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Assessment of the efficacy of a local steroid rescue treatment administered 2 days after a moderate noise-induced trauma in guinea pig

A local treatment after noise trauma in guinea pig

Elisabeth Mamelle, Naila El Kechai, Victor Adenis, Yann Nguyen, Olivier Sterkers, Florence Agnely, Amélie Bochot, Jean Marc Edeline, Evelyne Ferrary

Abstract

Objectives: Intratympanic injection of corticosteroids membrane after noise-induced hearing loss is an accepted alternative to general administration. We investigated the effect on hearing of a hyaluronic acid gel with liposomes loaded with dexamethasone (DexP) administered into the middle ear.

Methods: An acute acoustic trauma was performed to 13 guinea pigs for a period of 1 hour on Day₋₂. Two 2 days after the noise trauma, the animals were then assigned randomly to four experimental groups: control without gel, gel injection, gel containing free DexP, gel containing DexP loaded into liposomes. Auditory thresholds were measured with Auditory Brainstem Response before Day₋₂ and at Day 0, Day 7 and Day 30 after noise trauma.

Results: Seven days after, a complete hearing recovery was observed in the control group at all frequencies apart from 8 kHz, and no recovery was observed in the three groups receiving a gel injection. Thirty days after trauma, all of the animals had recovered normal hearing, apart from at the 8-kHz frequency, with similar auditory thresholds.

Conclusions: Local DexP administration 48 h after a mild acoustic trauma did not improve hearing recovery, even with a sustained release in a specific gel formulation designed for inner ear therapy.

Key words: Dexamethasone phosphate; guinea pigs; hearing loss; noise-induced; hydrogel; liposomes

Introduction

Exposure to intense noise is frequently associated with non-genetic (acquired) hearing loss in adult subjects. The traumatizing noise may be continuous, repetitive impulsive (industrial noises), or pure impulsive (explosions, gunshots)[1]. Acute acoustic trauma leads to mechanical and metabolic damage to the cochlea. The mechanical damage is due to excessive movements of the basilar membrane leading to loss of hair cells, impaired stereocilia, and collapse of supporting cells[2]. The subsequent metabolic damage includes ionic disorders involving an intracellular influx of potassium or calcium, excitotoxicity following excessive release of glutamate, and oxidative stress due to the production of free radicals[3]. These mechanisms can lead to relatively rapid cell death by necrosis and/or apoptosis. Part of the initial noise-induced hearing loss is only temporary, and may recover as a result of cochlear repair mechanisms[3, 4]. However, exposure to impulse noise typically results in substantial permanent hearing loss.

At a clinical level, common treatments after noise-induced hearing loss consist of resting in a silent environment, administration of corticosteroids which may or may not be associated with vasodilator drugs, antioxidant drugs, hyperbaric oxygen therapy or normovolemic hemodilution[1]. Various pharmacologic compounds such as corticosteroids, apoptosis inhibitors, and antioxidants have been tested in animal models with various levels of noise-induced hearing loss[1, 2]. In these noise trauma animal models, therapies have been administered either before or after noise exposure, and these were effective to varying degrees with regard to hearing protection.

In an earlier study, we demonstrated that a liposomal hyaluronic acid gel loaded with dexamethasone phosphate (DexP) injected into the middle ear of guinea pigs resulted in a high drug concentration in the perilymph as well as a sustained release of the drug for up to 30 days[5]. The aim of the present study was to determine whether this local treatment could have a therapeutic effect in guinea pigs when applied 2 days after a moderate acoustic trauma,

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2 a clinically relevant condition.
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6 **Materials and methods**

7 *Animals*

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10 Thirteen male Hartley albino guinea pigs (Charles River, L'Arbresle, France) were used in the
11 study weighing 300–500 g with auditory thresholds in the normal range[6]. Animal care and
12 experimental procedures were conducted in accordance with the guidelines established by the
13 European Communities Council Directive (2010/63/EU Council Directive Decree), and were
14 approved by the local ethics committee (N° AP 2014-23).
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22 *Study design (Fig. 1)*

