**Supplementary Introduction**

Recently, intra-tympanic (IT) steroid injection has been widely used as it shows fewer systemic adverse effects and a high degree of uptake across the round window (RW) membrane. According to a systematic review that analyzed 29 prospective randomized controlled trials, the authors recommended IT steroid injection as an adjunct therapy for idiopathic sudden sensorineural hearing (ISSNHL) and noise-induced hearing loss [Lavigneet al., 2016]. Moreover, the clinical practice guidelines of the American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) state that “clinicians should offer IT steroid perfusion when patients have incomplete recovery from ISSNHL” [Stachleret al., 2012]. It seems that IT steroids have beneficial effects in acute hearing loss with few adverse effects. Some studies have concluded that the treatment effects of IT steroids may be superior to those of systemic steroids. In a human study that compared the steroid concentration between IT and intravenous (IV) administration, it was found that the concentration of steroid in the perilymph was significantly higher (88 times) in the IT group [Birdet al., 2007, Birdet al., 2011]. However, locally administered steroid is usually in a liquid formulation, such as dexamethasone phosphate (D), and is readily eliminated from the middle ear through the Eustachian tube. Also, loss of injected steroid is not only from the eustachian tube but also via the high clearance inside the cochlea: distribution, metabolism, and elimination [Salt and Plontke, 2009]. Accordingly, vehicles that slowly release steroid will be beneficial in overcoming this intra-cochlear clearance. It is known that a steroid delivered via the IT route peaks at 1–2 h after injection and lasts in the perilymph for only several hours [Yanget al., 2008]. To deliver steroid over a longer duration, repeated injections are usually required; most reported studies indicated 3–10 injections per patient [Seggaset al., 2011]. To overcome this weakness, sustained biomaterial-based drug-delivery vehicles have been studied. For example, chitosan [Paulsonet al., 2008, Saberet al., 2010], poloxamer 407 [Wanget al., 2009], gelatin [Inaokaet al., 2009], and collagen [Endoet al., 2005] have been considered as materials that could release the drug over a long duration.

HA has been used in medicine for treating osteoarthritis and atopic dermatitis. It is also used as a common cosmetic ingredient in skin-care products. The United States Food and Drug Administration (US-FDA) has approved HA for the use in eye surgery, and osteoarthritis pain in the knee, ankle, and shoulder [Fallacaraet al., 2018]. Because HA can retain and slowly release various medications, it has been used as an inner ear drug-delivery vehicle in several studies [El Kechaiet al., 2015, El Kechaiet al., 2016].

Traditionally, gels are defined as a substantially dilute cross-linked system, which exhibits no flow when in the steady-state [Ferry, 1980]. Hyaluronic acid can have both sol and gel properties, depending on the concentration and temperature. In this study, the hyaluronic acid was studied in a concentration of 2wt% and at body temperature. In this condition, HA tends to be similar to a liquid formula: it easily flows by gravity. In this study hyaluronic acid was in a sol condition, but it may be different in other experimental conditions**.**

