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Protective effects of local administration of ciprofloxacin on the risk of pneumococcal meningitis following cochlear implantation

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Abstract

Objectives—To determine if ciprofloxacin retains its antimicrobial activity after storage with Healon® at ambient temperature and at 37°C over 5 weeks and then to establish whether the application of ciprofloxacin/Healon® onto scala tympani electrode arrays reduces the risk of meningitis in implanted rats inoculated with *S. pneumoniae*.

Study design—*in vitro* laboratory and *in vivo* animal studies

Methods—The antibacterial activity of three concentrations of ciprofloxacin/Healon® (7.5, 75 and 750 µg/ml) was examined over 5 weeks at both ambient temperature (23°C) and body temperature (37°C). Thirty-six rats (18 implanted with ciprofloxacin (750 mg/ml)/Healon®-coated electrode array and 18 without the coating) were infected with *S. pneumoniae* 4 weeks after implantation via three different routes of infection (hematogenous, middle ear and inner ear) and observed for the development of meningitis.

Results—The antibacterial activity of ciprofloxacin/Healon® was maintained over 5 weeks at both 23°C and 37°C. The implanted rats with the ciprofloxacin/Healon-coated electrode array were protected from meningitis when the bacteria were given via the hematogenous route (Fisher's exact test $P = 0.008$, but not when the bacteria were inoculated directly into the middle or inner ear. However, the time to develop meningitis was significantly longer in rats implanted with a coated array, irrespective of the route of inoculation ($P < 0.05$, log rank test).

Conclusion—Our animal model demonstrated that a ciprofloxacin-coated electrode array can protect healthy implanted rats from meningitis when the route of infection is hematogenous, and can delay the onset of meningitis when bacteria are inoculated directly into the middle or inner ear.

Keywords

Ciprofloxacin; Sodium hyaluronate; pneumococcal meningitis; cochlear implantation; *Streptococcus pneumoniae*

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Introduction

There has been an increase in the number of reported cases of cochlear implant related meningitis since 2002¹. Clinical studies have shown that meningitis occurring after cochlear implantation is more common than previously thought, and that most cases are caused by *S. pneumoniae*^{2,3}. In a controlled laboratory environment, the presence of a cochlear implant in healthy rats has been shown to reduce the threshold of bacteria required to induce pneumococcal meningitis⁴. Although the risk of meningitis following cochlear implantation is still extremely low, every effort should be made to minimize this risk. As one third of clinical reported cases of meningitis occur within 4 weeks of implantation^{1,2}, the use of a local antibacterial agent in conjunction with a carrying medium during cochlear implantation may reduce the risk of peri-operative infection.

Ciprofloxacin is a broad spectrum antibacterial agent that inhibits bacterial DNA-gyrase, an enzyme necessary for coiling the bacterial chromosome⁵. It was chosen for this study because it is relatively non toxic to the hair cells within the inner ear⁵⁻⁸ and is effective against a wide range of Gram-negative and Gram-positive bacteria including bacteria found in acute and chronic otitis media⁹⁻¹¹. However, there are no current data to determine whether ciprofloxacin will maintain antibacterial activity or a therapeutic level within the cochlea over a long period of time (> 4 weeks). Healon® is a sterile, non-immunogenic, non-inflammatory, non-pyrogenic viscoelastic preparation of a highly purified, high molecular weight fraction of sodium hyaluronate. Sodium hyaluronate is a physiological substance that is widely distributed in the extracellular matrix of connective tissues in both animals and humans. It is widely used during cochlear implantation as a lubricant on the electrode array allowing easier and deeper insertion of the electrode into the inner ear^{12,13}. Healon® does not cause any adverse effects to sensory epithelium¹⁴ or spiral ganglion¹⁵. Therefore, Healon® is a potential carrier medium for antimicrobial agents during cochlear implant surgery.

