Assessment of the Protective Effect of Pneumococcal Vaccination in Preventing Meningitis After Cochlear Implantation

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Objectives: To examine if a 23-valent pneumococcal capsular polysaccharide vaccine (PPV23) reduces the risk of meningitis in healthy rats after cochlear implantation.

Design: Interventional animal study.

Interventions: Thirty-six rats (18 immunized and 18 unimmunized) received cochlear implantations and were then infected with *Streptococcus pneumoniae* via 3 different routes (hematogenous, middle ear, and inner ear) in numbers sufficient to induce meningitis.

Results: The rats with implants that received PPV23 were protected from meningitis when the bacteria were delivered via the hematogenous and middle-ear routes (Fisher exact test *P* < .05). However, the protective effect of the vaccine in the rats with implants was only moderate when the bacteria were inoculated directly into the inner ear.

Conclusions: Our animal model clearly demonstrates that immunization can protect healthy rats with a cochlear implant from meningitis caused by a vaccine-covered serotype. This finding supports the notion that all current and future implant recipients should be vaccinated against *S pneumoniae*.


PREVIOUS CLINICAL STUDIES suggest that pneumococcal meningitis is more common in recipients of cochlear implants than in the age-matched general population. However, it is difficult to determine to what extent the cochlear implant contributes to this increased risk because many implant recipients have preexisting risk factors for meningitis. In a controlled laboratory environment, the presence of a cochlear implant has been shown to increase the risk of pneumococcal meningitis in healthy rats by reducing the threshold of infection for 3 different routes of inoculation: hematogenous, middle ear, and inner ear.

Pneumococcal vaccine has been shown to be an effective method for reducing the burden of disease caused by *Streptococcus pneumoniae* in the general population. Currently, 2 types of vaccine that generate anticapsular antibodies in human subjects are available for clinical use. The presence of anticapsular antibodies directed against a particular serotype of *S pneumoniae* following vaccination protects subjects from invasive disease caused by that serotype. One of these vaccines, Pneumovax 23 (Merck Sharp & Dohme Pty Ltd, South Granville, New South Wales, Australia), is a 23-valent pneumococcal capsular polysaccharide vaccine (PPV23) and was used in the present study. In healthy adults, PPV23 has been shown to reduce type-specific invasive pneumococcal disease by up to 90%. To treat younger patients, PPV23 is recommended only for children older than 2 years because younger children and infants are immunologically immature and might not respond to vaccines containing pure capsular antigen.

An example of the second vaccine is a heptavalent pneumococcal conjugated vaccine (PCV7) that contains purified capsular polysaccharides of 7 pneumococcal serotypes conjugated to a nontoxic variant of diphtheria toxin. Protein-conjugated vaccines are recommended for infants and children younger than 2 years because high immunogenicity is required for young children to induce a T cell–dependent response. Since 2000, the routine use of PCV7 in children younger than 5 years in the United States has resulted in a significant decline in the incidence of invasive pneumococcal disease in the age group targeted for vaccination, as well as among nonvaccinated older children and adults through indirect effects on pneumococcal transmission. Of note, PCV7 also reduces the carriage of serotypes contained in the vaccine and there-

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The number of meningitis cases based on our group’s previous studies (Table 1). The bacterial inoculum for each route has been shown not to induce meningitis, and 18 of these rats were randomly selected to undergo cochlear implantation 4 weeks prior to bacterial inoculation to evaluate the effects of vaccination in the prevention of pneumococcal meningitis in implant recipients for all routes of inoculation from the upper respiratory tract mucosa to the central nervous system.

The aim of this animal study was to provide clinicians with new knowledge relating to the efficacy of PPV23 in preventing pneumococcal meningitis in implant recipients for all routes of inoculation from the upper respiratory tract mucosa to the central nervous system.

