Published in final edited form as:

Otol Neurotol. 2006 September ; 27(6): 844–854.

Pneumococcal meningitis: development of a new animal model

Benjamin P.C. Wei, MBBS^{1,2}, Robert K. Shepherd, PhD^{1,2}, Roy M. Robins-Browne, MB, BCh, PhD, FRCPath, FRCPA³, Graeme M. Clark, MB, MS, PhD, FRCS(Edin & Eng), FRACS¹, and Stephen J. O'Leary, MBBS, PhD, FRACS^{1,2}

¹ Bionic Ear Institute

² Department of Otolaryngology, University of Melbourne, Melbourne, Victoria, Australia

³ Department of Microbiology and Immunology, University of Melbourne, Melbourne, Victoria, Australia

Abstract

Hypothesis—The rat is a suitable animal to establish a model for the study of pneumococcal meningitis post cochlear implantation

Background—There has been an increase in the number of cases of cochlear implant-related meningitis. The most common organism identified was *Streptococcus pneumoniae*. Whether cochlear implantation increases the risk of pneumococcal meningitis in healthy subjects without other risk factors remains to be determined. Previous animal studies do not focus on the pathogenesis and risk of pneumococcal meningitis post implantation and are based on relatively small animal numbers, making it difficult to assess the cause and effect relationship. There is, therefore, a need to develop a new animal model allowing direct examination of the pathogenesis of meningitis in the presence of a cochlear implant.

Methods—Eighteen non-implanted rats were infected with 1×10^6 and 1×10^8 colony forming units (CFU) of a clinical isolate of *S. pneumoniae* via three different inoculation routes (middle ear, inner ear and intraperitoneal) to examine for evidence of meningitis over 24 hours. Six implanted rats were infected with the highest amount of bacteria possible for each route of inoculation (4×10^{10} CFU intraperitoneal, 3×10^8 CFU middle ear, 1×10^6 CFU inner ear) to examine for evidence of meningitis with the presence of an implant. Histological pattern of cochlear infections for each of the three different inoculating routes were examined.

Results—Pneumococcal meningitis was evident in all 6 implanted animals for each of the three different routes of inoculation. Once in the inner ear, bacteria were found to enter the central nervous system either via the cochlear aqueduct or canaliculi perforantes of osseous spiral lamina, reaching the perineural and perivascular space then the internal acoustic meatus. The rate, extent and pattern of infection within the cochleae depended on the route of inoculation. Finally, there was no evidence of pneumococcal meningitis observed in 18 non-implanted rats inoculated at a lower concentration of *S. pneumoniae* when observed for 24 hours post-inoculation.

Conclusion—Meningitis in implanted rats following inoculation with a clinical isolate of *S. pneumoniae* is possible via all three potential routes of infection via the upper respiratory tract. The lack of meningitis observed in the 18 non-implanted rats suggests longer post-inoculation monitoring periods are required to ensure whether or not meningitis will develop. Based on this work we have developed a new animal model that will allow quantitative risk assessment of meningitis post cochlear implantation, and the assessment of the efficacy of potential interventional strategies in future studies.

Introduction

Since 2002 there has been an increase in the number of reported cases of meningitis associated with cochlear implants. The most common organism identified was *Streptococcus pneumoniae*^{1,2}. The incidence of pneumococcal meningitis in cochlear implant recipients was found to be greater than that of an age-matched cohort in the general population². Identified risk factors included an implant with a positioner, inner-ear malformations, the presence of a cerebral spinal fluid (CSF) leak after cochlear implantation and a history of ventriculoperitoneal-shunt placement. In addition, signs of otitis media have been present in implanted children with meningitis^{2,3}. It remains to be determined whether cochlear implantation *per se* increases the risk of meningitis in subjects with no existing risk factors for acquiring the disease.

The exact routes by which the bacteria reach the meninges in the presence of a cochlear implant are subject to debate⁴. Due to the potential compromise of both mucosal soft tissue and bony barriers between the inner and middle ear as a result of cochlear implantation, the direct spread of infection from the middle to the inner ear and then to the CNS has been proposed as a major route of infection⁵. Others have postulated that pneumococcal meningitis is caused by the bacteremia that follows colonization of the upper respiratory tract^{4,6}. The interventional strategies to reduce implant related infection will depend on the route of spread of bacteria from the middle ear to the meninges. Therefore, it is important to develop experimental models designed to study all the potential routes of infection leading to meningitis.

This paper describes the development of an animal model of pneumococcal meningitis with and without cochlear implantation. Three different inoculation routes were established to help differentiate between the potential routes of infection described above. In the presence of acute otitis media (AOM) as a result of direct middle ear inoculation, *S. pneumoniae* can reach the meninges either via the hematogenous route or via direct inner ear invasion to the CNS. Both the hematogenous and direct routes of infection need to be studied independently to assess their relative importance in cochlear implant related meningitis. Therefore two additional routes of inoculation were established to study pneumococcal meningitis for both hematogenous and direct spread (via the inner ear) of the bacteria to the meninges. Hematogenous seeding of the bacteria in the CNS can be achieved by direct intraperitoneal (i/p) inoculation⁷. Direct spread of bacteria to the CNS can be achieved by inoculation of the bacteria directly into the inner ear as described in this paper.