In this study, the antibacterial stability of ciprofloxacin/Healon® mixtures was examined *in vitro* over a period of 5 weeks. The substance was then tested in animals using a previously established model of pneumococcal meningitis in rats with cochlear implantation^{4,16}. As *S. pneumoniae* can reach the central nervous system from the middle ear mucosa via different routes^{4,16,17}, this study also examined whether a ciprofloxacin/Healon® mixture applied to the electrode array during implant surgery can reduce the risk of meningitis following *S. pneumoniae* inoculation by three different routes of infection: hematogenous, middle ear and inner ear.

Methods

In Vitro Healon® and Ciprofloxacin testing: Sample preparation and storage

To determine if ciprofloxacin retained its antimicrobial activity after storage with Healon® at ambient temperature (23°C) and body temperature (37°C), three samples containing different concentrations (7.5, 75 and 750 µg/ml) of ciprofloxacin (CiloQuin 0.3%, Ioquin, NSW, Australia) in 0.4 ml Healon® (Pharmacia, NSW, Australia) were prepared using sterile techniques in a laminar flow hood and stored in sterile polypropylene tubes for 5 weeks at 23°C or 37°C. The antimicrobial activity of the samples was assessed weekly for 5 weeks (described below) and the results were compared to freshly diluted control solutions of ciprofloxacin/Healon® and ciprofloxacin/water. The concentrations of ciprofloxacin used for this study were derived from previous studies and have been shown to have minimal effects on the hearing sensory neurons^{6,8,18}.

Analysis of antimicrobial activity

Disk diffusion tests were performed using the protocol recommended by the National Committee for Clinical Laboratory Standards (NCCLS) with *Escherichia coli* ATCC 25922 as the indicator organism.¹⁹ Horse blood agar (HBA) plates (Columbia agar base supplemented with 0.2% Special-Peptone, 0.1% Agar No.1, and 5% horse blood [Oxoid Ltd., Basingstoke, Hampshire, England]) were streaked with *E. coli* ATCC 25922 and incubated in air at 37°C overnight. The next day, 5 to 10 *E. coli* colonies were selected and suspended in Mueller-Hinton broth (Oxoid) at a density equivalent to 0.5 McFarland standard (approximately 1.5×10^8 colony-forming units [CFU]/ml). Plate counts were used to verify that bacterial numbers were within acceptable limits.

A sterile cotton swab was dipped into the bacterial suspension and used to streak several Mueller-Hinton agar plates to cover the entire surface of the plate with *E. coli*. Using sterile forceps, antibiotic susceptibility disks (Oxoid) impregnated with 10 µl of a test solution of ciprofloxacin in Healon®, were placed on duplicate agar plates. Control disks, impregnated with either Healon® or sterile water alone, were placed at the center of each plate. Plates were incubated in air at 37°C for 18 hours, and the diameter of the zone of inhibition (in millimeters) around each disk was measured. The antimicrobial activity of Healon®-ciprofloxacin was read off a standard curve prepared by triplicate measurements of the zone of growth inhibition around disks containing 9 different concentrations of ciprofloxacin, ranging from 7.5 to 750 µg/ml.

In vivo testing of Healon® and ciprofloxacin: Source of animals

In total, 36 otologically normal adult Hooded Wistar rats (10 to 16 weeks old) weighing between 100 and 450 g were used for this study. All of the experimental animals were bred and housed in the animal house within the Department of Otolaryngology, University of Melbourne, and were free of endogenous pathogens, including *S. pneumoniae*. All procedures and animal handling were conducted in accordance with guidelines set by the Animal Research & Ethics Committee of the Royal Victorian Eye and Ear Hospital and “The Australian code of practice for the care and use of animals for scientific purposes” from the Australian National Health and Medical Research Council 2004.

