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Systemic Immunity Influences Hearing Preservation in Cochlear Implantation

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Abstract

Hypothesis—To determine whether a systemic immune response influences hearing thresholds and tissue response after cochlear implantation of hearing guinea pigs.

Methods—Guinea pigs were inoculated with sterile antigen (Keyhole limpet hemocyanin) 3 weeks before cochlear implantation. Pure-tone auditory brainstem response thresholds were performed before implantation and 1 and 4 weeks later. Dexamethasone phosphate 20% was adsorbed onto a hyaluronic acid carboxymethylcellulose sponge and was applied to the round window for 30 minutes before electrode insertion. Normal saline was used for controls. Cochlear histology was performed at 4 weeks after implantation to assess the tissue response to implantation. To control for the effect of keyhole limpet hemocyanin priming, a group of unprimed animals underwent cochlear implantation with a saline-soaked pledget applied to the round window.

Results—Keyhole limpet hemocyanin priming had no significant detrimental effect on thresholds without implantation. Thresholds were elevated after implantation across all frequencies tested (2–32 kHz) in primed animals but only at higher frequencies (4–32 kHz) in unprimed controls. In primed animals, dexamethasone treatment significantly reduced threshold shifts at 2 and 8 kHz. Keyhole limpet hemocyanin led to the more frequent observation of lymphocytes in the tissue response to the implant.

Conclusion—Systemic immune activation at the time of cochlear implantation broadened the range of frequencies experiencing elevated thresholds after implantation. Local dexamethasone provides partial protection against this hearing loss, but the degree and extent of protection are less compared to previous studies with unprimed animals.

Keywords

Cochlear implant; Hearing loss; Innate immunity; Keyhole limpet hemocyanin; Systemic immune system

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Protection of auditory and vestibular function during surgical manipulation of the inner ear is a cornerstone of otology. More recently, cochlear implantation aims to preserve residual hearing (1-3). After cochlear implantation, inflammation, oxidative stress, and ultimately apoptosis of hair cells have been shown to contribute to the widespread loss of hearing that frequently results from electrode insertion into the basal turns of the cochlea. The application of anti-inflammatory medications to the inner ear can ameliorate inner ear dysfunction. This leads to better preservation of hearing after experimental cochlear implantation (4-6), strongly implicating inflammation as a cause of insertion-related hearing loss. Similarly, local application of antiapoptotic agents, such as Jnk inhibitors, can protect hair cells and reduce postoperative hearing loss (7). Cochlear inflammation can occur as a result of both a local and a systemic immune response to injury. Local injury increases venule permeability within the modiolus and lateral cochlear wall and upregulates the expression of cell adhesion molecules, leading to the transendothelial migration of circulating immune competent cells into the cochlea. The result is an amplification of the local inflammatory response (6,8-10). Although some aspects of the local cochlear inflammatory response to implant surgery have been studied, the influence of systemic immunity on its modulation has not. Here we investigate whether the state of the systemic immune system affects cochlear dysfunction after inner ear surgery.

Systemic immunity can be modulated by inoculation with sterile antigens that generate a cognate immune response, especially in combination with adjuvants such as complete Freund adjuvant (CFA) that also engage innate immunity (11,12). Complete Freund adjuvant has been shown to activate circulating leukocytes and enhance their production of proinflammatory T_H1 cytokines and other mediators (13,14). It is thought that the activation state of leukocytes is a major determinant of subsequent immune and inflammatory responses, even at distant sites (15). Activated leukocytes respond with greater affinity to local injury signals, such as the increase in endothelial expression of cell adhesion molecule noted above. Within the cochlea, “priming” of the immune system has resulted in increased inflammation and, consequently, greater hearing loss after cochlear stress induced by either noise exposure (9) or adaptive immunity (8).

Here we test whether inner ear surgery shows a similar effect. Specifically, we explore whether priming of the immune system will lead to a greater elevation of auditory thresholds after a cochlear implantation procedure. Glucocorticosteroids have been shown to ameliorate the loss of hearing in response to cochlear implantation (4,16,17) presumably by blocking inflammation and local tissue injury signaling. We postulate that local dexamethasone will be less effective in blocking the systemic response to local injury signaling when the immune system has been primed, resulting in poorer hearing protection.