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24 **Fig. 1** Experimental protocol near here
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29 After the initial determination of the auditory threshold by Auditory Brainstem Responses
30 (ABR), all of the animals were subjected to noise-exposure at D₋₂. Two days later, at D₀, the
31 audiogram was re-tested with ABR to evaluate the hearing loss induced by the acoustic
32 overexposure. Following this post-trauma ABR test, animals were assigned randomly to four
33 experimental groups:
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- 40 1. control without gel injection but with the bulla opened (eight ears),
- 41 2. hyaluronic acid gel injection without DexP (HA; six ears),
- 42 3. hyaluronic acid gel containing free DexP (HA-DexP; six ears),
- 43 4. hyaluronic acid gel containing DexP loaded into liposomes (HA-DexP-Lip; five ears).
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49 In all the animals in these groups, both ears were used and each ear was considered to be an
50 independent measurement. In the HA-DexP-Lip group, one animal had only one ear injected
51 with the gel (which explains the odd number of ears in this group). The hearing thresholds
52 were recorded again at Day 7 (D₇) and Day 30 (D₃₀) for all groups.
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58 *Materials*

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Dexamethasone sodium phosphate (DexP, Mw 516.4 Da, purity 98%) was obtained from Fagron (Amsterdam, The Netherlands). Egg phosphatidylcholine (EPC, purity 96%) was provided by Lipoid GmbH (Ludwigshafen, Germany). Cholesterol (Chol) was purchased from Sigma–Aldrich Co. (St Louis, MO, USA). 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy-poly-(ethyleneglycol) 2000] (DSPE-PEG2000, purity > 99%) was obtained from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Sodium hyaluronate (HA, Mw 1.5 MDa, purity 95%) was provided by Acros Organics (New Jersey, USA). All other chemicals were of analytical grade.

Preparation of hyaluronic acid (HA) gel formulations

DexP-loaded PEGylated liposomes (DexP-Lip) were prepared by the thin film method as previously described[7]. Briefly, lipid film composed of EPC:Chol:DSPE-PEG2000 (60:35:5 mol%) was prepared using rotary evaporation. The lipid film was hydrated with 100 mg/mL DexP solution in Milli-Q water. The liposome suspension was extruded to achieve a final size of 140 nm. Non-encapsulated DexP was eliminated by dialysis using cellulose dialysis membranes with a molecular weight cut-off of 15 kDa (Spectrum Laboratories, Inc, CA, USA) against HEPES/NaCl buffer (10/115 mM, pH 7.4) at 4°C for 48 h with repeated changes of buffer and protection from light. The mean encapsulation efficiency for DexP-Lip was 182 ± 30 mg DexP/g lipid.

HA and HA-DexP gels were prepared by dissolving HA (2.28% w/v) in HEPES/NaCl buffer (10/115 mM, pH 7.4) and in 10 mg/mL of DexP solution, respectively (DexP dissolved in HEPES/NaCl buffer 10/115 mM, pH 7.4). For the preparation of liposomal gel (HA-DexP-Lip), HA (2.28% w/v) was dissolved in the liposome suspension at 80 mM final lipid concentration with a DexP concentration of 10 mg/mL. The gels were then homogenized by vortex mixing for 5 min, maintained at room temperature for 1 h and then stored at 4°C for at least 12 h before *in vivo* injection.

Exposure to traumatic sound

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Animals, placed individually in a wire mesh cage (23 x 23 x 15 cm), were exposed for 1 h to a traumatic tone (5 kHz pure tone, 100 dB SPL) in an acoustically isolated chamber (IAC model AC2). The pure tone was generated by a wave generator (Hewlett-Packard, model HP 8903B), amplified (Prism-Audio, model LA-150M) and sent to two piezoelectric tweeters (Motorola, model KSN 1005) located on each side of the cage. The sound delivery system was calibrated to obtain 100 ± 5 dB at various locations in the cage, verified by a calibrated type I precision sound level meter (B&K model 2235). As in a previous study[8], visual inspection of the animal during exposure indicated that there was no preferred orientation of the animal with regard to the speakers.