A pneumococcal meningitis model examining all potential routes of infection spread from the upper respiratory tract to the meninges is the first key step to allow a quantitative investigation of the risks of CNS infection posed by cochlear implants for subsequent pneumococcal infection and to promote methods for preventing the spread of infection from the middle ear to the cochlea and CSF.

Methods and materials

A total of 24 otoscopically-normal adult Wistar rats (150 to 300g, 10–16 weeks of age) were used in the experiment. All procedures and animal handling were approved by the Animal Research Ethics Committee of the Royal Victorian Eye and Ear Hospital (project #04-105A) and conducted in accordance with guidelines set by the “The Code of Practice for the Care and Use of Animals in Research in Australia” from the National Health and Medical Research Council.

Preparation of the inoculum

S. pneumoniae strain 447A, which carries the type 2 capsular antigen, and was originally isolated from the CSF of a child with meningitis, was selected for the study based on our previous experience with it in a cat model of otitis media^{8,9}. The virulence of this strain was confirmed by passaging it through mice, after which it was stored at -70°C in aliquots of nutrient broth containing 30% glycerol. The inoculum for each experiment was prepared from bacteria grown on horse blood agar (HBA) at 37°C overnight in air containing 5% CO_2 . Isolated colonies from these plates were thoroughly emulsified in sterile pre-warmed phosphate buffered saline (PBS) until they matched a turbidity standard equivalent (McFarland Equivalence Turbidity Standards¹⁰ of the desired concentrations. The number of viable bacteria in the inoculum was determined by making serial ten fold dilutions in PBS for quantification just prior to inoculation¹¹. The bacteria were spotted onto HBA plates (10 μl / dilution), allowed to dry and incubated at 37°C overnight. Colonies were counted following incubation and the actual inoculum calculated as colony-forming units/ml (CFU/ml).

The number of bacteria chosen for the present experiment (Table 1) was based on previous experience using *S. pneumoniae*^{12–16}. The maximum deliverable volumes of inoculum for the three different routes of inoculation were: 1 ml for i/p, 10 μl for middle ear inoculation and 1 μl for direct inner ear inoculation.

A cohort of implanted animals was also used to create a “worst-case” scenario to examine whether pneumococcal meningitis could be achieved in rats with cochlear implants. The animals were inoculated four weeks after cochlear implantation in order to allow the development of a soft tissue capsule around the electrode array. The highest numbers of bacteria for each inoculation site were employed (Table 1).

Surgical anaesthesia

Rats were anesthetized with an intraperitoneal (i/p) injection of a mixture of 8mg/kg xylazine and 75mg/kg ketamine. The animals were then placed on a heated pad maintained at 37°C throughout the surgery.

Cochlear implantation

Six of the 24 animals used in this study received a unilateral cochlear implant 4 weeks prior to inoculation with *S. pneumoniae* (Table 1). The dummy electrode arrays for implantation into the rat scala tympani were manufactured in house from 5 mm lengths of 0.10 mm OD polyimide tubing. The tube was coated with a layer of medical grade silicone to a diameter of 0.15 mm (Fig. 2). The dummy electrodes were cleaned with absolute alcohol in an ultrasonic cleaner, rinsed with MilliQ water (x3) and bathed in MilliQ water for 10 minutes before drying, packaging and sterilizing using H_2O_2 sterilization.

Using sterile technique, a post auricular skin incision was made to expose the round window membrane (RWM). The bulla cavity was inspected for any abnormality of the middle ear mucosa. The stapedial artery, which is located just below the round window niche, was cauterized with a Zencor MF1 bipolar coagulator. A cochleostomy was performed just below the round window niche over the site of the cauterized stapedial artery¹⁷. All care was taken to clear away bone dust and blood from the cochleostomy before placement of the dummy electrode array, which was inserted 2–3 mm into the scala tympani (Fig. 1). The cochleostomy was sealed with temporalis fascia and the wound sutured in two layers.

Following surgery, the implanted animals received two doses of prophylactic enrofloxacin 10 mg/kg subcutaneously (s/c) diluted 1:1 with saline. One dose immediately after surgery and the second dose 12 hours later. Antipamezole hydrochloride 5mg/ml i.m. was given to reverse

the effects of anesthesia and 0.03–0.05 mg/kg s/c buprenorphine for analgesia. All animals were given 10 ml/kg of normal saline s/c during recovery from the surgery for fluid replacement and assessed for signs of post-operative pain and discomfort. Additional buprenorphine was given on an 8 to 12 hourly basis as required.

Bacterial inoculation

Intraperitoneal inoculation—Following anesthesia, 1 ml of the desired concentration of *S. pneumoniae* 447A was inoculated into the intra-peritoneal cavity using a sterile 20 gauge needle and 1 ml syringe.

Middle ear inoculation—Under general anesthesia, the left bulla of each rat was exposed using sterile surgical techniques. The bulla cavity was filled with Gelfoam® into which 10 µl of the inoculum was injected. The bulla was then covered with temporalis fascia and the wound sutured in two layers.

Inner ear inoculation—Under general anesthesia, the left bulla was exposed and a cochleostomy was performed close to the round window niche using a straight Kirschner wire. Using a 5 µl microsyringe connected to a micropump, 2 µl of perilymph was removed and 1 µl of inoculum was then gradually injected into the scala tympani. The cochleostomy and the bulla were covered with temporalis fascia and the wound sutured in two layers.