MATERIALS AND METHODS

Experimental Design

The study tested 2 main hypotheses:

Hypothesis 1—Keyhole limpet hemocyanin (KLH)–primed animals will lose more hearing during cochlear implantation than unprimed animals.

Hypothesis 2—Dexamethasone applied locally to the round window will offer less protection against hearing loss after cochlear implantation in KLH-treated compared with unprimed animals.

For all experiments, guinea pigs with normal preoperative hearing underwent cochlear implantation in 1 ear, preceded by the local application of a drug to the round window.

Animals in the KLH-primed group were randomly allocated to receiving a local application of dexamethasone to the round window before surgery (20% dexamethasone applied for 30 min) or were treated with normal saline as the control. The main outcome measures were pure-tone auditory brainstem response (ABR) threshold shifts at 1 and 4 weeks after implantation. The status of the foreign body reaction (FBR) 4 weeks after implantation was a secondary outcome measure.

To test Hypothesis 1, ABR threshold shifts after cochlear implantation in KLH-primed saline treated controls (5 animals) were compared with threshold shifts in implanted, unprimed saline controls (7 animals). Keyhole limpet hemocyanin priming involved a subcutaneous inoculation with KLH in CFA and then a booster of KLH in incomplete Freund adjuvant 2 weeks later. We also expected that ABR thresholds would not differ in the ears not operated on (i.e., ears contralateral to the cochlear implant) of KLH-primed animals because of an absence of injury signally within these intact cochleae. To test Hypothesis 2, ABR threshold shifts of KLH-primed dexamethasone-treated animals (5 animals) were compared with the KLH-primed saline controls (5 animals).

Animals

Experiments were conducted on tricolor guinea pigs ($n = 10$) with a minimum weight of 300 g. All animal procedures were approved by the Animal Research and Ethics Committee of the Royal Victorian Eye and Ear Hospital 07/155. All surgical procedures and ABRs were conducted under a general anesthesia induced and maintained with 4 mg/kg of ketamine and 60 mg/kg xylazine in a 3:1 mixture injected intramuscularly. Subcutaneous injections of sterile antigen were performed under a gaseous anesthesia of isoflurane, which was administered with oxygen at a concentration of 0.5% to 1% at a rate of 500 ml/min.

Keyhole limpet hemocyanin was injected subcutaneously into the central upper back. The first inoculation consisted of 1.0 mg of KLH diluted in 0.5 ml of CFA. The second inoculation of 1.0 mg of KLH in 0.5 ml of incomplete Freund adjuvant was 2 weeks later. The injection site was inspected daily for signs of ulceration or severe local inflammatory reaction. Cochlear implantation followed 1 week after the second inoculation. Controls for KLH inoculation underwent the same surgical procedures and ABR recordings, except the inoculation with KLH.

Cochlear Implantation and Electrodes

The electrodes used were specifically designed for the guinea pig cochlear implantation model and have been described previously (4). Briefly, each is a silastic electrode with 3 platinum rings ranging in diameter from 0.41 mm at the tip to 0.45 mm at the base. The distance between each of the 3 rings is 0.75 mm, making a full insertion of all 3 rings a total of 2.25 mm.

Cochlear implantation was performed in the left ear via a postauricular incision. The temporalis muscle was incised, and the bulla was exposed. A bullostomy was made with a high-speed drill, allowing exposure of the round window, cochlea, and facial nerve. Seprapack (Genzyme Corporation, Cambridge, MA, USA) adsorbed with either normal saline or dexamethasone 20% was applied to the round window for 30 minutes before drilling a 0.7-mm-diameter cochleostomy. In all cases, the electrode was inserted into the basal turn to a depth of 2 to 3 mm using a “soft surgery” technique (18,19) until resistance was met. Electrode insertion to this depth carries the tip of the implant to the upper cochlear turn. The cochleostomy was sealed with fibrous tissue. The electrode was trimmed and secured within the bulla before the skin was closed with sutures or glue.