MPEG-PCL (MP) is a biocompatible material developed by our group that consists of polyethylene glycol (PEG) and ε-polycaprolactone (PCL) block copolymer with amphiphillic properties [Kimet al., 2001]. While polylactic-co-glycolic acid (PLGA) can provoke inflammation due to the acid environment formed by its degradation [Kimet al., 2012], MP is easier to prepare and additionally is believed to have a lower chance of causing inflammation. MP solution is a thermosensitive material; that is, it exhibits solution-to-gel transitions according to temperature. MP is in the sol state at room temperature, but as soon as it is injected into the body (middle ear), it transitions to a gel state [Bagger-Sjobacket al., 1993, Kimet al., 2012]. MP has an advantage over HA in that MP is in a gel state in the middle ear while HA is always in the sol state. The gel state in the middle ear makes it more resistant to drainage through the Eustachian tube. However, because it is in a sol state in the syringe, MP can be injected through very small needles in a minimally invasive manner. The clinical use of MP has been studied in various fields, such as voice disorders [Choiet al., 2016], bone regeneration [Kwonet al., 2014, Kwonet al., 2015], and intra-tumoral chemotherapy [Seoet al., 2013]. It has been reported that gel integrity persists for at least 10 months within subcutaneous tissue and that the probability of immunoreactivity and inflammation is low [Kimet al., 2012]. However, it has not been studied in the ear. The middle and inner ear are unique in several aspects. While the middle ear is a cavity deep in the temporal bone, it is actually an extracorporeal space. It is relatively sterile; however, normal flora reside there, and exogenous organisms can also enter via the Eustachian tube [Masantaet al., 2015]. The middle ear is isolated from the external world by a thin membrane that is fragile and vulnerable to infection. The subcutaneous space is a sterile intracorporeal space that is covered by durable subcutaneous tissue and skin. Accordingly, IT injection of MP may show different outcomes from previous studies. In this study, we sought to assess whether MP would be a good drug-delivery vehicle in the ear.

**Supplementary Material and Methods**

***Acute acoustic trauma***

White noise was amplified using a power amplifier (MA-620, Inkel, Incheon, Korea). A Beyma CP800TI (Polígono Industrial Moncada, Valencia, Spain) was used. The animals were anesthetized deeply with a mixture of zoletil (33 mg/kg) and xylazine (8 mg/kg) via intramuscular administration before noise exposure.

***Vehicles and injection procedure***

The rats were anesthetized with zoletil and xylazine and the tympanic membrane (TM) was examined with a surgical microscope (Opmi Pico; Zeiss, Oberkochen, Germany). A 1-mL syringe (Kovax-Syringe 1 mL, Korea Vaccine Co., Seoul, Korea) was combined with a 24 G needle (Angiocath Plus, BD, Sandy, UT, USA) and a mini-extension tube (Mini-Volume Line, Insung Medical, Seoul, Korea). While an experienced otology surgeon held the needle and perforated the eardrum, the assistant carefully pushed the syringe at low speed (~40 µL/10 s). An air vent was first made in the anterior superior quadrant of the TM, and the vehicle/drug was injected in the posterior superior quadrant of the TM. The injection was stopped when the vehicle/drug completely filled the middle ear (bulla) or the vehicle/drug leaked out through the air vent. Thus, ~30–60 μL of vehicle/drug was injected in each ear.

We achieved good or fair quality of injection in 92.5% of cases; these ears were included in the study. After injecting the vehicle/drug into one ear, the other ear was also immediately injected with a different vehicle/drug; the time interval between first and second injections was < 5 min. To prevent position effects, the animals were placed in a straight prone position without leaning toward the left or right side until the conclusion of the experiment. Because four different groups were studied, the animals were always injected with two different vehicle/drugs. The vehicle/drug that was injected and the order in which the vehicle/drug was injected were performed in a random order.

***Hearing threshold measurement with auditory brainstem response (ABR)***

Prior to ABR measurement, animals were anesthetized, as mentioned above. Sub-dermal needle electrodes were inserted at the vertex (active electrode) and behind the injected ipsilateral ear (reference electrode) and contralateral ear (ground electrode). The speaker was aligned with the external auditory canal and the earphone tube was inserted gently into the ear canal. Click auditory stimuli were delivered to the target ear. Hearing thresholds were determined by evaluating the lowest stimulus level for waves III/V and SN10 (slow, negative wave) recognition, from 90 dB SPL with a 5-dB SPL decrease.

***Endoscopic examination of the TM and Hair cell count***

The surface integrity, healing of the perforation, and transparency of the TM were evaluated. Also, the presence of inflammation, infection, and otorrhea was evaluated.