Surgical anesthesia

Rats were anesthetized with a mixture of 8 mg/kg xylazine (Ilium Xylazil-20®, Troy Laboratories Pty. Ltd. NSW, Australia) and 75 mg/kg ketamine hydrochloride (Ketamine®, Parnell Laboratories, NSW, Australia) injected intraperitoneally (IP). A local anesthetic agent (0.1 ml of lignocaine hydrochloride with 0.0182 mg/ml of adrenalin tartrate, Troy Laboratories, NSW, Australia) was injected subcutaneously (SC) around the surgical incision. The animals were then placed on a heated pad maintained at 37°C throughout the surgery. The animals were given 0.03–0.05 mg/kg SC buprenorphine (Temgesic®, Reckitt Benckiser, NSW, Australia) for analgesia immediately after surgery. They were assessed continually over 48 hours for signs of post-operative pain and discomfort, which was treated with buprenorphine 8 to 12 hourly when necessary. Animals received 10 ml/kg of normal saline SC for fluid replacement during recovery from the surgery.

Animal surgery: Scala tympani electrode array design and cochlear implant surgery

The dummy scala tympani electrode design for infection study in rats was established in our previous study^{4,16}. In brief, 4 mm of polyimide tubing (Cole-Parmer®, Illinois, USA) with outer diameter of 0.10 mm was coated with a layer of silicone (Dow Corning Medical Grade

Elastomer MDX4-4210, Factor II, AZ, USA) to a diameter of 0.15 mm. The distal 1 mm of the electrode was covered with PVC tubing as an aid to insertion allowing the surgeon to hold the dummy electrode array with forceps. The dummy electrodes were cleaned with absolute alcohol in an ultrasonic cleaner then rinsed with MilliQ water 3 times and bathed in MilliQ water for 10 minutes before drying, packaging and sterilizing using H₂O₂ sterilization (STERRAD® 100S).

Thirty-six adult Hooded Wistar rats were implanted in the left cochlea with a dummy electrode. One microliter of freshly made ciprofloxacin/Healon® mixture (750 µg/ml) was applied to the electrode surface of the implant before inserting into the left inner ear of 18 rats. This volume which was chosen not to exceed the inner ear volume as determined by the dimension of perilymphatic space in rats^{20,21}. The surgical insertion technique has been described in our earlier work^{4,16}. All animals had two doses of prophylactic enrofloxacin (Baytril 50®, Bayer Australia Ltd., NSW, Australia) 10 mg/kg SC diluted 1:1 with saline. One dose was given immediately after surgery and another 12 hours later.

Animal infection study

Four weeks after surgery, all 36 animals were infected with *S. pneumoniae* 447A, which carries type 2 capsular antigen. This strain was selected for this study based on our previous experience with it in a rat meningitis model^{4,16,17}. The preparation of the bacterial inoculum has been described previously^{4,16,17}. The size of the inoculum used for each route of inoculation was determined from our previous studies^{4,17}. Viable counts of the inoculum confirmed that each rat received the desired number of bacteria. The minimum inhibitory concentration of the test strain to ciprofloxacin was 0.5 – 1.0 µg/ml. and showed no increase in pneumococci re-isolated from infected rats.

Three routes of bacterial inoculation (IP, middle ear, inner ear) were used to study the effect of different routes of infection on the risk of meningitis in implanted rats with and without the application of ciprofloxacin/Healon® mixture to the dummy electrode. Bacteremia as a result of IP inoculation was introduced to study hematogenous spread of infection without the possible confounding effect of a direct invasion of the meninges via the inner ear following the middle ear infection. The methods of IP, middle and inner ear inoculation have been reported previously^{4,16,17}.

Post infection monitoring

Following inoculation, each animal was examined at least twice daily for clinical signs of meningitis over 5 days. Clinical assessment was recorded on a 12-point monitoring sheet described previously^{4,16,17}. Animals were euthanized if one of the following conditions was met: a score greater than 9; weight loss of greater than 25%; or a score of 5 – 9 with rectal temperature above 41°C. Animals without clinical evidence of meningitis were euthanized at the end of the fifth day after infection.