ABR Recordings

Auditory brainstem responses were recorded before implantation, as well as at 1 and 4 weeks afterward. The ABR recording system and the positioning of the recording electrodes have been described previously (4,20,21). Tone pips (5-ms duration, 1-ms rise/fall times) of 2, 8, 16, 24, and 32 kHz were tested in the left (implanted) ear, and frequencies of 2, 8, and 32 kHz were tested in the right ear. Attenuation of the contralateral ear was achieved using an ear mold compound (Otoform, Dreve, Germany). Responses were filtered between 150 Hz and 3 kHz, amplified 100,000 (DAM-5A; W-P Instruments, Inc., New Haven, CT, USA) and digitized (Tucker-Davis Technologies, Alachua, FL, USA). Auditory brainstem responses were tracked from high to low intensity in 5-dB steps, until the wave form signal was lost in the background noise. The wave forms were analyzed using a specifically designed software program written in Igor Pro by Dr. James Fallon and adapted by Prof. Stephen O'Leary. The investigators were blinded to the experimental group during the analysis. Threshold was defined as the lowest level of the acoustic stimulus to evoke a wave III response greater than 0.4 μ V in amplitude as detected by the software and confirmed by visual inspection. Normal hearing before implantation was defined as an ABR threshold of less than 48 dB SPL (peak-to-peak equivalent) in response to a 100- μ s rarefaction click.

Histology

Four weeks after implantation, Guinea pigs were killed with an overdose of pentobarbitone and perfused with heparinized normal saline and then 10% neutral-buffered formalin. Both cochleae were harvested, trimmed and preserved in formalin, and decalcified in 4% (wt/vol) ethylenediaminetetraacetic acid before embedding. Cochleae were oriented using agar and then paraffin embedded. Three midmodiolar sections of 10- μ m thickness were mounted, cover slipped, and stained with hematoxylin and eosin. Three sections were examined by light microscopy (Carl Zeiss, Gottinger, Germany) by an independent pathologist blinded to the experimental and treatment groups. Both the inflammatory cell types present and the extent of acute or chronic inflammatory process in response to the electrode were recorded.

Data Analysis

Statistical analysis of ABR thresholds and threshold shifts was undertaken by univariate general linear modeling, with factors including tone-pip frequency, the time of implantation after priming, the time of ABR testing after surgery, and dexamethasone treatment. In case of significant main effects ($p < 0.05$), multiple pairwise comparisons were performed using the Holm-Sidak correction for multiple tests. Significant interactions between factors were further probed by performing an analysis of variance on the relevant subgroups of data. The histological findings of the cochlea in the saline and dexamethasone groups were compared qualitatively. All calculations were performed using the software package SPSS version 16 (SPSS, Inc., Chicago, IL, USA).

RESULTS

ABR Analysis

Hearing levels are reported as threshold shifts between preimplanted levels at both 1 and 4 weeks after cochlear implantation.

Similar ABR threshold shifts were evident in the KLH-primed and unprimed (control) guinea pigs at all frequencies except 2 kHz. At 2 kHz, the magnitude of threshold shift was significantly greater in the KLH-primed group (Fig. 1). This is reflected in there being a significant main effect of stimulus frequency, and a significant interaction between stimulus

frequency and KLH priming, on analysis of variance (ANOVA, $p = 0.01$, univariate analysis). Thresholds were similar at 1 and 4 weeks after surgery (Fig. 1).

In KLH-primed animals, at 2 kHz, dexamethasone treatment resulted in a reduction in threshold shift of implanted ears when compared to the saline-treated animals. At higher frequencies, there was not a significant treatment effect of the dexamethasone, except at 8 kHz after 4 weeks (Fig. 1).

Thresholds in the contralateral (unimplanted) ears of KLH-primed animals did not differ significantly from pre-implantation thresholds across all frequencies measured at both 1 and 4 weeks after implantation (Fig. 2) because ANOVA failed to reveal significant main effects of either frequency or dexamethasone treatment.