On the last day of the experiment, cardiac perfusion and cochlea harvesting were performed. After anesthesia, cardiac perfusion was performed with phosphate-buffered saline (PBS) and 4% paraformaldehyde (PFA). Then, the cochlea was harvested. The specimens were fixed for 24 h at 4°C and rinsed for 5 min, three times, with PBS. Organ of corti surface preparation was performed under a stereoscopic microscope (DSZ-40T, Dongwon Systems, Anyang, Korea). The cochlea was stained with phalloidin (Alexa Fluor 546, Life Technologies, Oregon, USA) and rinsed for 5 min, three times, with PBS. Three rows of outer hair cells were counted within the length of 200 μm in each turn. Two separate samples of each turn were used for measurements. When the hair cells were empty or invisible on photomicrographs, the hair cells were considered to be non-living.

***Middle ear histology***

The bulla together with the cochlea was harvested. Fixation and washing were performed as described above. The bullae were decalcified in 10% EDTA solution for 4 weeks. Consistent location of the TM was identified and evaluated by finding the section in which the malleus head and its fibrous connection to the TM could be identified. Bulla mucosa was evaluated at two different locations: mucosa near the RW and mucosa at the base of the bulla (BB). The thickness of the mucosa was quantified and the presence of residual vehicle/drug was evaluated on an ordinal scale, between 1 and 3 (1, no residual vehicle/drug; 2, residual vehicle/drug partially filling the cavity; and 3, residual vehicle/drug fully filling the cavity)

**Supplementary Results**

***Endoscopic examination of the TM***

The biocompatibility of three IT drug-delivery vehicles for dexamethasone phosphate (D) and the associated hearing outcomes were compared in Sprague Dawley rats with normal hearing; details are presented in the Materials and Methods section. In some ears, chronic discharge due to inflammation was observed (Supplementary Fig. 1). The incidence of inflammation was 0.0% in the saline+D and HA+D groups and 70.8% in the MP+D group. Thus, inflammation was found only in the MP+D group. The onset of inflammation was 7.5 ± 2.4 days after MP+D injection and the offset of inflammation was 22.6 ± 9.1 days after MP+D injection. Even after full recovery, a thickened and opaque-looking TM was frequently the result in this group (Supplementary Fig. 1). In the HL control group, no inflammation was expected because no intervention was made. However, opportunistic inflammation of the TM was found in two ears. Because this inflammation in the HL control group was unrelated to any IT injection, these ears were excluded from further analysis.

***Micro computed tomography of the bulla air space***

The duration of vehicle/drug visualized in the bulla was significantly longer for the MP+D group versus the saline+D (*p* < 0.001) and HA+D groups (*p* < 0.001). The vehicle/drug also lasted longer in the HA+D group compared with the saline+D group, but there was no statistically signifnicant difference. (*p*= 0.074).

***Histology of the middle ear***

The thickness of the RW was 8.4 ± 2.6 μm in the saline+D group, 9.0 ± 4.4 μm in the HA+D group, 11.3 ± 2.9 μm in the MP+D group, and 12.5 ± 2.8 μm in the HL control group. There was no significant difference in RW thickness among the four groups.

The thickness of the mucosa near the RW was 3.6 ± 2.3 μm in the saline+D group, 4.9 ± 2.3 μm in the HA+D group, 5.0 ± 1.0 μm in the MP+D group, and 4.1 ± 1.1 μm in the HL control group. There was no significant difference in the thickness of the mucosa near the RW among the four groups.

The thickness of the mucosa at the base of bulla (BB) was 19.4 ± 16.7 μm in the saline+D group, 15.4 ± 1.6 μm in the HA+D group, 21.5 ± 11.5 μm in the MP+D group, and 14.5 ± 7.1 μm in the HL control group. There was no significant difference in the thickness of the BB mucosa among the four groups.