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 microscopy and culture as described previously^{4,16,17}. The serotype of *S. pneumoniae* isolated from the cultures was determined using commercial typing sera (Statens Serum Institute, Copenhagen, Denmark). Animals were then given a lethal IM dose of pentobarbitone sodium, 120 mg/kg body weight (Lethabarb®, Virbac Pty. Ltd. NSW, Australia) and were transcardially perfused with 0.9% saline, then 10% neutral buffered formalin (NBF) pH 7.4 at 4°C. The brain, meninges and cochleae were harvested and placed in 10% NBF for further processing.

Thirty-six brains with meninges were harvested, stored in 10% NBF for 48 hours and then embedded in paraffin. The specimens were sectioned, 10 μ m thick, stained with hematoxylin and eosin (H&E) and Gram stain and examined under light microscopy for presence of inflammation and bacteria.

Six pairs of randomly selected cochleae were harvested from the temporal bones and fixed in 10% NBF. 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's resin. The embedded cochleae were orientated in the mid-modiolar plane and two sets of twenty-one 2- μ m sections were collected at 126- μ m intervals throughout the cochlea. One set of 21 sections was stained with H&E and the other set with Gram stain. The brain and meninges were examined for the presence of inflammatory cell response and bacteria within the subarachnoid space and brain tissue, and thickening and hyperplasia of the meningeal cells. The cochleae were examined for the presence of bacteria and inflammatory cells.

Statistical analysis

Statistical analysis was performed using Prism version 4.02 and InStat version 3.05 (GraphPad Software Inc., Dan Diego, CA). A P value of less than 0.05 was considered to be significant.

Results

In vitro study

During *in vitro* testing of ciprofloxacin/Healon®, the three different concentrations of ciprofloxacin tested retained antibacterial activity over a 5 week period at both ambient (23°C) and body temperature (37°C). The highest concentration of ciprofloxacin (750 μ g/ml) was used in the subsequent *in vivo* testing as the aim of the study was to achieve maximum protection against pneumococcal meningitis in rats with a cochlear implant.

In vivo study

Rats implanted with a dummy electrode array coated with the ciprofloxacin/Healon® mixture (750 μ g/ml) remained healthy during the 4 week period prior to inoculation with *S. pneumoniae*. When rats developed signs of meningitis, they appeared tired, lethargic, unresponsive to stimulation by sound or light, exhibited a hunched body posture, poor grooming, weight loss and had a rectal temperature above 38°C. When these signs were present, the histology of the brain consistently showed evidence of meningitis with inflammatory cells and Gram-positive diplococci in the subarachnoid space. The correlation between the clinical signs and histopathological evidence of meningitis was established in our previous work^{4, 16,17}. All 18 implanted rats without the ciprofloxacin/Healon® coated electrode developed both clinical and histological evidence of meningitis regardless of the route of inoculation (Table 1). On the other hand, the preventative effect of ciprofloxacin/Healon® on meningitis was statistically significant in rats inoculated IP (P=0.008, Fisher's exact test, one-tailed), but was not significant in rats inoculated via the middle ear (P>0.2) or the inner ear (P=0.09) (Table 1). However, comparing to the non-treated cohort, the development of meningitis was significantly delayed in rats inoculated via the middle and inner ear. (P=0.04, log rank test; Fig. 1). The time required for implanted rats to develop meningitis for both the ciprofloxacin/Healon® treated and untreated groups is summarized in Figure 1.

Microbiology

The results of the microbiological examination of CSF, blood and middle ear swab are summarized in Table 2. All of the pneumococci isolated from rats were serotype 2, i.e., the

same as the initial inoculum, and their susceptibility to ciprofloxacin was the same as that of the strain used for inoculation (MIC= 0.5 to 1.0 µg/ml).