Post inoculation monitoring

The post-operative management of the animals followed that described following cochlear implantation (above) except that no prophylactic enrofloxacin were given to animals after bacterial inoculation. Following inoculation each animal was examined at least twice daily for clinical signs of meningitis. The clinical assessment was recorded based on a 12-point scoring system developed in conjunction with a veterinarian. This assessment included the animal's weight and a quantitative monitoring of the animal's general status as indicated by alertness (1, normal; 2, tired but still responded to light, sound and tactile stimuli; 3, very lethargic with no movement to stimuli); grooming (1, normal; 2, abnormal); posture (1, normal; 2, hunched); movement (1, spontaneous; 2, only in the presence of light, sound or tactile stimuli; 3, no movement with stimuli); rectal temperature (1, normal; 2, above 37°C). Animals were euthanized if one of the following conditions was met: a score of 10 or above, a weight loss of greater than 25%; or a score of 5–10 with rectal temperature of greater than 41°C. Regular monitoring of the rats using this technique allowed early detection of meningitis.

Specimen collection

Animals were deeply anesthetized with a mixture of isoflurane and oxygen 24h after inoculation (non-implanted cohort), or once they developed clinical signs of meningitis (implanted cohort; Table 1). A 30 gauge needle with a 1 ml syringe was used to perform a cisternal puncture to aspirate the CSF^{7,15}. Blood (1 ml) was aspirated from an intracardiac puncture via a 23G needle and cultured using an automated blood culture system. Finally, bacterial swabs were taken from the left bulla using a strict aseptic technique.

Tissue preparation

Each animal was euthanized with a lethal dose of pentobarbitone sodium (120 mg/kg) and transcardially perfused with normal saline and 10% neutral buffered formalin (NBF) pH 7.4 at 4°C. The brain, meninges and the cochleae were harvested. Brain specimens including meninges were taken from 24 rats and were stored in 10% NBF for 48 hours then embedded in paraffin. The specimens were sectioned at 10 µm, stained with hematoxylin and eosin (H & E) or Gram stain. Cochleae of the 6 implanted rats were decalcified in a solution of 10%

ethylene diamine tetra-acetic acid in 0.1M phosphate buffer (pH 7.4), and then processed and embedded in Spurr's resin. The embedded cochleae were orientated in the mid-modiolar plane, and 2 sets of twenty-one 2- μ m sections were collected at 126 μ m intervals throughout the cochlea. One set of sections were stained with H & E and the other with Gram stain.

Examination of the specimens

The main outcome measures for the study were the clinical and histological detection of meningitis. CSF, blood culture and middle ear cultures were taken to detect the presence of bacteria. The cochlea and meninges were harvested for histological analysis and were used to confirm the culture results. Serotyping of *S. pneumoniae* isolated from the cultures was performed to ensure that the strain causing the pathology matched the inoculum. The presence of bacteria in the CSF was used to confirm the aetiology of meningitis. Because only a small volume of CSF was available from this species contamination is a potential problem. Therefore the histology of the brain was considered the gold standard test to detect meningitis.

Histological sections were examined under a light microscope. The brain and the meninges were examined for the presence of an inflammatory cell response within the subarachnoid space and brain tissue, as well as for thickening and hyperplasia of the meningeal cells, and Gram-positive cocci within the subarachnoid space and brain tissue. The implanted cochleae were examined for fibrous tissue response to cochlear implantation, and insertion trauma. All turns of both implanted and non-implanted cochleae were examined for the presence of inflammatory cells and bacteria.

Results

The eighteen non-implanted rats receiving either 1×10^6 CFU or 1×10^8 CFU of *S. pneumoniae* via three different routes of infection did not develop meningitis over a 24 hour monitoring period. The inner ear inoculum of 1×10^8 CFU in 1 μ l was too viscous to be infused using the microsyringe pump consequently no infection was observed in this group of rats (Table 1).

In contrast, all 6 rats with cochlear implants acquired meningitis when given the highest doses of bacterial inocula via the three different routes of infection. Clinical evidence of meningitis developed within 12–48 hours following inoculation and depended on the route of inoculation. In these animals the meninges were thickened with inflammatory cells and Gram-positive cocci were seen within the subarachnoid space (Fig. 2). The development of the infection in the central nervous system was successfully monitored and predicted by use of the daily clinical monitoring sheet. The histopathological evidence of meningitis correlated closely with the clinical symptoms. Two rats exhibited cerebral oedema and meningitis within 12 hours of i/p inoculation of 4×10^{10} CFU of *S. pneumoniae*; two animals exhibited clinical and histological evidence of meningitis within 24 hours following middle ear inoculation of 3×10^8 CFU of *S. pneumoniae*; and two animals exhibited clinical and histological evidence of meningitis 48 hours after direct inner ear inoculation of 1×10^6 CFU of *S. pneumoniae*.