And we performed a histologic evaluation of the round window (RW) membrane and measured the thickness after 45 days. This figure summarizes the thickness of the RW. The thickness of the RW membrane was 8.4 ± 2.6 μm in the saline+D group, 9.0 ± 4.4 μm in the HA+D group, 11.3 ± 2.9 μm in the MP+D group, and 11.9 ± 4.2 μm in the HL control group. There was no significant difference in the thickness of the RW between the four groups. It seems that the RW is not affected by the drug/vehicle. This point is important, since thickening of the RW may have a big effect on the intra-cochlear penetration of drug from the middle ear into the inner ear. (Supplementary Fig. 5).

***Auditory brainstem results (ABR) for hearing threshold***

To examine the hearing threshold change of the animals, ABRs were performed before exposure to noise, 1 h after noise exposure, 3 h after vehicle/drug injection, and 4, 8, 30, and 45 days after injection. Prior to ABR measurement, animals were anesthetized, as mentioned above. Before the acute acoustic trauma, the hearing threshold was normal (≤35 dB SPL) in all animals.

The proportion of ears with good treatment outcomes (hearing threshold ≤ 35 dB SPL at PID 45) was 27.3%(3/11) in the saline+D group, 63.6%(7/11) in the HA+D group, 25.0%(4/16) in the MP+D group, and 25.0%(1/4) in the HL control group.

***Hair cell count***

The number of hair cells in the HL control group was significantly fewer than in the MP+D group (*p* = 0.040). The number of hair cells in the HL control group was also smaller than that in HA+D group (*p* = 0.061) and the saline+D group (*p* = 0.079), but these differences were statistically marginal.

**Supplementary Discussion**

Considering that MP has been reported to be biocompatible in subcutaneous tissue [Kimet al., 2012], it seems that the IT drug delivery is more precarious and vulnerable to adverse reactions than subcutaneous drug delivery. Various drug-delivery vehicles, such as gelatin, collagen, and poloxamer, have been reported to be biocompatible inside other body parts. Now, they are being used as IT drug delivery vehicles. However, it seems these materials should be assessed again in the ear, before use as an IT drug-delivery vehicle in humans.

Why MP caused inflammation in the ear but not in subcutaneous tissue is unclear. However, one reason may be that the TM is very thin. The use of copolymers, such as PLGA, in humans has been approved by the US-FDA; however, it is known that PLGA can produce tissue-specific macrophages and foreign-body giant-cell responses [Thevenotet al., 2010]. MP was developed by our group to overcome this problem. We have reported that the inflammatory reaction caused by MP is significantly less than that observed in response to PLGA [Kimet al., 2012]. However, despite significant control of the inflammatory reaction with MP, this may not be sufficient in the ear. The subcutaneous tissue and skin is a thick and durable layer, optimized for efficient sequestration and self-protection. The TM is a very thin membrane, optimized for efficient vibration. Accordingly, a foreign body reaction that is negligible within subcutaneous tissue may be considered significant in the TM. Another reason may be related to the long persistence of the material in the ear. PCL is known to have a slow degradation rate. Various copolymeric systems have been researched to improve the slow degradation inherent in PCL[Cohn and Salomon, 2005, Hyunet al., 2006, Zhaoet al., 2007]. Copolymers of PCL with poly-L-lactic acid (PLLA) have produced substances with more rapid degradation rates, because the introduction of PLLA segments into PCL segments decreased the crystallinity[Kanget al., 2010]. The use of MP, a MPEG-b-(PCL-ran-PLLA) gel, also partially solves this problem. However, the degradation speed of MP is still very slow (>1 year in some formulations). Because MP originates from PCL[Kanget al., 2010], it may cause some small degree of adverse reaction. Moreover, the long contact time between MP and the TM may increase the chance of inflammation. From our results, we found that the onset of the inflammatory reaction was not acute, but rather delayed, up to 7.5 ± 2.4 days after injection. Thus, if the copolymer vehicle biodegrades within 1 week, the chances of inflammation may decrease.