Audiograms determined from Auditory Brainstem Response (ABR)

The audiograms from the guinea pigs were evaluated by recording the ABR following an auditory stimulus. The ABR test was performed on both ears before acoustic trauma (D_{-2}) and after trauma (D_0 , D_7 , and D_{30}), under gas general anesthesia (isoflurane). Tone bursts of 50 ms duration were delivered at 1, 4, 8, 16, 24, and 32 kHz, by a high frequency transducer (Etymotic Research ER1) connected to an external ear canal probe. The ABR were recorded via subcutaneous electrodes (SC25, Neuro-Services) placed subcutaneously in the leg, the vertex, and the ipsilateral retro-auricular side of the analyzed ear. The ABR were collected using a commercial interface and software (RT Otophy Lab, Echodia, France), and were determined from 80 dB, with 5 dB decreasing steps. The thresholds corresponded to the minimal intensity for which an ABR wave (usually wave 3) was clearly detected. To minimize the use of animals (according to the 3R rule), both ears from each animal were used, and each ear was considered to be an independent repetition.

Injection procedure

Gel injection into the middle ear (HA, HA-DexP or HA-DexP-Lip) was performed at D_0 , under general gas anesthesia (isoflurane). Body temperature was maintained around 37°C by a controlled heating tap. To guarantee precise application of the gel on the round window

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2 under microscopic view, the gel was injected into the middle ear after a surgical approach
3 instead of a direct intratympanic injection. The retro-auricular skin was shaved and cleaned
4 with povidone-iodine. Local anesthesia was administered by the injection of lidocaine
5 hydrochloride (0.5 mL, 1%) into the subcutaneous tissues to complete the general anesthesia.
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7 After a retro-auricular incision, the muscles were gently pushed aside from the bone and the
8 tympanic bulla was carefully opened using a 2-mm diamond bur to drill the bone (Collin,
9 NSK, Nakashima, Japan) allowing visualization of the cochlea as well as the round window.
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11 The gel was injected using a 1 mL syringe equipped with a 29 G needle (diameter = 0.16 mm,
12 length = 88 mm, B. Braun). The volume of gel injected was $150 \pm 20 \mu\text{L}$ (DexP dose: $1.5 \pm$
13 0.2 mg), depending on the size of the bulla. This procedure was performed under binocular
14 loupe to inject the gel on the round window, and then to completely fill the bulla. The muscle
15 and skin were then sutured with a 3.0 Vicryl suture (Ethicon, Cincinnati, OH, USA). After the
16 injection procedure, the guinea pigs received an intramuscular injection of antibiotic
17 prophylaxis (Enrofloxacin Bayer, Leverkusen, Germany). A general analgesic (Tolfedine 1
18 mg/kg i.p.) was delivered at the end of surgery. In control animals, the same surgical
19 procedure was performed but without any gel injection.
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37 ***Statistical analysis***

38 Results are expressed as mean \pm SEM. GraphPad Prism 7 software was used for statistical
39 analysis. Non-parametric tests were performed, and statistical analysis of the hearing
40 thresholds was conducted using two-way or one-way analysis of variance (ANOVA), with
41 Tukey's post-hoc test. A p value < 0.05 was considered to be statistically significant.
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51 **Results**

52 Before noise exposure (D_2), auditory thresholds determined between 1 and 32 kHz were in
53 the range of what has previously been described in guinea pigs[6]. Auditory thresholds, before
54 noise exposure, were equivalent among the individuals and between groups (two-way
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2 ANOVA, ns; **Fig. 2**). Two days after noise exposure, a noise-induced threshold shift (D_{-2} vs.
3 D_0) was observed at all frequencies apart from 1 kHz, and was similar between the different
4 experimental groups (**Fig. 2**). The mean hearing loss recorded at 8 kHz was 29 ± 3 dB, and at
5 16 kHz, it was 24 ± 3 dB. For 4, 24 and 32 kHz, a hearing loss around 15 dB was observed
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Fig. 2 Pre- and post-trauma ABR thresholds near here

Seven days after noise-exposure (D_7), a total recovery of the thresholds was observed in the control group at 4, 16, 24 and 32 kHz (**Fig. 3**). However, a 23 ± 4 dB hearing loss was still observed at 8 kHz (two-way ANOVA, $p < 0.0001$). In the other groups, in which gel was applied in the bulla, whatever the formulation (HA, HA-DexP or HA-DexP-Lip), no recovery was observed at D_7 : ABR thresholds were similar to the post-trauma ABR thresholds measured at D_0 at all frequencies (two-way ANOVA, ns; **Fig. 3**).