### METHOD

#### SOURCE OF THE ANIMALS

All experimental animals were bred and housed in the animal house in the Department of Otolaryngology, University of Melbourne. Animals were free of endogenous pathogens, including *S pneumoniae*. All procedures and animal handling were approved by the animal research ethics committee of the Royal Victorian Eye and Ear Hospital (project No. 05-112A) and conducted in accordance with guidelines set by the Code of Practice for the Care and Use of Animals in Research in Australia.

In total, 84 otologically normal adult Hooded-Wistar rats were used in this study. There were no significant differences in the age and weight of the animals used in this study. One cohort of 18 rats received a cochlear implant to the left ear 4 weeks prior to inoculation with the bacteria. Previous work showed that rats undergoing cochlear implantation had a lower threshold for pneumococcal meningitis than control rats. The second cohort of 26 rats was given pneumococcal vaccination, and 18 of these rats were randomly selected to undergo cochlear implantation 4 weeks prior to bacterial inoculation to evaluate the effects of vaccination in the prevention of meningitis. The third cohort of 40 unimmunized rats that did not undergo implantation served as a control for the measurement of baseline levels of antibody to *S pneumoniae*.

Thirty-six rats that underwent implantation from the immunized and unimmunized cohorts were allocated to receive bacterial inoculation by 1 of the 3 different routes (intraperitoneally [IP], via the middle ear, or via the inner ear) (Table 1). The number of *S pneumoniae* bacteria chosen for each route was based on our group’s previous studies (Table 1). The bacterial inoculum for each route has been shown not to induce meningitis in control rats with a cochleostomy only, but consistently caused meningitis in rats with a cochleostomy and a cochlear implant.

### SURGICAL ANESTHESIA

The rats were anesthetized with an IP injection of a mixture of 8 mg/kg of xylazine (Ilum Xylazil-20; Troy Laboratories Pty Ltd, Smithfield, New South Wales, Australia) and 75 mg/kg of ketamine hydrochloride (Ketamine; Parnell Laboratories, Mascot, New South Wales, Australia). A local anesthetic agent (0.1 mL of lignocaine hydrochloride with 0.0182 mg/mL of adrenalin tartrate [Troy Laboratories Pty Ltd]) was injected subcutaneously (SC) around the surgical incision. The animals were then placed on a heated pad maintained at 37°C throughout the procedure. The animals were given 0.05 to 0.05 mg/kg buprenorphine SC (Temgesic; Reckitt Benckiser, West Ryde, New South Wales, Australia) for analgesia immediately after surgery. They were assessed continually for signs of postoperative pain and discomfort, and buprenorphine was given every 8 to 12 hours if there were signs of pain or discomfort. Animals received 10 mL/kg of isotonic sodium chloride solution SC for fluid replacement during recovery from the surgical procedure.

### SCALA TYPANI ELECTRODE ARRAY DESIGN AND COCHLEAR IMPLANTATION PROCEDURE

The dummy scala tympani electrode array was described in previous reports by our group. In brief, 5 mm of polyimide tubing (Cole-Parmer, Vernon Hills, Illinois) with an outer diameter of 0.10 mm was coated with a layer of silicone (Dow Corning Medical Grade Elastomer MDX4-4210, Factor II; Lakeside, Arizona) to a diameter of 0.15 mm. The dummy arrays were cleaned with absolute alcohol in an ultrasonic cleaner, rinsed with MilliQ water for 10 minutes before drying, packaging, and sterilizing using hydrogen peroxide sterilization (STERRAD 100S; Advanced Sterilization Products, Irvine, California).

Thirty-six adult Hooded-Wistar rats underwent implantation with a dummy scala tympani electrode array in the left cochlea. The surgical insertion technique has been described in our group’s earlier work. In brief, the dummy array was inserted 2 to 3 mm into the scala tympani, and the cochleostomy was sealed with temporalis fascia. After cochleostomy implantation, all 36 rats received 2 doses of 10 mg/kg enrofloxacin SC (Baytril 100; Bayer Australia Ltd, Pymble, New South Wales, Australia) diluted 1:1 with isotonic sodium chloride, one dose immediately after surgery and the second dose 12 hours later.

