Division of the stapedial tendon results in noise-induced damage to the inner ear

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Source of support: Departmental sources

Background: The effect of division of the stapedial tendon on susceptibility to noise-induced inner ear damage has not been previously studied. This study aimed to evaluate the effects of noise exposure following division of the stapedial tendon in guinea pigs.

Material/Methods: Ten adult albino guinea pigs were used. The stapedial tendon of each right ear was cut. The stapedial tendon in each left ear was left intact and these ears served as a control group. DPOAEs and ABR tests were performed before and 10 days after noise exposure. The animals were exposed to a 110-dB noise stimulus for 6 h in a silent room a week after surgery. Cochleas of the animals were removed, and inner and outer hair cells were examined under a light microscope.

Results: We found that noise exposure adversely affected DPOAE measurements at all frequencies except 2 KHz in experimental ears. Noise exposure also produced significantly elevated ABR thresholds in experimental ears at 2, 4, 8, and 16 KHz. On histopathological examination, we found a significantly greater prevalence of apoptotic cells in the experimental ears.

Conclusions: Based on these findings, we can conclude that after division of the stapedial tendon, noise exposure may cause damage to the inner ear. This is the first study in the English literature that demonstrates the potential protective effect of the stapedial tendon against acoustic damage.

MeSH Keywords: Stapes Surgery • Noise – adverse effects • Stapedius • Otoacoustic Emissions, Spontaneous

Full-text PDF: http://www.medscimonit.com/download/index/idArt/890158
Background

Stapedectomy and stapedotomy are surgical techniques currently used in the treatment of otosclerosis. Surgery for otosclerosis involves an exposed inner ear and this puts the patient at risk of developing tinnitus, vertigo, sensorineural hearing loss, and even complete deafness. There is a risk of sensorineural hearing loss given that the stapedial tendon, which protects the inner ear from noise damage, is routinely cut in stapes surgery. This has not been investigated previously.

Exposure to high-intensity noise can cause irreversible hearing damage. The harmful noise may be continuous (e.g., concerts), receptive impulsive (e.g., industrial noise), or pure impulsive (e.g., explosion, gunshot noise). The damage mechanisms differ depending on the type of the noise and are of dual origin: mechanical and metabolic. Mechanical damage develops when movement of the basilar membrane is excessive, inducing detachment of hairs from the tectorial membrane. Metabolic disorders have multiple origins: ionic, excitotoxic, and production of cochlear free radicals [1].

Otoacoustic emission (OAE) testing is a suitable tool for objective non-invasive assessment of inner ear function, particularly that of the organ of Corti [2]. The micromotility of the outer hair cells following acoustic stimulation provides the OAE response [3]. It was established early on that noise exposure caused diminished OAEs [4]. This study aimed to evaluate the effects of noise after dividing the stapedial tendon in guinea pigs using OAE testing, auditory brainstem response (ABR) measurement, and histological examination.

Histopathological demonstration of apoptotic changes in the inner and outer hair cells of the cochlea is undoubtedly the most objective way to determine cochlear damage. The apoptotic cells can be easily seen under a light microscope following TdT-mediated dUTP-biotin nick-end labeling (TUNEL) staining [5].

To the best of our knowledge, this is the first study in the English literature that demonstrates the protective effect of the stapedial tendon against acoustic trauma.

Material and Methods

Ten adult albino guinea pigs (600–900 g) were used. They ranged in age from 2 to 15 months. The stapedial tendon in each right ear was cut and the left ears served as the control in each animal. Tympanometric examination, OAE testing, and ABR measurements were performed before surgery. Tympanometric examinations were performed to exclude middle ear pathology, which can affect the OAE results. One week after surgery, a 4-kHz octave band noise stimulus at an intensity of 110 dB SPL was presented by the audiometer (Interacoustics AC 40) in a silent room for 6 h. The animal cage was put exactly half-way between 2 loudspeakers, positioned at 40 cm from each loudspeaker. Noise intensity was measured using a Precision sound level meter. The noise level variation was less than 3 dB within the space available to the animal. Measurement of OAEs, tympanometric examination, and ABR measurements were repeated after noise exposure. Tympanometric examinations were performed using a low-frequency (226 Hz) probe tone (AZ-26 Interacoustics). The experimental protocol was approved by the Hacettepe University Animal Care and Use Committee (protocol no. 2005/15-2).

Surgical technique

General anesthesia with ketamine (50 mg/kg) and xylazine (5 mg/kg) was administered intramuscularly. Prophylactic antibiotics were administered before surgery and immediately after surgery. A parallel incision to the posterior of the bony ear canal 1–2 mm from the tympanic membrane was performed to the right ear of the guinea pigs with an otomicroscope. The tympanomeatal flap was elevated and the stapedial tendon was located posteroinferiorly and divided. After the completion of the procedure, all guinea pigs were euthanized.