Fig. 3 Post-trauma thresholds at D_7 (solid black line) near here

One month after the trauma (D_{30}), total recovery was observed in all groups compared to pre-trauma thresholds (apart from 8 kHz frequency in each group), with no differences among all groups (**Fig. 4**). In the control group, at 4, 16, 24 and 32 kHz frequencies, ABR thresholds remained stable at D_{30} , compared to D_7 , with no difference compared to pre-trauma ABR thresholds (two-way ANOVA, ns; **Fig. 4**). At 8 kHz, the hearing loss was 20 ± 3 dB for the control group, 15 ± 6 dB for the HA group, 14 ± 7 dB for the HA-DexP group, and 13 ± 5 dB for the HA-DexP-Lip group.

Fig. 4 Post-trauma thresholds at D_{30} (solid black line) near here

Discussion

In the present study, acoustic traumas were performed on guinea pigs to test the efficacy of DexP when delivered from a local gel 2 days after noise exposure. Guinea pig is a model commonly used to study hearing disabilities because hearing thresholds are age-dependent when evaluated 1 month after birth (**Table 1**)[6, 9]. However, there are differences in hearing between pigmented and albino guinea pigs. Albino guinea pigs usually have lower hearing than pigmented guinea pigs, especially in the midfrequencies[10]. Thus, the albino model might experience poorer recovery probably related to the properties of the *stria vascularis* which lacked melanin and showed a different glutathione distribution[10, 11]; however, this point remains under debate because similar noise trauma experiments performed in both models showed higher hearing loss in pigmented guinea pigs (**Table 1**)[11–14].

With the acoustic trauma performed in the present experimental protocol, a temporary threshold shift was observed at most frequencies apart from 8 kHz, which showed a permanent threshold shift of 20 dB at 7 and 30 days in the control group. These data are consistent with those of Gourévitch et al., who demonstrated that a single trauma could produce a temporary threshold shift restricted to 6–9 days[8]. Indeed, initial noise-induced hearing loss can partially recover with cochlear repair mechanisms that promote cell recovery, and antioxidant intracellular reactions of defense mechanisms[4].

The acoustic trauma delivered in this study was centered on the 5 kHz frequency which usually leads to a threshold shift an octave above the frequency of the traumatic sound[15]. More severe noise traumas (>105 dB) were usually performed in other studies to test corticosteroid efficacy in albino guinea pigs, with various duration times from 20 min to 48 h (**Table 1**). Harrop-Jones et al. found greater temporary ABR thresholds with a 2 h acoustic trauma (narrow bandpass 4–8 kHz) at 105 dB, similar to the present noise trauma, especially at 4 and 16 kHz, with hearing losses around 40–50 dB and 40–20 dB, respectively [16].

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2 Interestingly, they demonstrated a benefit from the OTO-104 gel (6% dexamethasone in
3 poloxamer gel) 10 days after the trauma at 4 kHz but not 8 kHz, when it was injected 2 days
4 after noise exposure, and at 8 kHz when OTO-104 was injected 3 days after trauma. When the
5 OTO-104 gel was applied before noise exposure, otoprotection was demonstrated at 4, 8 and
6 16 kHz frequencies with the two-noise trauma paradigms tested: 2 h at 105 dB or 110 dB. In
7 their study, only 50 μ l gel was injected through the tympanic membrane whereas the present
8 protocol used a retroauricular approach to fill the whole bulla (around 150 μ l).