Histological Analysis

A histological analysis was performed on cochleae harvested 1 month after implantation with the results displayed in Table 1. The tissue reaction was similar to that observed previously. The electrodes are removed before tissue processing, and their location is apparent as a circular filling defect in the FBR. Here cochlear implantation involves the insertion of a thin electrode, via a cochleostomy, into the scala tympani. The electrode just passed into the upper basal turn. Half way along the lower basal turn (where the histology was performed), the dummy electrode occupied approximately 20% of the area of scala tympani and usually was situated in the upper half of the scala (Table 1). The lateral position of the electrode varied; most frequently, it was situated nearer the outer cochlear wall, but at times, the electrode was found medially, adjacent to the osseous spiral lamina. Therefore, electrode position observed in this animal model resembled that observed with a straight electrode array. We have not yet explored round window insertion, so do we not have comparable data for this surgical approach.

The FBR was characterized as either a loose areolar fibrous tissue occupying much of the scala tympani or a denser fibrosis. Lymphocytes were present within the FBR in most KLH-treated animals, but none of the controls ($p < 0.001$, χ^2). Multinucleated giant cells were present within the fibrotic tissue of most animals. The percentage of scala tympani that occupied the FBR was calculated and recorded in Table 1. On ANOVA, priming did not affect the extent of the FBR ($F_{15,1} = 0.77$, $p = 0.40$) but the treatment group did ($F_{14,2} = 10.4$, $p = 0.002$), with post hoc testing revealing that the dexamethasone group differed significantly from both the saline-treated, primed animals and the unprimed controls in exhibiting more extensive fibrosis.

DISCUSSION

The main predictions of this study have been borne out, namely that priming of systemic immunity affected hearing loss during cochlear implantation and that a single dose of dexamethasone applied to the round window afforded less protection in primed than has been observed previously in nonprimed animals. Above 2 kHz, the magnitude of the threshold shift was similar in both primed and unprimed animals and also previous studies from our laboratories (4,5,22), perhaps reflecting saturation of the injury effect. The effect of priming at 2 kHz manifested as a persistent threshold shift, not observed in the nonprimed group or in our previous studies where threshold shifts at this frequency were minimal (4,5,22). Therefore, priming broadened the range of frequencies experiencing an elevation in threshold.

Dexamethasone treatment was less effective in ameliorating the threshold shift in primed animals associated with implantation than had been observed in previous studies on

unprimed guinea pigs (4,5,22). In these earlier studies, local round window delivery of dexamethasone resulted in a reduction in threshold shift of 8 kHz or higher; however, in primed animals, a protective effect was only observed at 2 kHz.

The functional effects of cochlear implantation in the immunized animal were similar to those observed in a previous study, which demonstrated that priming of systemic immunity with a sterile antigen increased the magnitude of the hearing loss after noise exposure (9). Similarly, stimulation of innate immunity through the systemic administration of bacterial lipopolysaccharide led to increased hearing loss when the inner ear was later exposed to lipopolysaccharide (11). In both of these cases, the greater hearing loss was mediated by an increase in the cochlear inflammatory response. In the absence of a local (cochlear) trigger, priming of the immune system alone did not cause a cochlear inflammatory response sufficient to degrade auditory function (8,9,11,12,23). Our results are consistent with this interpretation because thresholds did not change significantly in ears that were not implanted on. It is not possible from the present experiment to identify the step(s) of the implant procedure that elicited the systemic immune response, but the presence of the electrode within scala tympani seems likely to be the most significant. This is suggested by an observation of Ye et al. (24) that cochleostomy per se causes only minimal elevation of ABR thresholds.

The reason why dexamethasone afforded poorer protection in primed animals than has been reported in unprimed animals is not clear. It may be that insufficient drug was present within the cochlea or that the drug was not present for long enough to prevent an inflammatory response to injury signaling. We think that the former is unlikely because priming would not be expected to influence the kinetics of locally applied drug and because an earlier study in unprimed animals demonstrated that locally delivered dexamethasone was able to protect hearing arising from a second turn implant (25). Alternatively, the recruitment of inflammatory cells from the systemic circulation could have been exaggerated in the antigen-primed animals, overwhelming the protective response of the drug. This seems possible because leukocytes were observed significantly more frequently in KLH-treated animals. Another possibility is that the leukocytes recruited from the circulation were in a higher state of activation. This seems plausible given that immunization activates circulating leukocytes, enhancing their production of cytokines and other biological mediators once recruited from the circulation. These more activated cells may have been less affected by dexamethasone than the cells of unprimed animals. However, it should be noted that dexamethasone did reduce threshold shift at 2 and 8 kHz at 4 weeks. It is possible that injury signaling was weaker in the 2-kHz region (the second turn) because it is more distant from the site of implantation, and this may explain why dexamethasone had a protective effect here but not at higher frequencies where the inflammatory response was presumably more intense.