Cochlear histology

The pattern and distribution of bacteria and inflammatory cells within the cochleae of rats with clinical and histological evidence of meningitis were consistent with our previous studies^{4, 16,17}. In brief, Gram-positive cocci and inflammatory cells were present in both cochleae of rats with meningitis. The amount and distribution of the inflammatory cells were symmetrical in both cochleae in rats inoculated IP. However, in rats inoculated via the middle or inner ear, the distribution of the bacteria and inflammatory cells was asymmetrical in that labyrinthitis was more severe in the ipsilateral cochlea (Figure 2 and 3). When inflammation was present, more inflammatory cells were found in the scala tympani than in the scala vestibuli in the contralateral cochlea of rats receiving bacteria via the inner ear or middle ear. Inflammatory cells and bacteria were also found to infiltrate the fibrous connective tissue seal surrounding the intra-cochlear portion of the dummy electrode array in rats inoculated via the middle or inner ear (Figure 4). In IP-inoculated rats without meningitis, the histological appearance of the cochleae was normal (Figure 5). In rats without meningitis following the middle or inner ear inoculation, few inflammatory cells and serofibrinous exudates were seen in the basal turn of the ipsilateral cochleae. However, there were no signs of inflammation in the contralateral cochleae.

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

Discussion

This study demonstrated that the use of ciprofloxacin/Healon® mixture during cochlear implantation reduced the risk of pneumococcal meningitis especially when the bacteria were introduced into the blood circulation via an IP inoculation 4 weeks after implantation. Although no significant reduction in the incidence of meningitis was observed when bacteria were inoculated into implanted rats via the middle or inner ear, the onset of meningitis was significantly delayed in rats which received coated implants. A possible explanation for these findings is that the numbers of bacteria which reach the cochleae via the blood are likely to be lower after IP inoculation than when they are inoculated directly into the middle or inner ear. In addition, Healon® may not have been able to retain the ciprofloxacin at sufficiently high concentrations within the inner ear over a 4 week period, following dispersion of the drug through the perilymph and the CSF via the cochlear aqueduct or via the peri-neural and perivascular spaces within the inner ear. Accordingly, concentrations of ciprofloxacin which may be sufficient to protect against bacteria invading the blood-brain barrier from the systemic circulation may be inadequate when larger numbers of bacteria access the CSF via the cochlear aqueduct.

It is also likely that the presence of a foreign body such as an electrode array in the inner ear altered the antimicrobial susceptibility of *S. pneumoniae*. Although the in vitro susceptibility of the bacteria to ciprofloxacin was unchanged after infecting the rats, the antimicrobial susceptibility of bacteria may be reduced when they are in contact or in close proximity to a foreign body^{22–25}. For example, the susceptibility of *S. aureus* to antibiotics was reported to decrease by 2 to 10 fold when the bacteria were adherent to a silicone catheter²². The resistance observed was not genetic, or due to the presence of a resistant subpopulation of bacteria, as the

antibiotic resistance disappeared when the bacteria were removed from the foreign body, subcultured and retested^{22,23}.

Ciprofloxacin was chosen for this study because it is non-ototoxic and is active against a wide range of bacteria including *S. pneumoniae*⁵. Ciprofloxacin is not associated with any cochlear damage when administered systemically^{8,11,26–28}, and its local application to the round window membrane (RWM) in animals shows no evidence of ototoxicity^{5–8}. In chinchillas, approximately 1/600 of 0.1 mg/ml ciprofloxacin applied to the RWM enters the inner ear over a period of 75 minutes without any adverse effects to the sensory organs⁸. In guinea pigs, twice daily application of a much higher concentration of ciprofloxacin (7.5 mg/ml) over the RWM for 7 consecutive days showed no adverse morphological or electrophysiological effects in the inner ear⁶. The choice of a non-ototoxic antimicrobial agent is important as a number of cochlear implant recipients have some residual hearing. The concentrations of ciprofloxacin that were examined in this study were derived from previous animal studies with direct application of ciprofloxacin to the RWM^{6,8} and from a previous *in vitro* study with direct application of ciprofloxacin to the outer hair cells¹⁸. As expected, our *in vitro* experiment demonstrated that ciprofloxacin at a concentration of 750 µg/ml had the highest antibacterial activity and this concentration was subsequently used in the *in vivo* experiment.