In the implanted rats that developed meningitis as a result of bacteremia via i/p injection, bacteria were found along the perivascular and perineural spaces within the internal acoustic meatus (IAM) and modiolus bilaterally. Bacteria were also seen in the stria vascularis on both sides of cochleae. Although there was no evidence of inflammatory cells or bacteria in the scalae throughout the cochleae, bacteria were present in the lumen of the right stapedial artery and also found to infiltrate the thick, well-organized, arterial wall (Fig. 3).

In implanted rats with meningitis following a middle ear inoculation, bacteria and inflammatory cells were found within the dense, well-formed and matured peri-implant fibrous

seal (Fig 4). There were no bacteria or inflammatory cells in the interface between the fibrous seal and the implant. In the ipsilateral inner ear, bacteria and inflammatory cells were found within the scala tympani, scala vestibule, Rosenthal's canal and the canaliculi perforantes within the wall of osseous spiral laminae (OSL), adjacent to the scala tympani. Bacteria were located along the peri-neural and peri-vascular spaces within the OSL, modiolus and the internal acoustic meatus (IAM). Extensive degeneration of spiral ganglion cells were seen within the OSL infiltrated by bacteria and inflammatory cells (Fig. 5). Few bacteria were isolated within the scala media. Serofibrinous or proteinaceous exudate was seen in some sections of scalae tympani and vestibule, while endolymphatic hydrops was also evident in some sections.

The contralateral cochlea of animals that received a middle ear inoculation that resulted in meningitis exhibited bacteria and inflammatory cells within the IAM, modiolus and scala tympani. In the scala tympani, bacteria and inflammatory cells were prominent in the basal turn, very close to the undersurface of the OSL and infiltrating the contralateral round window membrane in some specimens (Fig. 6). In one animal, the cochlear aqueduct was examined in the contralateral cochlea; bacteria and inflammatory cells were found to traverse the aqueduct and within the scala tympani (Fig. 6).

In implanted rats with meningitis as a result of direct inner ear inoculation, bacteria and inflammatory cells were found to infiltrate within the fibrous tissue seal around the dummy implant. They also infiltrated the canaliculi perforantes of the OSL to enter Rosenthal's canal. The distribution of the bacteria and inflammatory cells in the ipsilateral and contralateral cochlea was similar to the middle ear inoculated cohort (Fig. 7).

Finally, the non-implanted/non-meningitic cohort exhibited a number of histological features that contrasted with the implanted/meningitic animals described above. First, there were no inflammatory cells or bacteria within the inner ears following i/p inoculation. Second, there was only a mild inflammatory response in the ipsilateral cochleae following middle ear inoculation (Fig. 8). Finally, although bacteria and inflammatory cells were seen in the basal turn scala tympani of cochleae subject to direct inner ear inoculation, the contralateral cochleae showed no evidence of infection (Fig. 8).

Discussion

The present study describes a new pneumococcal meningitis model we have established in rats using a clinically relevant strain of *S. pneumoniae*. The model is unique in that meningitis is achieved via three different routes of inoculation to resemble all potential routes of pneumococcal infection from the upper respiratory tract to the CNS. The histological pattern of spread of pneumococci from the inner ear to the CNS and then to the contralateral ear in the presence of a cochlear implant is delineated in this new model.

The cochlear pathophysiology of both anterograde (middle ear to inner ear) and retrograde (meninges to the inner ear) spread of infection is not well understood. The use of the Gram stain before (the un-implanted cohort) or following clinical evidence of meningitis (the implanted cohort) provides a means of identifying Gram positive pneumococci and to trace the possible route of spread of the bacteria.

In the two implanted rats that acquired early meningitis as a result of bacteremia following i/p inoculation, bacteria were found to locate symmetrically in the perivascular and perineural spaces of the IAM and modiolus. No bacteria were seen within the scalae of both cochleae - probably due to the early stage of the disease. Both the cochlear aqueduct and IAM have been proposed to be the potential routes of infection from the meninges to the inner ear based on post-mortem human temporal bone studies^{18–20}. Previous animal studies of meningitic

suppurative labyrinthitis also demonstrated the cochlear aqueduct as a pathway of infection from meninges to the labyrinth^{21–23}. Our study also demonstrates that bacteria can infect the inner ear via perivascular and perineural spaces of the IAM to the modiolus of the cochleae.

The histology of anterograde spread of infection from the middle ear to the meninges then to the contralateral cochlea has a distinct appearance compared to cochlear infection as a result of meningitis from a haematogenous route of infection. Specimens from rats with either direct inner ear inoculation or middle ear inoculation demonstrated a more severe degree of labyrinthitis in the ipsilateral cochlea compared to the contralateral cochlea. Bacteria were found to reach the subarachnoid space from the ipsilateral cochlea by traversing the canaliculi perforantes of OSL to enter neural structures within Rosenthal's canal and then following the perineural and perivascular spaces of cochlear nerve within the modiolus and IAM to reach the CNS. This finding is consistent with a previous study in the chinchilla in which the bacteria were inoculated directly into the middle ear cavity²⁴. However, bacteria can also reach the CNS and the contralateral cochlea via the cochlear aqueduct as observed in the present study (Fig. 6).

A well formed peri-implant fibrous seal was present around the electrode array and cochleostomy site 4 weeks after implantation consistent with previous animal studies⁵. The presence of bacteria infiltrating the well formed fibrous seal to enter the scala tympani suggests infection can spread from the middle ear into the inner ear. Histological evidence from the present study also indicated that in cases of severe infection, bacteria have the ability to invade and track through the thick, organized and condensed fibromuscular wall of the stapedial artery, suggesting that the body's natural fibrous seal may not be sufficient in preventing either inner ear or CNS infection following a severe middle ear infection.