Keyhole limpet hemocyanin priming neither increased the extent of fibrosis within the cochlea nor promoted a delayed or progressive hearing loss. The question of delayed hearing loss is of clinical interest because, despite successful preservation of residual hearing in cochlear implant patients, auditory function can be lost weeks or months after implantation (26-28). Although the reasons for this delayed hearing loss are unclear, progressive fibrosis and damping of the motion of the basilar membrane have been postulated as potential causes (29). The histopathology of the tissue response to implantation varies between subjects, with the number of foreign body giant cells, the extent of fibrosis, and the presence of neo-ossification differing between temporal bones of patients who have undergone cochlear implantation (30). We had thought it possible that KLH priming would increase fibrosis and, consequently, promote delayed hearing loss, given the indirect evidence for greater intracochlear inflammation after KLH priming, namely, the extended frequency range over

which hearing loss was observed and the reduced protective effect of local steroids. This was not the case in the cohort studied here, suggesting that any effect of KLH priming is not particularly large. It is possible that other mechanisms, such as the site and/or nature of cochlear injury signals, will be more important than the status of the systemic immune system in determining the extent of fibrosis.

Electrode impedance has been viewed as being influenced by the nature of interactions between the cochlear electrode and the foreign body response that surrounds it (31). There would seem to be a relationship between the initial tissue response and impedance, as evidenced by the observation from several trials (32,33), including a recent randomized controlled trial (34) that electrode impedance is reduced when a single dose of steroids is applied locally to the cochlea at the time of implantation. There is insufficient experimental evidence available to propose with confidence a mechanism by which the reduced electrode impedance may arise; however, it has been proposed by several authors reviewing clinical data that the extent of fibrosis may be minimized by the presence of steroids (35,36). Other experimental results, on the other hand, suggest a delay in the rate of development but not a reduction in the extent of fibrosis (37) after steroid application. An alternative is that it is the cellular content of the foreign body that is influenced by the steroids, and the present experiment gives a hint that this is plausible. Fewer animals receiving steroids were found to have lymphocytes within the foreign body response, so it is conceivable that, in the clinic, when electrode impedances are found to increase transiently in association with vertigo (36), the presumed “labyrinthitis” manifests as a change in the number of inflammatory and/or immune competent cells within the tissue surrounding the implant rather than as a “serous labyrinthitis” involving the perilymph. Further research into the effects of steroids on the foreign body response associated with implantation, and its relationship to systemic immunity, may help to resolve these issues.

This study provides some insights into pharmacological hearing protection of the inner ear of relevance to determining the best route of administration for drug delivery. Both local and systemic routes of administration have been tested experimentally and in clinical trials for protection and/or rescue of hearing, but their mechanisms of action may not be equivalent. Pharmacological protection may act through either damping of local cochlear signaling or minimizing the recruitment of inflammatory cells from the systemic circulation. Local delivery can only act through suppression of local cochlear signaling, whereas systemic delivery could act through both mechanisms. As we have argued previously, the poor protection afforded by local steroid delivery in the KLH-primed animals may be due to a lack of suppression of the recruitment of activated leukocytes (38) into the inner ear. This possibility highlights the importance of continuing research to gain a better understanding of the mechanisms of action of local and systemic delivery because this will inform the most appropriate route for drug delivery in clinical practice. Another factor that may influence this debate includes the practicality of administering steroids via each of these routes. Local therapy is made convenient given that the round window is directly accessed during surgery (4) and that, with local therapy, high intracochlear drug concentrations can be achieved (39). However, diffusion of steroids through the cochlea is a slow process and introduces impractically long waiting times within the operating theater to achieve therapeutic levels in the upper turns of the cochlea where residual hearing resides in patients (5). Systemic administration, on the other hand, can achieve good protection with flexible timing relative to electrode insertion, but very high doses are required to have a therapeutic effect (40). Although this may raise concerns about systemic adverse effects, this potential risk is mitigated by the experimental evidence that suggests that a single dose may be sufficient to afford protection.