### PNEUMOCOCCAL VACCINATION AND SEROCONVERSION

Twenty-six adult rats received pneumococcal vaccination, and 18 of these rats underwent cochlear implantation 24 to 48 hours after vaccination. The use of polyvalent pneumococcal vaccine in rats has been established previously. We administered 0.25 mL of PPV23 (a mixture of purified capsular poly-

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**Table 1. Effect of Immunization With PPV23 on the Frequency of Meningitis in Rats With Cochlear Implants**

<table>
<thead>
<tr>
<th>Route of Inoculation</th>
<th>Infective <em>Streptococcus pneumoniae</em> Dose, CFU</th>
<th>Unimmunized Group</th>
<th>Immunized Group</th>
<th>( P ) Value ( ^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraperitoneal</td>
<td>( 4 \times 10^9 )</td>
<td>6/6</td>
<td>0/6</td>
<td>.002</td>
</tr>
<tr>
<td>Middle ear</td>
<td>( 3 \times 10^9 )</td>
<td>6/6</td>
<td>1/6</td>
<td>.02</td>
</tr>
<tr>
<td>Inner ear</td>
<td>( 1 \times 10^9 )</td>
<td>6/6</td>
<td>3/6</td>
<td>.18</td>
</tr>
</tbody>
</table>

Abbreviations: CFU, colony-forming units; PPV23, 23-valent pneumococcal capsular polysaccharide vaccine.

* Unless otherwise indicated, data are reported as number of rats with clinical and histologic evidence of meningitis/number of rats inoculated.

\( ^b \) Two-tailed Fisher exact test
saccharides from 23 prevalent serotypes of *S. pneumoniae*, including serotype 2) to individual rats both intramuscularly (IM) and SC. The same vaccine has been given to human subjects IM or SC for protection against invasive pneumococcal disease. Serum from the 26 vaccinated rats was collected 4 weeks later and assayed for antibodies to pneumococcal serotype 2 polysaccharide by enzyme immunoassay (EIA). The results were compared with those from 40 age-matched healthy unimmunized rats that served as a control group.

### QUANTIFICATION OF *S. pneumoniae* SEROTYPE 2–SPECIFIC IgG IN RATS

Serotype 2 polysaccharide IgG levels were measured using an EIA based on a previously described method amended to test rat serum. All samples were diluted in phosphate-buffered saline with 0.05% Tween-20 plus 1% (wt/vol) casein (Sigma-Aldrich, St Louis, Missouri). Volumes of 100 µL per well were used, except when blocking, when 200 µL of phosphate-buffered saline plus 1% (wt/vol) casein was used for 1 hour. All incubations were performed at 37°C in a humidified chamber.

Briefly, medium-binding microtiter plates (Microlon; Greiner Bio-One, Frickenhausen, Germany) were coated with purified type 2 pneumococcal capsule (ATCC, Manassas, Virginia) at a concentration of 5 µg/mL in 0.9% sodium chloride for 5 hours. Rat serum diluted 1:50 was absorbed with 50 µg/mL of pneumococcal cell wall polysaccharide (C-P; Statens Serum Institut, Copenhagen, Denmark) at 4°C overnight. Two-fold serial dilutions of serum samples were incubated with antigen for 3 hours and washed extensively. Bound antibody was detected using 1:20,000 diluted, peroxidase-conjugated, goat antirat whole-molecule IgG (Sigma-Aldrich), for 2 hours. After washing, the wells were incubated with TMB (3,3',5,5'-tetramethylbenzidine) substrate (Kirkegaard & Perry Laboratories Inc, Gaithersburg, Maryland) and stopped with 30 µL of 2.5M sulfuric acid after 9 minutes. Absorbance was measured at 450 nm with a reference filter at 620 nm. The resulting optical densities were converted to arbitrary EIA units by reading them off a standard curve generated using serum from 40 age-matched healthy unimmunized rats that served as a control group.