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18 **Table 1** near here

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20 In contrast to invasive systems such as an osmotic pump, which could deliver high
21 dexamethasone concentrations locally for outer hair cell protection[1, 17], the local drug
22 delivery system tested in the present study was specific HA gels delivering DexP. They were
23 designed for inner ear therapy, with two different kinetic release mechanisms. Indeed, the HA
24 gels could achieve longer residence times in the middle ear in contact with the round window
25 whereas the liquid solution usually injected is rapidly evacuated through the Eustachian
26 tube[7]. Furthermore, one gel injection could ensure a longer release delivery into the bulla
27 and thus could avoid repeated injections. DexP loaded into liposomes contained within HA
28 gel has been shown to ensure a sustained release with elevated concentrations for up to 30
29 days, compared to free DexP within HA gel, which could be released for up to 15 days[5].

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31 Nevertheless, in the present noise-induced hearing loss procedure, similar spontaneous
32 hearing recovery was observed in the treated animals compared to non-treated ones.
33 Moreover, in animals with gel injections, a delayed recovery was observed at D₇, compared to
34 the control group, which could be due to a conductive hearing loss induced by the gel
35 viscosity that filled the whole bulla and surrounded the traumatized ossicular chain. These
36 rheological behaviors and the presence of the gel at D₇ around the ossicular chain that was
37 overexposed to noise[5] may explain the delay in recovery observed in the three experimental
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The use of corticosteroids for hearing protection during noise over-exposure in animals has been widely described in the literature (**Table 1**); however, there was some discrepancy with regard to their success in hearing recovery. When dexamethasone was administered locally at different concentrations before noise exposure, efficient hearing thresholds could be observed 7 days after trauma[16, 17]. At variance, 14 days or 30 days after noise exposure, immediate local administration of steroid in a continuous delivery form (mini-osmotic pump, intratympanic injection, gelatin sponge) was shown to reduce hair cell loss (mostly outer hair cells), accelerate hearing recovery, but demonstrated various impacts on functional hearing outcomes (**Table 1**)[1, 13, 14]. Indeed Sendowski et al showed 16% of outer hair cell loss when methylprednisolone was administered with an osmotic pump before and after acoustic trauma compared to a control group (46%) with an earlier hearing recovery but finally, similar auditory thresholds on all frequencies at 14 days[1]. In opposition, at similar severe acoustic trauma, Chi et al demonstrated at 3 and 4 weeks a stable hearing recovery and greater outer hair cell loss (67%) with a local dexamethasone administration. The correlation between functional and histological outcomes remains unclear.

With regard to delayed injection, from 24 h up to several days after noise exposure, a significant decrease in treatment efficiency was seen in albino guinea pigs[13, 16]. As previously described, early administration of corticosteroids can prevent anticipated acoustic trauma such as gunshot in the military operating field or in noise trauma surgery. However, Tran Ba Huy and Sauvaget found that the mean delay between first symptoms and medical visit was 3.1 days for idiopathic sensorineural hearing loss in 321 patients at emergency care[18]. Therefore, a 48-h delay between the acoustic trauma and dexamethasone injection was chosen in the present study to replicate patient clinics which present unpredictable noise-induced hearing loss. Patients presenting unexpected acoustic trauma could be considered to have a sensorineural disorder. In this context, it is reasonable to assume that patients with acoustic trauma will not benefit from such local treatment. Indeed, dexamethasone locally

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2 applied may act more like an otoprotective drug with a potential impact on temporary
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4 threshold shift rather than a permanent threshold shift after noise exposure.
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9 **Conclusion**

10 The present study demonstrated a permanent hearing loss, at 8 kHz, 1 month after a mild
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12 noise trauma of 105 dB in albino guinea pigs. Dexamethasone in liposomes included in HA
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14 gel passively diffused through the round window 48 h after the acoustic trauma failed to
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16 promote additional or faster hearing recovery at 4, 16, 24 and 32 kHz compared to
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18 spontaneous recovery. The drug concentration in the inner ear and both the time and form of
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20 drug delivery must be carefully considered after acoustic trauma to ensure a potential benefit.
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Figure Captions

Fig. 1 Experimental protocol

Fig. 2 Pre- and post-trauma ABR thresholds

ABR thresholds in all animals were recorded before (dashed line, D₋₂) and after (solid line, D₀) noise exposure (5 kHz, 100 dB, 1 h) and are shown for comparison (two-way ANOVA; **** p<0.0001 Tukey's post-hoc test). Values are mean ± SEM (25 ears).