Recording of distortion product otoacoustic emissions

For recording of the distortion product otoacoustic emissions (DPOAEs), a biological system (Audi Scout Sport) was used. All animals were anesthetized. The acoustic probe was handheld at the opening of the external ear channel with a slight pressure. Both ears were measured at a stimulus frequency of f1/f2=1.22. The stimulus level of both frequencies was 70 dB SPL, and the noise to peak level of cubic DPOAEs 2f1-f2 was measured. The background noise level in the exposure room was below 50 dB SPL and room temperature was controlled at approximately 25°C. A total of 24 measurements at 1, 1.5, 2, 3, 4, and 6 kHz were performed in each ear of each animal before and after noise exposure.

ABR recording

Animals were anesthetized with ketamine and xylazine. Hearing thresholds of the guinea pigs were assessed using ABR. The sound stimulus consisted of a 15-ms tone burst, with a rise-fall time of 1 ms at 2, 4, 8, and 16 kHz. The intensity was varied in 5-dB steps. Responses for 1024 sweeps were averaged at each intensity level. In all cases, thresholds were defined as the lowest intensity required to produce a reproducible ABR wave form. ABR measurements were performed before surgery and 10 days after noise exposure.
Histological preparations

The temporal bones of the animals (n=20) were removed and processed for histological examination. The specimens were fixed with 4% formaldehyde and then decalcified using 0.1 mol/L ethylenediamine tetra-acetic acid (EDTA) [6]. Then the cochlea was removed. A microtome was then used to obtain 13–15 slices of 1 µm in 650–700 µm per block. Experimental and control slides were evaluated by a blinded histologist using TUNEL staining. Light microscopic evaluation was undertaken for the inner and outer cochlear hair cells. Then, the percentages of apoptotic cells were determined in the experimental and control groups using light microscopy.

Statistical analysis

The study data were analyzed using a paired sample t-test. P<0.05 was accepted as statistically significant.

Results

High inter-individual differences were found due to age differences between animals. Distinct change in DPOAEs was found at 1 kHz (p=0.033), 1.5 kHz (p=0.031), 2 kHz (p=0.428), 3 kHz (p=0.025), 4 kHz (p=0.010), and 6 kHz (p=0.003). The only frequency at which there was no statistically significant difference after noise exposure was 2 kHz. The mean values of DPOAEs in the experimental and control groups before and after noise exposure are shown in Figure 1. In the control group, there was a statistically significant difference at 6 kHz only (p=0.034). There was no difference in tympanometric examination before and after surgery. At the start of the experiment, mean ABR thresholds ranged from 23.5 to 31 dB SPL across frequencies for the right ears and from 23 to 31 dB SPL for the left ears. There was no statistically significant difference between right and left ear measurements before noise exposure. Thresholds measured 10 days later were higher in right ears, and ranged from 40 to 56 dB SPL. The noise exposure produced significant ABR threshold elevations for right ears at 2, 4, 8, and 16 kHz (p=0.07, p=0.01, p=0.001, and p=0.001, respectively). Thresholds measured 10 days after noise exposure ranged from 24 to 30.5 dB SPL for left ears, and this difference was not significant (p=0.8, p=0.5, p=0.9, p=0.1, respectively). TUNEL-positive cells were nearly absent in the inner and outer hair cells of the cochleas of the control group; however, the prevalence of TUNEL-positive cells was high in the experimental group (Table 1, Figure 2).

Discussion

In this study, we examined the effect of acoustic trauma on the cochleas of guinea pigs using DPOAEs, ABR thresholds, and histopathological examination, and we attempted to demonstrate the protective effect of the stapedial tendon. We have demonstrated increased damage in the ear with divided stapedial tendons. To our knowledge, there are no other studies in the literature that have investigated the extent of acoustic damage following division of the stapedial tendon.

Various animal models have been used for experimental middle ear surgery, including primates, rats, dogs, and cats [7]. The cat has been favored in studies of the histological and audiological effects of stapedectomy. Due to ethical and financial considerations, the guinea pig model is established at the preferred model in many areas in otologic research. One study described the guinea pig model as an efficient and low-cost alternative to the standard feline model for stapedectomy [8]. For these reasons, we used guinea pigs.

The effect of noise on hearing has traditionally been studied using auditory brain stem response (ABR) on guinea pigs. In these studies, noise-induced ABR threshold shifts were observed and it was reported that dexamethasone, geranylgeranylacetone, and tocopherol had protective effects [9–11]. In our study, DPOAEs and ABR thresholds were found to be more appropriate, as these tests reflect the condition of the outer ear.