There are several reasons why we consider the rat is a suitable animal model of pneumococcal meningitis post cochlear implantation: (i) cochlear implantation is possible¹⁷; (ii) the relatively low cost of the animals make it feasible to perform relatively large scale quantitative studies; (iii) previously established pneumococcal meningitis in infant and adult rats via direct intracisternal, i/p or nasopharyngeal inoculation^{7,15}; (iv) previously established pneumococcal otitis media models^{25,26}; and (v) the middle ear mucosa^{27,28} and function of the Eustachian tube^{29–31} resembles that of humans.

However, a rat model is only relevant if the animal can acquire meningitis with *S. pneumoniae*, preferably via the three possible infectious routes in order to resemble CNS infection in humans. An ideal strain of *S. pneumoniae* for the infection study should be able to induce disease both in humans and in rats. The route(s) by which infection spreads from upper respiratory tract mucosa (including middle ear cavity) to the CNS in rats, must also closely resemble that of a human infection. These two characteristics are important to ensure that the results obtained in the rat model are also clinically applicable. However, this model is developed in rodents with normal cochlea anatomy and is therefore not suitable to evaluate the risk of meningitis that is associated with an inner ear malformation.

The selection of the correct serotype of *S. pneumoniae* was essential for the establishment of the new rat model. The virulence of several pneumococcal serotypes has been found to be dissimilar between human and some animal species. Not all clinical CSF isolates can induce meningitis in rats²³. Most studies involving the use of type 3 and 6A *S. pneumoniae* in rats have shown that animals develop acute otitis media but not meningitis from direct middle ear inoculation³². Although Rodriguez et al²³ were able to show hematogenous pneumococcal meningitis in the infant rat following i/p inoculation of a *S. pneumoniae* type 6 clinical isolate, bacteremia and subsequent invasion of the central nervous system could not be achieved in adult rats from either i/p or intranasal inoculation¹⁵. The present study showed that *S.*

pneumoniae serotype 2 is a suitable strain because it was isolated from the cerebrospinal fluid of a child with meningitis and caused meningitis in implanted adult rats via three different methods of inoculation. Moreover, in the present model both the hematogenous and otologic routes can be studied, allowing us to determine whether an intervention such as cochlear implantation influences the risk of developing meningitis via either or both routes.

The variation in number of bacteria administered and the post-inoculation monitoring period reflects the fact that the prime purpose of this work was to successfully develop an appropriate animal model of meningitis. A post-inoculation monitoring period of 24 hours was initially selected for the non-implanted rat cohort in this study because this period has been widely used in previous studies¹⁻⁴. A small number of bacteria were present in the inner ear of non-implanted animals 24 hours after direct inner ear inoculation. Bacterial meningitis might have developed had the non-implanted animals been monitored for longer periods, although it is also possible that the animals could eliminate the bacteria in the inner ear following extended post-inoculation monitoring periods.

After failure to observe meningitis in any non-implanted animal after 24 hours of monitoring, we extended the work to include a worst case scenario i.e. implanted animals inoculated with the highest number of bacteria possible for each route of infection. We sought to adjust the monitoring period in the implanted animals so that they were sacrificed at the first clinical sign of meningitis or a period of 5 days (whichever came first). As a result, clinical evidence of meningitis was evident at 12, 24 and 48 hours post-inoculation, depending on the inoculation site. The meningitis model as illustrated in this study emphasized the importance of a longer duration (>24 hours) of monitoring following the inoculation. However, a prolonged period of infection should be avoided when taking the welfare of animals into consideration. We propose that all future studies using this model should extend the post-inoculation monitoring period to 5 days while ensuring rigorous monitoring so that the animal is sacrificed at the first clinical sign of meningitis.

Many cochlear implant recipients have pre-existing risk factors (e.g. cochlear malformation, skull base fractures) for meningitis. Therefore, it is difficult to determine whether the presence of the implant in isolation, or in combination with pre-existing risk factors, contributes to post implant meningitis⁵. The advantage of the animal model presented here is that the effect of cochlear implant *per se* on the risk of meningitis can be examined in healthy animals with no pre-existing risk factors in a control laboratory environment.

There are two limitations to the present study that must be taken into consideration. First, cochlear implant electrode arrays differ from the array modelled in the present study, however the surface of both consist mainly of medical grade silicone. Second, it is important to point out that the present meningitis model does not provide a valid model for an immature immune system found in young children; currently there is no other model that does this. However, it may be possible to use the proposed model in conjunction with the use of immunosuppressant drugs to model the immature immune system.