Fig. 3 Post-trauma thresholds at D₇ (solid black line)

Pre-trauma ABR thresholds at D₋₂ (dashed gray line) and post-trauma ABR thresholds at D₀ (solid gray line) are shown for comparison. ABR thresholds at D₇ (solid black line) are represented by (A) control group (trapezoid, eight ears), (B) HA group (squares, six ears), (C) HA-DexP group (diamonds, six ears), (D) HA-DexP-Lip group (triangles, five ears). Values are mean ± SEM. Hearing recovery was observed in the control group (A) in ABR thresholds at 16 and 24 kHz (D₇) compared to post-trauma (D₀) (two-way ANOVA; *p<0.05, **p<0.002; Tukey's post-hoc test).

Fig. 4 Post-trauma thresholds at D₃₀ (solid black line)

Pre-trauma ABR thresholds at D₋₂ (dashed gray line) and post-trauma ABR thresholds at D₀ (solid gray line) are shown for comparison. ABR thresholds at D₃₀ (solid black line) are represented by (A) control group (trapezoid, eight ears), (B) HA group (squares, six ears), (C) HA-DexP group (diamonds, six ears), (D) HA-DexP-Lip group (triangles, five ears). Values are mean ± SEM. Total recovery at D₃₀ was observed in all groups compared to pre-trauma thresholds (D₋₂), apart from 8 kHz frequency in each group, with no significant differences among all groups (two-way ANOVA; *p<0.02, **p<0.002, ***p<0.0001 Tukey's post-hoc test).

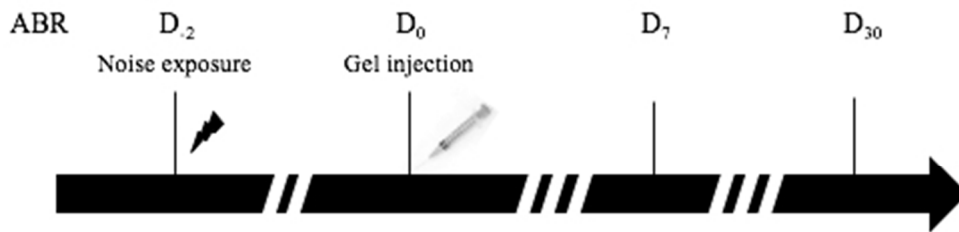


Fig. 1 Experimental protocol

172x44mm (72 x 72 DPI)

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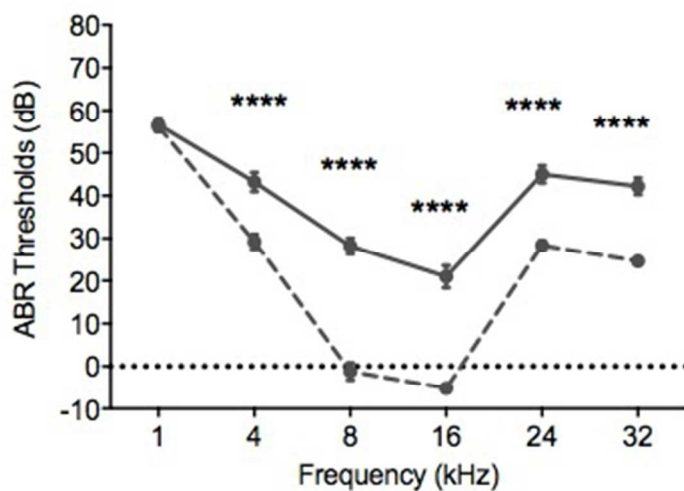


Fig. 2 Pre- and post-trauma ABR thresholds

ABR thresholds in all animals were recorded before (dashed line, D-2) and after (solid line, D0) noise exposure (5 kHz, 100 dB, 1 h) and are shown for comparison (two-way ANOVA; **** $p < 0.0001$ Tukey's post-hoc test). Values are mean \pm SEM (25 ears).