In terms of longevity, MP showed the longest duration. That is, when evaluated with CT, MP lasted for 47.5 days in the bulla, whereas saline lasted < 1 day and HA lasted for 1.8 days. Given that the drug (steroid) delivered via saline as a vehicle can last in the perilymph for only a few hours [Yanget al., 2008], maintaining the vehicle/drug in the bulla for a longer period may be preferable. However, this does not mean that simply longer sustainability is better. Vehicles that persist in the middle ear for an extended period may cause problems, including patient discomfort (aural fullness); moreover, there is a greater chance of inflammation. An empty vehicle may be worthless after it has fully released the drug. Furthermore, surgical intervention may be required in some cases to remove the vehicle/drug when it is no longer needed. A vehicle that lasts in the middle ear for an optimal drug-delivery period and that degrades readily after that period would be ideal. However, this optimal period is unknown and may differ among hearing disorders. For example, when treating a patient with ISSNHL loss, a vehicle that lasts for several days may be ideal, as a full dose of systemic steroids (60–75 mg) is usually prescribed for 3–10 days [Xenelliset al., 2006, Koltsidopouloset al., 2013]. When trying to prevent ototoxic hearing loss in a patient who is undergoing chemotherapy, a vehicle that lasts for several weeks, similar to a routine chemotherapy cycle, may be preferred. The optimal period of drug delivery is unclear for acute acoustic trauma, but it seems obvious that the duration is too short for saline and too long for MP. The sustainability of HA was much longer than for saline, ~2 days. Previous studies showed that it takes 1–5 days for HA to be removed in the guinea pig bulla [Engstromet al., 1987, Chandrasekharet al., 2000]; thus, the sustainability of HA is probably closest to the optimal period for treating acute acoustic trauma. The HA used in this study was 2 wt% and the molecular weight was 1000 kDa. Considering that HA with a higher molecular weight and viscosity is also available, the duration of HA may be tailored to meet the required treatment duration in future studies (e.g., lasting for 3–10 days in the bulla).

CT imaging has the advantage of visualizing the air space deep within the bulla. Using endoscopic images, the air space immediately behind the TM can be evaluated, but not the air space deep inside the bulla. CT images can also sharply differentiate vehicle/drug from air. Because the normal bulla is filled with air, CT seems to be a good method to evaluate the presence of vehicle/drug within the bulla. However, a CT image does not differentiate the vehicle/drug from fluid collection caused by adverse inflammation. As such, our CT data could be misleading due to this limitation. However, as there was no case of inflammation in the saline+D or HA+D group, attenuation inside the bulla must be caused by the vehicle/drug in these two groups. In the MP+D group, the attenuation within the bulla may be a mixture of vehicle/drug and fluid collection caused by inflammation. We sought to differentiate the two by interpreting the CT image together with the endoscopic image. According to our endoscopy analysis, inflammation was present in the MP+D group from PID 7.5 ± 2.4 to PID 22.6 ± 9.1. Accordingly, the attenuation inside the bulla during this period may be a mixture of vehicle/drug and fluid collection. However, after PID 22.6, we presumed that the fluid collection had resolved; thus, the remaining attenuation was attributed to the vehicle/drug. Attenuation inside the bulla lasted for a much longer period (47.5 days) than the inflammation (22.6 days). Accordingly, we believe that our interpretation that the vehicle/drug lasted for 47.5 days is valid. However, we admit that CT imaging may not be an ideal method for evaluating the presence of a vehicle/drug when there is a chance of inflammation.

Although some results did not reach statistical significance, the hearing outcome was most favorable in the HA+D group in our study. Among the four groups, hearing threshold was lowest in the HA+D group during the whole post-treatment period (PID 4–45) and was significantly lower than that in the MP+D group. The proportion of ears with good hearing outcome was also highest (63.6%) versus the other groups (25.0–27.3%) and was significantly higher than the MP+D group.

The treatment outcome may have been less favorable in the saline+D group, because the drug was not present for long. Saline may have delivered the drug over a very short period, resulting in a short-term improvement (PID 8). However, due to the short drug delivery duration, the hearing results may have been similar to the control group over the long term (PID 30–45). To overcome this problem, repeated injections may improve the outcome in the saline+D group.