The small numbers of laboratory animals used in most previous infection studies has made it difficult to assess the relative efficacy of different surgical approaches in reducing the risk of cochlear infections. A quantitative study using this new rat model can overcome these limitations, and show cause and effect relationships. The development of a quantitative model using the rat will allow us to investigate whether cochlear implantation alters the risk of acquiring pneumococcal meningitis in healthy animals via both hematogenous and otologic routes. The model will provide a means to establish whether there is an association between infection risk and surgical trauma to the modiolus and osseous spiral lamina following cochlear implantation. A demonstration that intra-cochlear trauma predisposes to meningitis would have

implications for both the surgical technique of implantation and implant design. The effectiveness of the fibrous seal around the cochlear electrode to reduce the invasion of bacteria should also be examined further. It is clear from the present study that bacteria can track through the fibrous sheath. Modifications of the cochlear implant electrode array to prevent bacteria from tracking around the prosthesis into the inner ear and then into the CNS should also be further investigated.

Conclusion

An animal model in rats that allows quantitative assessment of CNS infection risk post cochlear implantation is described. Pneumococcal meningitis developed in these animals after inoculation of a clinical isolate of *S. pneumoniae* serotype 2, via the three most likely routes of infection: hematogenous spread, and direct spread from the middle or inner ear. This animal model will allow us to determine whether an intervention such as cochlear implantation influences the risk of developing meningitis and to develop intervention strategies to reduce the risk of infection associated with cochlear implants.

Acknowledgements

We would like to thank staff from the Departments of Otolaryngology, and Microbiology and Immunology, University of Melbourne and Bionic Ear Institute for their support and help in the research project. Helen Feng and Jin Xu for manufacturing the dummy implant; Prue Nielson and Maria Clarke for histology (Department of Otolaryngology); Dr. Sue Pierce for veterinary support and Elisa Borg for animal maintenance; Susie Germano and Kristy Azzopardi for preparation of the bacteria (Department of Microbiology and Immunology); and Dimitra Stathopoulos and Rachael Richardson for editorial comments.

References

1. FDA. Public health web notification: risk of bacterial meningitis in children with cochlear implants. Available at www.fda.gov/cdrh/safety/cochlear.html. Accessed March 14, 2006.
2. Reefhuis J, Honein MA, Whitney CG, et al. Risk of bacterial meningitis in children with cochlear implants. *N Engl J Med* 2003;349:435–445. [PubMed: 12890842]
3. Cohen NL, Roland JT Jr, Marrinan M. Meningitis in cochlear implant recipients : the north american experience. *Otol Neurotol* 2004;25:275–281. [PubMed: 15129105]
4. Meli DN, Christen S, Leib SL, Tauber MG. Current concepts in the pathogenesis of meningitis caused by *Streptococcus pneumoniae*. *Curr Opin Infect Dis* 2002;15:253–257. [PubMed: 12015459]
5. Clark, GM. Cochlear Implants Fundamentals & Application. New York: Springer-Verlag; 2003. p. 831
6. Ring A, Weiser JN, Tuomanen EI. Pneumococcal trafficking across the blood-brain barrier. Molecular analysis of a novel bidirectional pathway. *J Clin Invest* 1998;102:347–360. [PubMed: 9664076]
7. Vogel, U.; Frosch, M. Infant rat model of acute meningitis. In: Zak, O.; Sande, MA., editors. Handbook of animal models of infection : experimental models in antimicrobial chemotherapy. London: Academic Press; 1999. p. 619-626.
8. Dahm MC, Clark GM, Franz BK, Shepherd RK, Burton MJ, Robins-Browne R. Cochlear implantation in children: labyrinthitis following pneumococcal otitis media in unimplanted and implanted cat cochleas. *Acta Otolaryngol* 1994;114:620–625. [PubMed: 7879619]
9. Berkowitz RG, Franz B, Shepherd RK, Clark GM, Bloom D. Cochlear implant and otitis media: a pilot study to assess the feasibility of *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* infection in the cat. *J Otolaryngol Soc Aus* 1985;5:297–299.
10. McFarland J. Nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *JAMA* 1907;14:1176–1178.
11. Miles A, Misra SS. Miles and Misera technique. *J Hyg* 1938;38:372.
12. Hirst RA, Gosai B, Rutman A, Andrew PW, O'Callaghan C. *Streptococcus pneumoniae* damages the ciliated ependyma of the brain during meningitis. *Infec Immun* 2003;71:6095–6100. [PubMed: 14500537]