137x93mm (72 x 72 DPI)

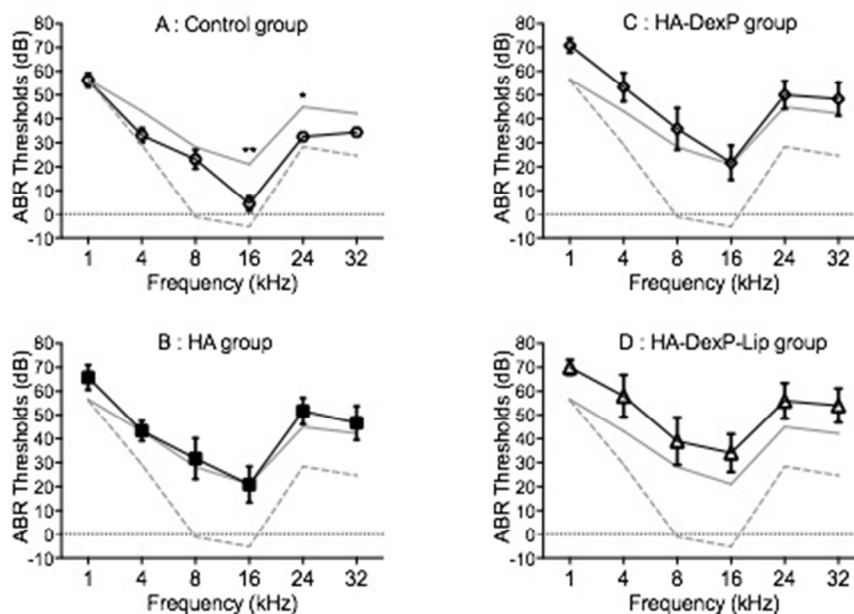


Fig. 3 Post-trauma thresholds at D7 (solid black line) Pre-trauma ABR thresholds at D-2 (dashed gray line) and post-trauma ABR thresholds at D0 (solid gray line) are shown for comparison. ABR thresholds at D7 (solid black line) are represented by (A) control group (trapezoid, eight ears), (B) HA group (squares, six ears), (C) HA-DexP group (diamonds, six ears), (D) HA-DexP-Lip group (triangles, five ears). Values are mean \pm SEM. Hearing recovery was observed in the control group (A) in ABR thresholds at 16 and 24 kHz (D7) compared to post-trauma (D0) (two-way ANOVA; * $p < 0.05$, ** $p < 0.002$; Tukey's post-hoc test).

161x118mm (72 x 72 DPI)