In summary, systemic immune system activation at the time of implantation had a significant negative impact on postoperative hearing thresholds, with the low frequencies being affected to a greater degree than observed in previous experiments in unprimed animals. Local application of a single dose of dexamethasone provided limited protection against the impact of systemic immune activation.

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FIG. 1.

Implanted ear: frequency-specific mean threshold shifts ($\pm$ SEM) in the implanted ear at both 1 and 4 weeks after surgery for control animals (○), KLH-primed animals with saline application to round window (◆), and KLH-primed animals with dexamethasone application to round window (■). * $p < 0.05$, t test.

FIG. 2.

Nonimplanted ear: frequency-specific mean threshold shifts ($\pm$ SEM) in the contralateral ear at both 1 and 4 weeks after surgery for KLH-primed animals with saline application to round window (◆) and KLH-primed animals with dexamethasone application to round window of contralateral ear (■).

TABLE 1
Summary of cochlea histology

	ID	Fibrosis (%)	Neutrophils	Lymphocytes	Giant cells	Ossification	Electrode	Electrode position (quadrants)	Fibrosis (quadrants)
KLH dexamethasone	HE0902	71	Yes	Yes	Yes	Yes	ST	1	24
	HE0910	26	No	Yes	Yes	No	ST	13	1234
	HE0852	31	No	Yes	Yes	Yes	ST	1234	24
	HE0848	71	No	Yes	Yes	Yes	ST	1	24
	HE0850	37	No	Yes	Yes	Yes	ST	1234	1234
KLH saline	HE0903	0	No	Yes	Yes	No	ST	24	1234
	HE0909	1	No	No	No	No	ST	No fibrosis	No fibrosis
	HE0847	19	No	No	Yes	No	ST	1	1
	HE0849	3	No	Yes	Yes	Yes	ST	2	24
	HE0851	0	No	Yes	Yes	Yes	ST	24	24
Control	HE0929	34	No	No	Yes	Yes	ST	1234	1234
	HE0930	2	No	No	Yes	Yes	ST	24	24
	HE0933	8	No	No	Yes	No	ST	2	2
	HE0934	2	No	No	Yes	No	ST	1	12
	HE0935	23	No	No	No	No	ST	4	4
	HE0936	23	No	No	Yes	Yes	ST	4	24

	ID	BM	OSL	OHC	IHC	Tunnel of Corti
KLH dexamethasone	HE0902	Intact	Intact	2	1	Present
	HE0910	Intact	Intact	1	1	Present
	HE0852	Intact	Intact	3		Absent
	HE0848	Intact	Intact	3	1	Present
	HE0850	Intact	Intact	3		Absent
KLH saline	HE0903	Intact	Intact	3	1	Present
	HE0909	Intact	Intact	3	1	Present
	HE0847	Intact	Fractured	3	1	Present
	HE0849	Intact	Intact	3	1	Present
	HE0851	Intact	Intact	3	1	Present
Control	HE0929	Intact	Fractured	0		Absent
	HE0930	Intact	Intact	3	1	Present
	HE0933	Intact	Intact	3	1	Present
	HE0934	Intact	Intact	3	1	Present
	HE0935	Intact	Intact	3	1	Present
	HE0936	Intact	Intact	0	1	Present

The electrode location and the location of the fibrosis were sited relative to 4 quadrants of scala tympani (1 – inner upper, 2 – outer upper, 3 – inner lower, 4 – outer lower). When describing the electrode position, it was often found that the electrode would overlap the boundaries of 2 (or more) quadrants, and in this case, all relevant quadrants were recorded. When describing fibrosis, the quadrants listed are those in which fibrosis was found.

BM indicates basilar membrane; ID, animal identifier; IHC, inner hair cell; OHC, outer hair cell number; OSL, osseous spiral lamina.