### MIDDLE-EAR INOCULATION

Under general anesthesia, the left bulla of 12 rats (6 from each group) was surgically exposed for direct inoculation of 10 µL of inoculum containing $3 \times 10^4$ CFU of *S. pneumoniae*. To retain the microorganisms in the bulla, the cavity was first filled with Gelfoam (Pharmacia & Upjohn Corporation, Kalamazoo, Michigan). After the inoculation of the bacteria, the opening of the bulla was covered with temporalis fascia, and the wound was sutured in 2 layers.

### INNER-EAR INOCULATION

Under general anesthesia, the left bulla of 12 rats (6 from each group) was surgically exposed, and a cochleostomy was performed with a straight Kirschner wire to access the scala tympani. Two microliters of perilymph were removed and 1 µL of a bacterial suspension containing $1 \times 10^3$ CFU was inoculated into the scala tympani over 1 minute using an infusion catheter, a 5-µL microsyringe (ILS, Stützerbach, Germany), and a microsyringe pump controller (World Precision Instruments Inc, Sarasota, Florida). After inoculation, the cochleostomy was covered with temporalis fascia. The opening of the bulla was also covered with temporalis fascia, and the wound was sutured in 2 layers.

### POSTINOCULATION MONITORING

Following the inoculation, each animal was examined a minimum of twice daily for clinical signs of meningitis over 5 days. The clinical assessment was recorded on a 12-point monitoring sheet as described previously. Animals were killed if 1 of the following conditions was met: a score of 10 or above or a score of 5 to 10 with a rectal temperature greater than 41°C.

### MICROBIOLOGICAL SPECIMEN COLLECTION AND TISSUE PREPARATION

Once early signs of meningitis were evident, rats were deeply anesthetized with isoflurane and oxygen to allow collection of CSF, middle-ear fluid, and blood for microscopic analysis and culture. The method of CSF collection has been described previously.

The animals were then given a lethal IM dose of pentobarbitone sodium, 120 mg/kg of body weight (Lethabarb; Virbac Pty Ltd, Peakhurst, New South Wales, Australia) and transcardially perfused with 0.9% isotonic sodium chloride then 10% neutral buffered formalin (NBF), pH 7.4 at 4°C. The brain, meninges, and the cochleae were harvested and placed in 10% NBF for further processing. Thirty-six brains with meninges were harvested and stored in 10% NBF for 48 hours and then embedded in paraffin. Representative portions of specimens were sectioned in 10-µm thickness, stained with hematoxylin-eosin and gram stain, and examined by light microscopy for the presence of inflammation and gram-positive cocci.

Nine pairs of randomly selected cochleae were harvested from the temporal bones and fixed in 10% NBF. The contralateral cochlea served as a control. They were decalcified in 10% ethylene diamine tetra-acetic acid in 0.1M phosphate buffer (pH 7.4) and then processed and embedded in Spurr resin. The embedded cochleae were oriented in the midmodiolar plane, and 2 sets of twenty-one 2-µm sections were collected at 126-µm intervals throughout the cochlea. One set of 21 sections was stained with hematoxylin-eosin and the other set with gram stain.

Blood, CSF, and middle-ear cultures were collected as an adjunct to the histologic analysis of the central nervous system to detect the presence of the bacteria. The serotype of *S. pneumoniae* isolated from the cultures was determined using commercial typing serum samples (Statens Serum Institut).


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The rats immunized with PPV23 did not exhibit any adverse local or systemic effect following vaccination. They remained healthy and steadily gained weight throughout the 4 weeks prior to inoculation with S pneumoniae type 2. Four weeks after immunization with PPV23, the mean ± SD EIA units to type 2 pneumococcal polysaccharide was 27.0 ± 1.8 in vaccinated rats compared with 18.0 ± 1.2 in unvaccinated rats (P < .001, unpaired 2-tailed t test). These results indicate that vaccination with a single divided dose of PPV23 given IM and SC was sufficient to induce a rise in the titer of specific antipneumococcal antibodies.