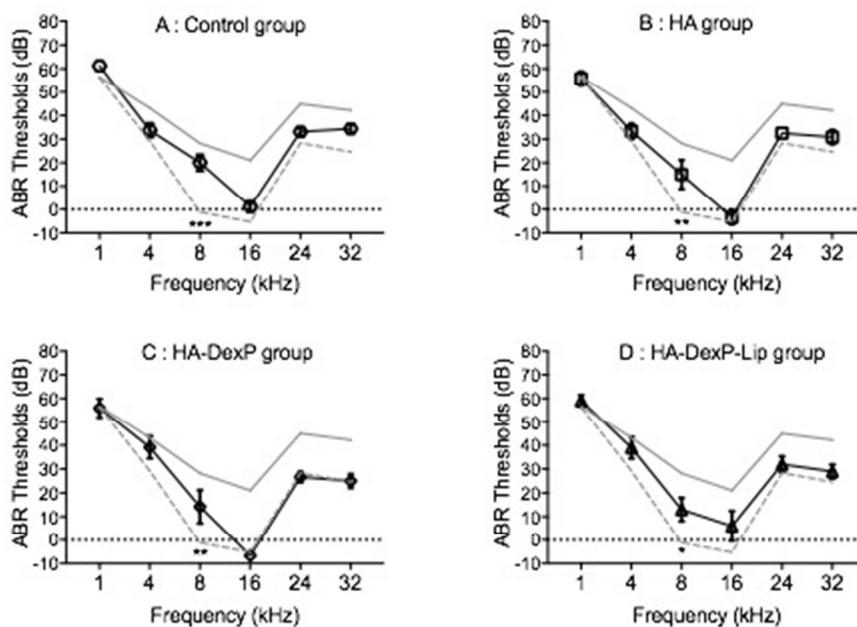


Fig. 4 Post-trauma thresholds at D30 (solid black line) Pre-trauma ABR thresholds at D-2 (dashed gray line) and post-trauma ABR thresholds at D0 (solid gray line) are shown for comparison. ABR thresholds at D30 (solid black line) are represented by (A) control group (trapezoid, eight ears), (B) HA group (squares, six ears), (C) HA-DexP group (diamonds, six ears), (D) HA-DexP-Lip group (triangles, five ears). Values are mean \pm SEM. Total recovery at D30 was observed in all groups compared to pre-trauma thresholds (D-2), apart from 8 kHz frequency in each group, with no significant differences among all groups (two-way ANOVA; * p <0.02, ** p <0.002, *** p <0.0001 Tukey's post-hoc test).

160x118mm (72 x 72 DPI)

Table

Table 1 Summary of the methods and results in noise-induced hearing loss guinea pig animal model studies testing corticosteroid efficacy

Acoustic trauma Intensity (dB)	Drug delivery		Noise-induced hearing loss in control group		Therapeutic efficacy	Reference		
	Duration	Drug	Form	Time			ABR	Time
or 110	2 h	Dexamethasone 50 µL OTO-104	IT	or 1 or 2 or 3 or 4 d after	1 5-20 dB (105 dB) 3 0-35 dB (110 dB) 4, 8, and 16 kHz	7 d	Efficacy on HL of 6% OTO-104 gel, before NE or 1, 2 d and 3 d after NE	[16]
90	1 h	Dexamethasone 0.8 mg	IT	14 h prior	30 dB clicks	2 h	Efficacy on HL	[19]
167	-	Dexamethasone 40 mg/ml drug	Gelatin soaked sponge	1 d after	50 dB clicks	30 d	Hearing recovery; HC protection	[13]
106	48 h	Methylprednisolone 20.48 mg	IT aerosol	Directly after	73 dB clicks	21 d	Efficacy on HL	[20]
120	24 h	Dexamethasone 1, 10, 100 or 1000 ng/ml	Mini-osmotic pump	4 d before to 7 d after	50 dB: 2, 4, 8 kHz 45 dB: 16, 20 kHz	7 d	Efficacy when ≥ 100 ng/ml outer HC protection	[17]
170	-	Methylprednisolone 200, 300, 500 or 1000 µM	Mini-osmotic pump	Starting 1 h after, 7 d	50 dB, 19 kHz	14 d	Accelerated hearing recovery	[1]
165	-	Methylprednisolone 40 mg/kg/d	- 1 h after, + /48 h,	4 times	80 to 90 dB 2, 4, 8, 16 kHz	30 d	Efficacy when applied IT 1 h after HC protection	[14]
115	3 h	Methylprednisolone 1.4 mg/ear	IT - 1 h after - 1 h after + /48h, - 4 IT 7 d after	4 times	25 to 40 dB 2-8 kHz	1 d	Efficacy on HL; OHC preservation	[12]
115	3 h	Dexamethasone 1 mg/kg/d	IP	5 d before	40 dB at 8 kHz 70 dB at 16 kHz 60 dB at 32 kHz	28 d	No efficacy	[11]

d: day; IT: intratympanic; IP: intraperitoneal; IM: intramuscular; HC: hair cell; ABR: auditory brainstem response; NE: noise exposure; HL: hearing loss; OHC: outer hair cell; np: not provided.

Animal model		Noise
pig	(g)	
Albino	200-300	Narrow band pass, 4-8 kHz
Pigmented	200-400	20 click stimuli/s
Albino	250	80 impulse noise
Albino	300-400	Broad-band noise
Albino	300-500	Octave band noise, 4 kHz
Pigmented	400-500	3 blank gunshots
Pigmented	250	60 impulse noise
Albino	300-350	White-band noise
Pigmented	>300	Octave band noise, 8-16 kHz