13. Kastenbauer S, Klein M, Koedel U, Pfister HW. Reactive nitrogen species contribute to blood-labyrinth barrier disruption in suppurative labyrinthitis complicating experimental pneumococcal meningitis in the rat. *Brain Res* 2001;904:208–217. [PubMed: 11406118]
14. Tsuboi Y, Kim Y, Paparella MM, Chen N, Schachern PA, Lin J. Pattern changes of mucin gene expression with pneumococcal otitis media. *Int J Pediatr Otorhinolaryngol* 2001;61:23–30. [PubMed: 11576628]
15. Townsend, GC.; Scheld, WM. Adult rat model of meningitis. In: Zak, O.; Sande, MA., editors. *Handbook of animal models of infection : experimental models in antimicrobial chemotherapy*. London: Academic Press; 1999. p. 627-629.
16. Strake JG, Mitten MJ, Ewing PJ, Alder JD. Model of *Streptococcus pneumoniae* meningitis in adult rats. *Lab Anim Sci* 1996;46:524–529. [PubMed: 8905585]
17. Lu W, Xu J, Shepherd RK. Cochlear implantation in rats: a new surgical approach. *Hearing Research* 2005;205:115–122. [PubMed: 15953521]
18. Igarashi M, Saito R, Alford R, Filippone M, Smith JA. Temporal bone findings in pneumococcal meningitis. *Arch Otolaryngol* 1974;99:79–83. [PubMed: 4809947]
19. Eavey RD, Gao YZ, Schuknecht HF, Gonzalez-Pineda M. Otologic features of bacterial meningitis of childhood. *J Pediatr* 1985;106:402–407. [PubMed: 3973777]
20. Schuknecht, HF. *Pathology of ear*. Malvern Pennsylvania: Lea & Febiger; 1993.
21. Kesser BW, Hashisaki GT, Spindel JH, Ruth RA, Scheld WM. Time course of hearing loss in an animal model of pneumococcal meningitis. *Otolaryngol Head Neck Surg* 1999;120:628–637. [PubMed: 10229585]
22. Bhatt SM, Lauretano A, Cabellos C, et al. Progression of hearing loss in experimental pneumococcal meningitis: correlation with cerebrospinal fluid cytochemistry. *J Infect Dis* 1993;167:675–683. [PubMed: 8440938]
23. Rodriguez AF, Kaplan SL, Hawkins EP, Mason EO Jr. Hematogenous pneumococcal meningitis in the infant rat: description of a model. *J Infect Dis* 1991;164:1207–1209. [PubMed: 1835472]
24. Schachern PA, Paparella MM, Hybertson R, Sano S, Duvall AJ 3rd. Bacterial tympanogenic labyrinthitis, meningitis, and sensorineural damage. *Arch Otolaryngol Head Neck Surg* 1992;118:53–57. [PubMed: 1728278]
25. Hermansson A, Emgard P, Prellner K, Hellstrom S. A rat model for pneumococcal otitis media. *Am J Otolaryngol* 1988;9:97–101. [PubMed: 3177766]
26. Hermansson A, Emgard P, Prellner K, Hellstrom S. A rat model for bacterial otitis media. *Acta Otolaryngol Suppl* 1989;457:144–147. [PubMed: 2929334]
27. Albiin N, Hellstrom S, Stenfors LE, Cerne A. Middle ear mucosa in rats and humans. *Ann Otol Rhinol Laryngol Suppl* 1986;126:2–15. [PubMed: 3094421]
28. Hellstrom S, Salen B, Stenfors LE. Anatomy of the rat middle ear. A study under the dissection microscope. *Acta Anat* 1982;112:346–352. [PubMed: 7113638]
29. White P, Hermansson A, Svinhufvud M. Surfactant and isoprenaline effect on eustachian tube opening in rats with acute otitis media. *Am J Otolaryngol* 1990;11:389–392. [PubMed: 2281840]
30. Albiin N, Hellstrom S, Salen B, Stenfors LE, Soderberg O. The anatomy of the eustachian tube in the rat: a macro- and microscopical study. *Anat Rec* 1983;207:513–521. [PubMed: 6650881]
31. Caye-Thomasen P, Tos M. Eustachian tube goblet cell density during and after acute otitis media caused by *Streptococcus pneumoniae*: a morphometric analysis. *Otol Neurotol* 2003;24:365–370. [PubMed: 12806285]
32. Prellner, K.; Hermansson, A.; White, P.; Melhus, A.; Briles, DE. Immunization and protection in pneumococcal otitis media studies in a rat model. In: Tomasz, A., editor. *Streptococcus pneumoniae molecular biology & mechanisms of disease*. New York: Mary Ann Liebert; 2000. p. 359-368.

Figure 1.

(a) Dimensions of dummy scala tympani electrode array. (b) Photomicrograph of a cochlea in spur resin showing a scala tympani electrode array inserted via a cochleostomy. ow: oval window with stapes footplate; rw: round window niche; S: Silicone coating; P: Polyimide tubing (Outer diameter of 0.10 mm and length 5.0 mm); ST: Silicone tubing. Scale bar: 0.15mm.

Figure 2.

Gram stain illustrating the meninges and subarachnoid space of (a) a normal control rat and (b) a rat with clinical evidence of meningitis induced by i/p inoculation. Gram positive cocci (arrows) and inflammatory cells with phagocytosed bacteria are located within the subarachnoid space. m: meninges; sb: subarachnoid space; cb: cerebral cortex. Scale bar: 10 μm.

Figure 3.

Lower power H & E photomicrographs illustrating the implanted (a) and contralateral control (b) cochleae of a rat 12 hours following i/p inoculation of 4×10^{10} CFU *S. pneumoniae*. This animal exhibited clinical and histological (CNS) evidence of meningitis. However the scalae of both cochleae were devoid of gross infection. Higher power photomicrograph of Gram stain from the modiolus (c), internal acoustic meatus (d) and the lateral wall of the scala media of the implanted cochlea (e), and the stapedial artery of the contralateral cochlea, (f) illustrates the presence of bacteria (arrows). The approximate location of the higher power micrographs (c–e) are illustrated in (a) and (b). bn: bone; sv: stria vascularis; lu: lumen of the stapedial artery; w: muscular wall of stapedial artery. Scale bar: (a) & (b) 200 μ m; (c–f) 10 μ m.

Figure 4.