Following inoculation with S pneumoniae all 18 rats with implants that were not immunized developed both clinical and histologic evidence of meningitis regardless of the route of inoculation (Table 1). When rats developed signs of meningitis, they appeared tired and lethargic; were unresponsive to sound and light stimuli; had a hunched body posture; and showed poor grooming, weight loss, and a rectal temperature above 38°C. When these signs were present, the histologic analysis of the brain consistently showed evidence of meningitis with inflammatory cells and gram-positive diplococci within the subarachnoid space. The correlation between the clinical signs of meningitis and histopathologic evidence of meningitis has been established in our group’s previous work. The time taken for rats in the various experimental groups to develop meningitis is shown in Figure 1.

In contrast, only 4 of the 18 rats with implants that were immunized with PPV23 developed meningitis (3 inoculated via the inner ear, 1 via the middle ear) (Table 1). The preventive effect of the vaccine against meningitis after cochlear implantation was statistically significant for IP (P = .002) and middle-ear inoculation (P = .02) analyzed using the 2-tailed Fisher exact test. However, the effect of immunization in rats with implants that were inoculated with pneumococci directly into the inner ear was not statistically significant (P = .18).

### HISTOLOGIC ANALYSIS

The brains and meninges were examined histologically for thickening and hyperplasia of the meningeal cells and for the presence of gram-positive cocci and/or inflammatory cell response within the subarachnoid space and the brain tissue. The cochleae were examined for the presence of bacteria and inflammatory cells.

### STATISTICAL ANALYSIS

Statistical analysis was performed using InStat version 3.05 (GraphPad Software Inc, San Diego, California). Unpaired 2-tailed t tests were used to compare serum antibody titers with type 2 polysaccharide in immunized and unimmunized rats. The Fisher exact test (2-tailed) was used to evaluate the effect of pneumococcal vaccination in preventing meningitis after cochlear implantation following the 3 different routes of inoculation. For all statistical analyses, a P value of less than .05 was considered significant.

### RESULTS

The rats immunized with PPV23 did not exhibit any adverse local or systemic effect following vaccination. They remained healthy and steadily gained weight throughout the 4 weeks prior to inoculation with S pneumoniae type 2. Four weeks after immunization with PPV23, the mean ± SD EIA units to type 2 pneumococcal polysaccharide was 27.0 ± 1.8 in vaccinated rats compared with 18.0 ± 1.2 in unvaccinated rats (P < .001, unpaired 2-tailed t test). These results indicate that vaccination with a single divided dose of PPV23 given IM and SC was sufficient to induce a rise in the titer of specific antipneumococcal antibodies.

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### MICROBIOLOGIC FINDINGS

The results of the microbiologic examination of CSF, blood, and middle-ear swabs are summarized in Table 2. Serotyping of pneumococci isolated from rats showed that the isolates were the same as those used for the initial inoculum.

### COCHLEAR HISTOLOGIC FINDINGS

The pattern and distribution of bacteria and inflammatory cells within the cochleae of the rats with clinical and histologic evidence of meningitis were consistent with our group’s previous findings. There were no gram-positive bacteria and inflammatory cells found within both cochleae of immunized rats inoculated IP (Figure 2). A more severe labyrinthitis was observed in the ipsilateral ears of rats inoculated with bacteria via the middle and inner ear (Figure 3 and Figure 4). In rats inoculated via the middle-ear cavity but not exhibiting meningitis, the histologic appearance of the cochleae was normal. In animals inoculated via the inner ear but without meningitis, a few inflammatory cells and serofibrinous exudate were seen in the basal turn of the cochlea.

Macroscopic examination of the ipsilateral middle-ear mucosa of the round window niche revealed evidence of middle-ear inflammation in animals inoculated via the middle ear. The contralateral control bullae showed no evidence of inflammation. There was no macroscopic evidence of inflammatory changes within the middle-ear mucosa in rats inoculated IP or directly into the inner ear.