In the MP+D group, it is more difficult to interpret the hearing results because there is a possibility of mixed hearing loss. From our CT results, residual MP, which may cause conductive hearing loss, was present inside the bulla. The exact amount of conductive hearing loss caused by residual MP in the bulla is unclear. We attempted to assess this conductive component by measuring the hearing threshold immediately before injection and immediately after MP injection on the same day. The difference between the two hearing measurements was 1.7 ± 10.8 dB SPL. MP in the bulla did cause additional conductive hearing loss but it was quite small. Even after compensating for the small conductive component, the hearing outcome in the MP+D group was not good; the deterioration in hearing from PID 30 to 45 was attributed to sensorineural hearing loss due to mild labyrinths after the inflammatory reaction. Because the residual MP in the bulla degraded progressively and the inflammation also resolved progressively during this period, it seems less likely that the conductive component increased. Our hypothesis is that persistent inflammation in the bulla for 2 weeks (from PID 7.5 ± 2.4 to PID 22.6 ± 9.1) may have resulted in inflammatory cytokine entry into the inner ear, subsequently leading to mild, progressive sensorineural hearing loss. However, no firm conclusion can be reached from this study alone. It remains unclear as to how much of a conductive component was mixed in the hearing outcome of the MP+D group over the experimental period.

Although, studies from Liberman have shown that level of noise from 112 dB SPL and above do generate loss of hair cells, it is true that some level of noise exposure can generate transient threshold shift and no hair cell loss. With Level of 116dB SPL, tonotopic damage is generated and exacerbated also in the middle frequencies and wide spread of outer hair cells loss is expected together with rupture of the organ of Corti structures [Chamberset al., 2012]. It may be possible that the lack of hair cell loss is coming from a protective effect of anesthesia that can attenuate sound transmission and induce less loss of hair cells [Kimet al., 2005, Wenet al., 2017].

The hook-base region may show a different pattern of damage compare to what we found. But In this study, we did not specifically evaluate the hook-base region. 32 kHz was the highest frequency that we evaluated by the ABR and cell count. According to the rat cochleogram [Viberg and Canlon, 2004], <32kHz represents 80% of the whole cochlea. We may have missed some interesting findings in this very high frequency region (20%). But if there was a gross difference in the whole cochlear, we should have found a difference. Loss of hair cell may be minimal because we did not specifically evaluate the hook-base region. The very high frequency region may show a different pattern of damage compare to what we found. And a protective effect of the anesthesia may have also played a role. Also, The level of acoustic trauma may have not reached exactly up to 120 dB for 3 hours. Hardware limitation may have produced a milder level of acoustic trauma.

In this respect, we should have analyzed the ribbon synapses as well as the hair cells to histologically quantify the amount of acoustic trauma. Unfortunately, we were unable to perform a ribbon synapse analysis; this may be an important shortcoming of the study. The number of hair cells was actually the lowest in the HL control group, significantly lower than that in the MP+D group, and marginally smaller than those of the saline+D and HA+D groups. Regardless of which vehicle was used, IT steroid treatment did have a beneficial effect in preserving hair cells. However, because the ABR and hair cell counts did not match, it is unclear whether the steroid had a beneficial effect on hearing outcome by helping the survival of hair cells. Mechanisms other than hair cell survival may also be involved in the therapeutic efficacy of IT steroid delivery. Also, we did not specifically evaluate the hook-base region, which damage with loss of cells can be noticed by noise exposure.

This study had several limitations. First, our results may not be applicable to humans. The permeability of the RW membrane and the volume of perilymph differ between rats and humans; thus, the results on humans may differ. Second, the sampling rate was not sufficiently high to verify the exact timing of inflammation, vehicle disappearance, or hearing improvement. Daily endoscopic, CT, and ABR measurements were not possible. Thus, if an important change occurred between these sampling time points, it may have been missed or recorded on the ‘wrong’ date. However, all four groups were evaluated on the same day with the same sampling rate schedule. Third, we did not directly measure the drug concentration in the perilymph for a potential correlation with hearing threshold; however, the primary aim of this study was to assess the biocompatibility and sustainability of the IT vehicles. The lack of pharmacokinetic information does not undermine the main goals of this study.