Photomicrograph illustrating Gram stain sections of an implanted rat cochlea 24 hours following middle ear inoculation with 3×10^8 CFU *S. pneumoniae*. This animal exhibited clinical and histological (CNS) evidence of meningitis.

Insertion trauma to the osseous spiral lamina (arrow heads) resulted in an extensive spread of bacteria (arrows) into Rosenthal's canal (rc). (b) higher power micrograph of the same section showing invasion of the bacteria (arrows) into the fibrous tissue sheath surrounding the electrode array. im: scala tympani electrode array. Scale bar: 10 μ m.

Figure 5.

Lower power H & E photomicrographs illustrating the implanted (a) and contralateral control (b) cochleae of a rat 24 hours following middle ear inoculation of 3×10^8 CFU *S. pneumoniae* ipsilateral to the implanted cochlea. This animal exhibited clinical and histological (CNS) evidence of meningitis. In this example there is severe labyrinthitis throughout the implanted ear (a) while the contralateral cochlea exhibits evidence of infection mainly localized to the scala tympani of the basal turn (b). Higher power micrograph of Gram stain of the OS (c & d) and modiolus (e) of the implanted cochlea, and the internal acoustic meatus (f) of the contralateral cochlea. Extensive bacterial invasion of Rosenthal's canal (c; arrows) appears to be associated with bacteria traversing the canaliculi perforantes (d; double arrow). Bacteria

were also located within the modiolus of the implanted cochlea (e), and the internal acoustic meatus of the contralateral cochlea (f). The approximate location of the higher power micrographs (c–f) are illustrated in (a) and (b). sgn: spiral ganglion cells. bn: bone. Scale bar: (a) & (b) 200 μm ; (c–f) 10 μm .

Figure 6.

(a) low power photomicrograph illustrating a Gram stain section from the right cochlea of an implanted rat 24 hours following inoculation with 3×10^8 CFU *S. pneumoniae* via the left middle ear. This animal exhibited clinical and histological (CNS) evidence of meningitis. In this example bacteria were predominately present in the scala tympani of the basal turn (b). Bacteria were also evident in the cochlear aqueduct (ca), (d) modiolus (m), and were also observed (e) traversing the round window membrane (rwm). (f) normal control round window membrane is illustrated for comparison. st: scala tympani. Scale bar: (a) 200 μ m; (b) 100 μ m; (c–f) 10 μ m.

Figure 7. Lower power H & E photomicrographs illustrating the implanted (a) and contralateral control (b) cochleae of a rat 48 hours following direct inner ear inoculation of 1×10^6 CFU of *S. pneumoniae*. This animal exhibited clinical and histological (CNS) evidence of meningitis. Extensive labyrinthitis of the inoculated ear (a) involved all three scalae. In contrast, the contralateral cochlea (b) exhibited bacteria predominantly within the scala tympani (arrows). Scale bar: 200 μ m.

Figure 8.

Lower power H & E photomicrographs illustrating representative cochlear histology in unimplanted non-meningitic animals. Left (a) and right (b) cochleae 24 hours after inoculation with 1×10^8 CFU to the left middle ear. Inflammatory cells were evident in the round window niche (rwn) of the left cochlea resulting in a small inflammatory response (ir) within the basal turn. The remainder of the cochlea appeared normal as did the contralateral control. Left (c) and right (d) cochleae 24 hours after direct inner ear inoculation with 1×10^6 CFU *S. pneumoniae*. Bacteria and inflammatory cells were evident in the scala tympani of the basal and middle turns (arrows) however the remaining scalae and the contralateral cochleae appeared normal. Scale bar: 200 μ m.

Table 1

Routes of infection	Number of bacteria (CFU)*	Number of Rats	Observation Period (Hours)**	Blood Culture (#)	CSF culture (#)	Middle ear Swab (#)	CNS Histology (x)
Rats without cochlear implantation							
Intraperitoneal (hematogenous)	1 × 10 ⁶	3	24	3	2	0	0
Intraperitoneal (hematogenous)	1 × 10 ⁸	3	24	3	2	0	0
Middle ear	1 × 10 ⁶	3	24	3	1	3	0
Middle ear	1 × 10 ⁸	3	24	2	0	3	0
Inner ear	1 × 10 ⁶	3	24	3	3	3	0
Inner ear	1 × 10 ⁸	3	24	n/a	n/a	n/a	0
Rats with cochlear implantation							
Intraperitoneal (hematogenous)	4 × 10 ¹⁰	2	12	2	2	0	2
Middle ear	3 × 10 ⁸	2	24	2	2	2	2
Inner ear	1 × 10 ⁶	2	48	2	2	2	2

* The volume of inoculum for each route of infection was 1 ml for i/p inoculation, 10 µl for middle ear inoculation, and 1 µl for inner ear inoculation.

** In rats receiving a cochlear implant this time indicates the post-inoculation period when meningitis became clinically evident.

Number of animal with positive culture for *S. pneumoniae*

(x) Number of rats with brain histology consistent with pneumococcal meningitis, namely, the presence of inflammatory cells and Gram positive cocci within the subarachnoid space.

n/a Specimens for bacterial cultures were not collected as the inoculation of 1 × 10⁸ CFU of *S. pneumoniae* via the inner ear was unsuccessful. The inoculum was too viscous to be delivered using micro-infusion pump system.