### COMMENT

Our group has previously shown that the presence of a cochlear implant significantly reduces the threshold of S pneumoniae required to produce meningitis in healthy rats for 3 different routes of inoculation. In the present study, we demonstrated that rats with implants vacci-
nated with a pneumococcal polysaccharide vaccine (PPV23) were protected against meningitis caused by a capsular type of bacteria covered by the vaccine. Protection was greatest when *S pneumoniae* was inoculated IP or into the middle ear but was not significantly reduced in rats inoculated directly into the inner ear. The PPV23 vaccination was selected for this study because it covers the clinical serotype 2 isolate, *S pneumoniae* 447A, that has been used to establish the pneumococcal meningitis model in rats with implants. The protective effect of PCV7, which evokes a more comprehensive immune response than PPV23 but does not cover serotype 2, was not examined.

The findings of protective efficacy of the PPV23 vaccine in this study are consistent with those of previous animal studies. In a rat model, PPV23 reduced mortality of splenectomized rats from systemic bacteremia following IP inoculation with *S pneumoniae* serotype 3. Pneumococcal capsular polysaccharide vaccine was also effective in the prevention of type-specific acute otitis media in chinchillas. This protection was associated with a high level of antibodies in both the serum and the middle-ear effusion.

However, elevated serum antibody titers against *S pneumoniae* did not significantly reduce the attack rate of meningitis following direct inner-ear inoculation. This might be explained by the fact that the antibody levels in the perilymph within cochleae is 1000 times less than in serum. Furthermore, the presence of a blood-labyrinthine barrier partially isolates the labyrinth from systemic immunity and may reduce the transit of antibodies from the serum into the inner ear. The direct inoculation of pneumococci into the inner ear resembles the clinical scenario of a direct spread of infection from the middle ear to the inner ear then to the meninges. Our results suggest that in the presence of middle-ear infections, the serum and mucosal antibodies derived from the vaccination can prevent infections from spreading to the inner ear and then to the meninges. However, if the bacteria cross over the bony and mucosal barrier to enter the inner ear (eg, after inner-ear surgery), the infection can still occur.

### Table 2. Results of Microbiologic and Pathologic Examinations of Rats With Cochlear Implants After Inoculation With *Streptococcus pneumoniae*

<table>
<thead>
<tr>
<th>Route of Bacterial Inoculation</th>
<th>Rats With Positive Findings, No.</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood Culture&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CSF Culture&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Middle-Ear Swab&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>Unimmunized 5</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Immunized 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Middle ear</td>
<td>Unimmunized 6</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Immunized 1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Inner ear</td>
<td>Unimmunized 6</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Immunized 3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of rats (of 6 inoculated with *S pneumoniae*) with a positive culture finding at necropsy.

### Figure 2. Low-power photomicrographs showing a cochlea with a scala tympani electrode array implant (im) (A) and the contralateral control cochlea (B) of an immunized rat 120 hours following intraperitoneal inoculation of $4 \times 10^6$ colony forming units of *Streptococcus pneumoniae*. This animal did not exhibit clinical or histologic central nervous system evidence of meningitis. The scalae of both cochleae were devoid of gross infection. For both panels, the scale bar indicates 200 µm (hematoxylin–eosin).
spread to the central nervous system even after the vaccination.

Although the effect of immunization in protecting rats with implants from meningitis following direct inner-ear bacterial inoculation was not statistically significant, some protective effect was observed in this experiment in terms of the incubation period and frequency of meningitis ($P = .06$, log-rank test for survival).

Findings from blood, CSF, and middle-ear cultures provided additional information to support the effectiveness of PPV23 immunization in preventing pneumococcal meningitis. Overall, there was a reduction in the number of culture-positive blood, middle-ear, and CSF specimens from the immunized rats compared with unimmunized rats.

In keeping with previous studies, the histologic appearance of the cochlea in rats with meningitis was dependent on the route of inoculation. There were no gram-positive bacteria and inflammatory cells within both cochleae of immunized rats inoculated IP. However, much higher numbers of bacteria and greater inflammation were observed in the cochlea ipsilateral to inoculation via the middle and inner ear compared with the contralateral controls.