Figure 1. Mean DPOAE amplitudes in control and experimental groups before and after noise exposure.
Preyer and Gummer have shown that the non-linearity of the mechanoelectrical transducer function of the outer hair cells, as seen in guinea pigs, is essential for the non-linear movement of the basilar membrane, a source of DPOAE generation [12]. In another study, DPOAEs were measured before and after noise exposure. Results of this study suggest that the ability of the cochlea to generate DPOAEs is associated with the condition of the outer hair cells. Industrial noise, even though it does not cause visible damage to the outer hair cells, or even loss of inner hair cells, can result in microdamage to stereocilia, leading to loss of hearing function, and this is reflected by diminished DPOAEs [2]. In another study, the effects of different types of realistic occupational noise (as well as impulse noise) on loss of DPOAEs in guinea pigs were tested. The study showed a link between changes in DPOAE amplitudes and the type of occupational noise exposure. Skellett et al. used 65-dB SPL broadband noise continuously for 3 or 11 days to evoke changes in DPOAEs in guinea pigs [13]. Clark and Pickles showed that exposure to high-intensity pure tones over a period of 5–30 min damaged outer hair cells according to intensity levels and exposure time [14]. A similar study with guinea pigs showed that exposure to realistic noise for 2 h resulted in changed DPOAEs [15].

Histopathological demonstration of damaged inner and outer hair cells is the most objective way to show cochlear damage.

### Table 1. Percentage of TUNEL-positive cells in the inner and outer hair cells.

| Control group | Experimental group |
|---------------|---------------------|
| Inner hair cells (TUNEL positive) | Outer hair cells (TUNEL positive) | Inner hair cells (TUNEL positive) | Outer hair cells (TUNEL positive) |
| Inner hair cells (TUNEL positive) | Outer hair cells (TUNEL positive) | Inner hair cells (TUNEL positive) | Outer hair cells (TUNEL positive) |
| 1. 1% | 3% | 70% | 86% |
| 2. 3% | 5% | 73% | 90% |
| 3. 7% | 4% | 86% | 79% |
| 4. 2% | 6% | 82% | 85% |
| 5. 1% | 2% | 75% | 87% |
| 6. 5% | 1% | 81% | 91% |
| 7. 3% | 3% | 72% | 88% |
| 8. 2% | 1% | 70% | 89% |
| 9. 1% | 6% | 79% | 94% |
| 10. 4% | 5% | 65% | 90% |

### Figure 2. (A) Control group spiral ligament and outer hair cells. SLig: Spiral ligament, BM: Basilar membrane, ι: Outer hair cells. TUNEL staining 400× (OM). (B) Experimental group spiral ligaments and outer hair cells. SLig: Spiral ligament, BM: Basilar membrane, ι: Outer hair cells. TUNEL staining 400× (OM).
TUNEL positivity is an indicator of nonspecific cellular damage, including necrosis [16].

It was noteworthy to demonstrate significantly more TUNEL-positive cells in the organs of Corti of the experimental animals when compared to controls. This result indicates that the damage due to acoustic trauma was significantly greater in ears with divided stapedial tendons.

The beneficial impact of stapedial surgery on improving hearing in otosclerotic patients is well proven; the immediate success rate is between 80% and 90% or even higher, and the air-bone gap can usually be reduced or closed [17]. However, there is controversy regarding sensorineural hearing loss in patients with otosclerosis. It has been argued that the high-frequency sensorineural hearing loss seen in postoperative stapedectomy patients is simply related to presbycusis and is not different from aged-matched controls [18]. Conversely, some have suggested that the increase in high-frequency thresholds is caused by trauma experienced during the surgery itself, or expansion of otosclerotic focus into the cochlea [19]. One known cause of hearing loss following stapes surgery is inner ear barotrauma. In a study investigating the effects of barotrauma after stapedectomy in guinea pigs, cochlear effects were determined using electrophysiological thresholds and cochlear hair cell counts. It is suggested that stapedectomy does not appear to predispose the guinea pig model of divided-barotraumas to cochlear squeal. There is no study that proves convincingly that noise is a cause of sensorineural hearing loss after stapes surgery. However, it is known that one of the most important mechanisms in protecting the ears from noise damage is the stapedial tendon, and this is cut during the surgery.

We found that noise significantly affected ABR thresholds at all frequencies and all DPOAE measurements except 2 kHz in guinea pigs that were exposed to a high degree of noise over a long time. This type of noise can cause permanent threshold shift. This noise altered all outer hair cells; although the change in DPOAE results at 2 kHz was not statistically significant, it still changed. The histopathological analysis was consistent with these results.

**Conclusions**

In light of these findings, it can be assumed that noise can cause damage to the inner ear after stapes surgery. In future studies, these findings may be further supported by the examination of defects in the outer hair cells using electron microscopy. In the clinic, patients should be advised to protect themselves from noise after stapes surgery.

**Acknowledgements**

The authors declare no competing interest. No financial support was received for this paper.