were totally lost. These changes were more marked in the outermost row (Figure 4). In addition, the inner hair cells (IHCs) showed abnormalities in the form of shortening, clumping, and collapse of their stereocilia.

clumped and inclined stereocilia (Figure 6).

Trauma-plus-steroid (TS) group

The DPOAEs mean amplitudes and S/N were statistically higher than the (TT) ($p < 0.05$) except at 1006 Hz although it was still significantly below the control values (Figure 5). The mean DPOAEs ranged from 12.3 dB at 1006 Hz to 36.7 dB at 8084 Hz and the S/N ratio ranged from 11.2 dB Hz at 1006 Hz to 44.1 dB at 8084 Hz. In SEM, almost complete recovery of the OHCs abnormalities was evident with barely perceptible stereociliary disarray. IHCs show marked recovery with only slightly shortened stereocilia (Figure 7).

Discussion

Drill-induced trauma during ear surgery or experimentation can damage the inner ear through several mechanisms: direct mechanical action, metabolic impairment and altered permeability of the cell membrane.¹⁵ Moreover, drill-induced mechanical vibrations have a synergistic action with sound energy.¹⁶ Vibrations may induce sympathetic stimulation in the inner ear with corresponding vasospasm.¹⁷

Intratympanic injection of corticosteroids is used mainly because it avoids the systemic effects of these powerful anti-inflammatory drugs. However, the amount of drug that actually passes through the round window membrane may vary depending on several factors such as anatomy of the region, mucosal bands, permeability of the round window membrane, and pharmaceutical form of the drug.¹¹

The permeability of the round window membrane in normal and diseased ears has been studied previously by Goycoolea *et al.*¹⁸ Use of facilitating agents, such as histamine, to increase round window membrane permeability was used experimentally.⁹ Development of the sustained release form of corticosteroids placed against the round window membrane was also suggested.¹⁹

- **High concentration intratympanic steroids can be applied to the inner ear without systemic effects**
- **This study tests the efficacy of intratympanic methylprednisolone acetate (IT-MPA) in the guinea-pig model after inner ear trauma induced by a drill**
- **IT-MPA was found to have no deleterious effect on the inner ear**
- **IT-MPA significantly improved drill-induced sensorineural hearing loss and inner ear morphological changes in guinea pigs**

Trauma-plus-time (TT) group

Two weeks following trauma, DPOAEs' amplitudes and S/N ratio were statistically lower than the control group ($p < 0.05$) except at 1006 Hz (Figure 5). The amplitudes ranged from 10.9 dB at 1006 Hz to 22.5 dB at 8084 Hz and the S/N ratio ranged from 9.5 dB at 1006 Hz and 30.4 dB at 8084 Hz. In SEM, stereociliary arrangement is better than the (T) group indicating some recovery. The innermost row of OHCs showed almost normal stereociliary bundles while the second and outermost rows had some stereociliary disarray with scar formation apparently replacing the lost cells. Some IHCs had

In the present study, MPA was chosen for intratympanic use because it is five times more potent than hydrocortisone and it has a better profile in inner ear fluid than dexamethasone.¹² In the steroid-only group (S), normal hearing as measured by DPOAEs 12 days after injection proves the safety of MPA for intratympanic use. This has been experimentally demonstrated by Silverstein and Choo, in guinea pigs. They found a 72 per cent increase in the cochlear blood flow from the baseline over the first two hours after intratympanic dexamethasone without decrease in cochlear microphones or compound action potentials.¹⁹

In the present study, SEM study of the organ of Corti immediately after drill-induced trauma confirms the damaging effect on the stereociliary bundle. This takes the form of fracture, collapse, clumping, and dissolved stereocilia in both OHCs and IHCs. Hamernik & Salvi²⁰ and Ye *et al.*²¹ reported similar results.^{20,21} Stereocilia are the most susceptible structures for damage as their position lies between the structure and function of the organ of Corti. Disarrayed hair bundles represent a 15–30 dB threshold shift.¹⁶ The OHCs are known to be more sensitive to acoustic trauma than the IHCs. Some changes are short-lived and stereocilia may start to return to their pre-exposure stiffness in about 15 minutes following mechanical stimulation.²²

In the trauma-plus-time group (TT), incomplete recovery of hearing and inner ear morphology, especially in the third row of OHCs, was evident 12 days after trauma. A longer period of follow up may be needed to document the final healing.¹⁵

In the present work, the statistically significant improvement in the DPOAEs amplitude and the more normalized inner ear morphology in the trauma-plus-steroid group (TS) confirm the value of IT-MPA following drill-induced trauma. The beneficial effect of IT steroids in sudden hearing loss has been reported previously by Silverstein *et al.*¹⁹ They found that the average SRT improvement was 15 dB and the average SDS improvement was 24 per cent after IT steroids in patients with idiopathic sensorineural hearing loss. Also, Parnes *et al.*¹² used IT steroids in 37 patients with various inner ear disorders. Patients with immune-mediated hearing losses showed the best results, with notable improvement also seen in cases with 'sudden deafness'.

In conclusion, IT-MPA significantly improves the drill-induced hearing loss and stereociliary changes in guinea pigs. However, extrapolation from this animal model to what may accidentally happen during ear surgery is not possible until controlled human studies are available. Until this time, drill-induced trauma should be avoided during surgery and hearing loss following this type of trauma should be regarded as irreversible. Having said that, the authors strongly recommend intra-operative IT-MPA instillation following all middle-ear drill work in the presence of an intact ossicular chain. It is safe and it probably improves post-operative hearing.

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