Healon® was selected as the carrying medium because it is a non ototoxic agent and is widely used during cochlear implantation to aid insertion^{14,15}. The viscoelastic property of Healon® also may also improve the localization of drugs such as ciprofloxacin around the implant within the scala tympani. Our *in vitro* study showed that ciprofloxacin diffused much more slowly from the susceptibility disc into the surrounding agar when dissolved in Healon® compared with distilled water. This observation suggested that ciprofloxacin might bind to Healon® and be released gradually over time. However, the kinetics of ciprofloxacin release from the Healon® have not been studied in detail, and will require further testing if the Healon®/ciprofloxacin mixture is to be used in clinical settings.

It is important to point out that this study only examined *S. pneumoniae*, which is the predominant bacterial cause of post implant meningitis. The bacteriology of peri-operative meningitis (within 4 weeks of surgery) is different from meningitis occurring more than 4 weeks post surgery. Although *S. pneumoniae* has been isolated in patients with peri-operative meningitis, other bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Streptococcus mutans* have also been isolated^{1,2}. It is likely that these bacteria were introduced at the time of surgery, and that a ciprofloxacin-coated electrode array may also protect against meningitis caused by them, given their susceptibility to ciprofloxacin^{11, 28}.

The use of local antimicrobial agents to prevent infection due to foreign bodies is not a new concept. Many medical devices impregnated with antimicrobial agents are available commercially. The use of antibiotic loaded bone cement, for example, can prevent infection of implanted orthopedic prostheses^{29–32}, while intravascular catheters impregnated with antiseptics such as chlorhexidine and silver sulfadiazine reduces the risk of microbial colonization^{33,34} and catheter related blood stream infections³⁵. Similarly, the use of antibiotic impregnated CSF shunts reduces infection during the early postoperative period^{36–38} and for up to 3 years later^{37,38}.

The recent increase in reported cases of meningitis after cochlear implantation suggests that cochlear implants coated with antimicrobials may be needed to reduce the risk of meningitis, especially within the first for week of the postoperative period, when approximately one third of implant related meningitis occurs^{1,2}. Although this study suggests that coating the electrode array with ciprofloxacin may reduce risk of cochlear implant-related infection, further research

is required before our findings can be applied to cochlear implantation of human subjects. For example, research into the pharmacokinetics of locally delivered ciprofloxacin is required as it is possible that ciprofloxacin/Healon® mixture may not be confined within the scala tympani over a period of 4 weeks. Pharmacokinetic analysis of ciprofloxacin release from the surface of an electrode array is also required to determine if prolonged release of ciprofloxacin at clinically relevant concentrations is achieved during the peri-operative period. Surface modifications of the electrode array with impregnation of antimicrobial agents may overcome this problem. Although ciprofloxacin appears to be non toxic to the neural elements of the inner ear, further work is required to determine the maximum permissible dose of ciprofloxacin in the inner ear and central nervous system.

Conclusion

In an *in vitro* study of ciprofloxacin with Healon® as a carrier, we have demonstrated that ciprofloxacin retained its antimicrobial activity after storage at ambient temperature and at 37° C over a period of 5 weeks. The application of a ciprofloxacin/Healon® mixture during cochlear implantation in rats reduced the risk of pneumococcal meningitis when the bacteria were inoculated IP 4 weeks after implantation. However, coating the electrode array with ciprofloxacin/Healon® did not reduce the risk of meningitis when bacteria were given via the middle or inner ear, but it did significantly prolong the incubation period of implant-associated meningitis. Although there is some evidence to suggest that ciprofloxacin-coated electrode arrays may reduce the risk of peri-operative meningitis, further research is required before devices of this type can be applied in clinical practice.

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Figure 1.

The time required for implanted rats to develop meningitis for both the ciprofloxacin/Healon® treated and untreated groups. Cumulative mortality (in percentage) over 5 days of ciprofloxacin/Healon® treated and untreated rats were implanted with cochlear implants. Route of infection: (IP: intraperitoneal), (ME: middle ear), (IE: inner ear).

Figure 2.