HA may last inside the bulla for a longer duration (1.8 days) compared with saline (<1 day). The hearing outcome was favorable when the drug was delivered via HA. In contrast, MP may not be a good candidate as a vehicle for IT drug delivery; the incidence of inflammatory reactions was high and histological changes, such as TM thickening, were observed. The time that MP lasted within the bulla was too long for treating acute hearing disorders. Although the possibility of mixed hearing loss should be considered, the hearing outcome was also significantly worse than in the HA+D group.

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**Supplementary Figure legends**

**Supplementary Fig. 1**. **Endoscopic examination of the tympanic membrane**.

The TM perforation made during the IT injection healed well in most animals at 45 days. Closure of the perforation was identified 24.2 ± 9.2 days after IT injection in the saline+D group, 24.2 ± 9.3 days in the HA+D group, and 11.3 ± 9.3 days in the MP+D group.

TM, tympanic membrane; IT, intra-tympanic; D, dexamethasone phosphate; HA, hyaluronic acid; MP, methoxy polyethylene glycol-b-polycaprolactone block copolymer (MPEG-PCL); HL, hearing loss; PID, post-injection day.

**Supplementary Fig. 2. Endoscopic images after IT injections.**

The TM perforation made during the IT injection appeared at endoscopic image in PID 0 (<2hr). The TM perforation was not verified in the HL control group. This is the equivalent baseline of TM perforations at PID 0 (2hr).

**Supplementary Fig. 3**. **Representative image of inflammation seen after intra-tympanic drug injection**.

The TM looked normal immediately after injection. After 1 week, bulging, swelling, hyperemic changes, and draining were observed for ~2 weeks. After 1 month, the inflammation subsided, but the TMs looked thick and opaque.

Pre indicates pre-injection;

**Supplementary Fig. 4**. **Histology of the middle ear**.

The TM was significantly thicker in the MP+D group than in the saline+D and HA+D groups and HL control group. The thicknesses of the RW and mucosa were similar among the four groups. In the MP+D group, a large amount of residual vehicle/drug was found in the RW niche and at the base of the bulla (\*). Almost no residual vehicle/drug was found in the other groups.

Asterisk indicates foreign material (residual vehicle/drug, MP+D in this figure). RW, round window.

**Supplementary Fig. 5**. **Thickness of the round window membrane.**

The thickness of the RW membrane was 8.4 ± 2.6 μm in the saline+D group, 9.0 ± 4.4 μm in the HA+D group, 11.3 ± 2.9 μm in the MP+D group, and 11.9 ± 4.2 μm in the HL control group. There was no significant difference in the thickness of the RW between the four groups.

**Supplementary Fig. 6**. **Loss of outer hair cells in the cochlea**.

In confocal microscopy images, the number of lost hair cells was quantified. More hair cell loss was identified in the HL control group than in the other groups that had been treated with dexamethasone phosphate; this was most prominent in the basal turn. Arrows indicate the loss of hair cell

**Supplementary Fig. 7. High power images of outer hair cells in the cochlea**.

In high power images of outer hair cells, regions of hair cell loss were clearly identified. The number of missing hair cells was highest in HL control group.

**Supplementary Fig. 8. conductive hearing loss after IT injections.**

There is <10 dB conductive hearing change immediately after IT HA injection in not-exposed rats. The amount of conductive hearing loss is presumed to be 1.7 dB at day 4 and 0.8 dB at day 8. We presume that the attenuation is 5 dB at most. The conductive component soon comes back to normal within 1 day and there is no evidence hearing loss thereafter.