The current recommendations from the US Food and Drug Administration, Centers for Disease Control and Prevention, and Advisory Committee on Immunization Practices are that all current and future cochlear implant recipients should receive age-appropriate vaccination with either PPV23 or PVC7. Our study strongly supports this recommendation.

In recipients of cochlear implants, the duration of protection following pneumococcal vaccination and protection against serotypes not covered by the vaccine remain to be determined. Studies in subjects without a cochlear implant have shown a gradual decline in specific antibody concentrations after a primary series of PPV23, and guidelines recommend booster immunization.

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**Figure 3.** Photomicrographs of rat cochlea specimens. A and B, Low-power photomicrographs (scale bar indicates 200 µm) illustrating the cochlea with the scala tympani electrode array implant (im) (A) and the contralateral control cochlea (B) of an immunized rat 120 hours following middle-ear inoculation of $3 \times 10^4$ colony forming units of *Streptococcus pneumoniae* (hematoxylin-eosin). This animal exhibited clinical and histologic central nervous system evidence of meningitis. In this example, there is a severe labyrinthitis apparent throughout the ear with the implant (A), while the contralateral cochlea (B) exhibits evidence of infection mainly localized to the modiolus (c) and internal acoustic meatus (d). The c and d areas in panel B are shown enlarged in panels C and D, respectively. C and D, Higher-power photomicrographs (scale bar indicates 10 µm) of gram-stained sections of the modiolus (C) and internal acoustic meatus (D) of the contralateral cochlea illustrate the presence of bacteria (arrows); bn indicates bone.
tion after 3 years in patients younger than 10 years and after 6 years in older patients. However, antibody levels may fall below a protective threshold earlier, thus leaving some cochlear implant recipients unprotected before the booster dose. The immunologic memory for long-term protection against pneumococcal disease in implant recipients requires further research. Currently, there are no clinical or animal data to indicate whether the non-invasive serotypes (not covered by PPV23) can cause meningitis in cochlear implant recipients. Therefore, future research should be carried out to study the effect of the cochlear implant on the pathogenesis of serotypes not covered by the PPV23 vaccine.

In conclusion, PPV23 protected rats with a cochlear implant from acquiring meningitis when bacteria were inoculated IP or into the middle-ear cavity. However, the vaccine was only moderately effective when the bacteria were introduced directly into the inner ear. Overall, the protective effect of pneumococcal immunization suggests that all current and future recipients of cochlear implants should be immunized against \textit{S} \textit{pneumoniae}.

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Author Contributions: Dr Wei had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Wei, Robins-Browne, Shepherd, Clark, and O’Leary. Acquisition of data: Wei, Robins-Browne, and Azzopardi. Analysis and interpretation of data: Wei, Robins-Browne, Shepherd, Azzopardi, Clark, and O’Leary. Drafting of the manuscript: Wei and Robins-Browne. Critical revision of the manuscript for important intellectual content: Wei, Robins-Browne, Shepherd, Azzopardi, Clark,

Figure 4. Photomicrographs of rat cochlea specimens. A and B, Low-power photomicrographs (scale bar indicates 200 μm) illustrating the cochlea with the scala tympani electrode array implant (A) and the contralateral control cochlea (B) of an immunized rat 86 hours following inner-ear inoculation of $1 \times 10^6$ colony forming units of \textit{Streptococcus pneumoniae} (hematoxylin-eosin). This animal exhibited clinical and histologic central nervous system evidence of meningitis. In this example, there is a severe labyrinthitis apparent throughout the ear with the implant (A), while the contralateral cochlea (B) exhibits a less severe infection (arrow points to the bacteria and inflammatory cells in the scala tympani). *Artifact from histologic preparation. The c and d areas in panels A and B, respectively, are shown enlarged in panels C and D, respectively. C and D, Higher-power photomicrographs (scale bar indicates 10 μm) of gram-stained sections of the modiolus of the ipsilateral cochlea (C) and internal acoustic meatus of the contralateral cochlea (D) illustrate the presence of bacteria (arrows); bn indicates bone.

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