Lower power H&E photomicrographs illustrating the implanted (a) cochlea with a ciprofloxacin/Healon® coated scala tympani electrode array and contralateral control (b) cochlea of a rat 108 hours following middle ear inoculation of 3×10^4 CFU *S. pneumoniae*. This animal exhibited clinical and histological (CNS) evidence of meningitis. In this example there is a severe labyrinthitis throughout the implanted ear (a) while the contralateral cochlea exhibited a less severe labyrinthitis (b). Scale bar: (a) & (b) 200 μ m.

Figure 3.

Lower power H&E photomicrographs illustrating the implanted (a) cochlea with a ciprofloxacin/Healon® coated scala tympani electrode array and contralateral control (b) cochlea of a rat 80 hours following inner ear inoculation of 1×10^3 CFU *S. pneumoniae*. This animal exhibited clinical and histological (CNS) evidence of meningitis. In this example there is a severe labyrinthitis throughout the implanted ear (a) while the contralateral cochlea exhibits a less severe evidence of infection (arrows)(b). Higher power photomicrographs of Gram stain of modiolus (c) of the ipsilateral cochlea and internal acoustic meatus (d) of the contralateral cochlea illustrates the presence of bacteria (arrows). The approximate location of the higher power micrographs (c,d) are illustrated in (a) and (b). bn: bone. Scale bar: (a) & (b) 200 μ m; (c) & (d) 10 μ m.

Figure 4.

Lower power H & E photomicrograph of a horizontal section taken at the level of the round window niche of the implanted left (a) cochlea with a ciprofloxacin/Healon® coated scala tympani electrode array of a rat 102 hours following middle ear inoculation of 3×10^4 CFU *S. pneumoniae*. This animal exhibited clinical and histological (CNS) evidence of meningitis. Higher power photomicrographs of Gram stain of the scala tympani at the location of the scala tympani electrode array (b & c) illustrates the presence of bacteria and inflammatory cells within (arrows) and surround (double arrows) the peri-implant fibrous seal. bn: bone; fas: fibrous plug; fs peri-implant fibrous seal. im: scala tympani electrode array; ic inflammatory cells. Scale bar: (a) 200 μ m; (b) & (c) 100 μ m.

Figure 5.

Lower power H & E photomicrographs illustrating the implanted (a) cochlea with a ciprofloxacin/Healon® coated scala tympani electrode array (im) and contralateral control (b) cochlea of a rat 120 hours following IP inoculation of 4×10^6 CFU *S. pneumoniae*. This animal did not exhibit clinical or histological (CNS) evidence of meningitis. The scalae of both cochleae were devoid of gross infection. Scale bar: (a) & (b) 200 μ m.

Table 1

Effects of ciprofloxacin/Healon® on the frequency of meningitis in rats with cochlear implants

Route of inoculation (number of colony-forming units of <i>S. pneumoniae</i> administered)	Implanted without ciprofloxacin/Healon®	Implanted with ciprofloxacin/Healon®
Intra-peritoneal (4×10^6)	6/6	1/6 [*]
Middle ear (3×10^4)	6/6	4/6 ^{NS}
Inner ear (1×10^3)	6/6	3/6 ^{NS}

* The protective effect of ciprofloxacin/Healon was statistically significant $P=0.008$ (Fisher's exact test, one-tailed)

NS = not significant ($P > 0.05$)

Table 2

Results of microbiological and pathological examination of rats with cochlear implants after inoculation with *S. pneumoniae* via three different routes.

Route of inoculation	Blood culture	CSF culture	Middle ear swab	Histologically- confirmed meningitis
Intraperitoneal :				
Without Ciprofloxacin/Healon®	5*	4	0	6
With Ciprofloxacin/Healon®	3	1	1	1
Middle ear :				
Without Ciprofloxacin/Healon®	6	4	6	6
With Ciprofloxacin/Healon®	5	4	6	4
Inner ear :				
Without Ciprofloxacin/Healon®	6	6	5	6
With Ciprofloxacin/Healon®	5	3	6	3

* No. of rats (of 6 inoculated with *S. pneumoniae*) with a positive culture at necropsy.