Duration of protective activity of cerebrospinal fluid shunt catheters impregnated with antimicrobial agents to prevent bacterial catheter-related infection

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This study determined the protective effect of antibacterial processing of cerebrospinal fluid (CSF) shunt catheters against infection with staphylococci, which is an important complication following CSF shunt placement for hydrocephalus. Also examined is the effect of a conditioning film such as that seen on the luminal surface of shunts used in posthemorrhagic hydrocephalus. Conventional preventative measures, including antimicrobial prophylaxis, confer a temporary or unproven benefit. The authors have therefore developed a process for impregnation of CSF shunts with rifampicin and clindamycin, and this has been shown previously to achieve the target duration of 28 days of protective activity in vitro. The present study demonstrates the limit of the period of protection and the efficacy of the processing against a wide range of staphylococci, particularly in the presence of a plasma protein conditioning film. Five strains of Staphylococcus aureus and 17 coagulase-negative staphylococci, all clinical isolates, were inoculated into the shunts at 2-week intervals until failure of antimicrobial protection occurred. The results showed that the process protected against all strains for between 42 and 56 days and that the conditioning film did not diminish the protection. Catheters processed by this method show promise of significant reductions in the incidence of CSF shunt infections.

KEY WORDS • hydrocephalus • shunt infection • prevention • antimicrobial impregnation

Despite Modifications in Surgical Technique, the Incidence of Cerebrospinal Fluid (CSF) Shunt Infections Remains Unacceptably High, Especially in the Neonatal Period. The majority of these infections are caused by staphylococci, with coagulase-negative staphylococci predominating. In most cases the organisms infiltrate the shunt during surgical insertion or revision. Despite claims made in many reports, there is no clear evidence that conventional perioperative an antimicrobial prophylaxis offers any benefit. We have therefore developed a process that has been shown to confer antimicrobial protection against coagulase-negative staphylococci for a target period of 28 days. The present study was designed to assess the duration of protective activity against a range of strains of Staphylococcus aureus and coagulase-negative staphylococci and to determine whether the processed catheters retained their activity in the presence of a protein conditioning film.

Clinical Material and Methods

Processing of Catheters

The antimicrobial drugs were previously selected on microbiological, toxicological, and physicochemical grounds from a short list of antistaphylococcal agents. One major criterion, apart from antibacterial spectrum, was the ability to diffuse through the molecular matrix of silicone elastomer, and candidate compounds not fulfilling this criterion included aminoglycosides (for example, gentamicin) and glycopeptides (for example, vancomycin). After further testing, singly and in combinations, the drugs chosen were clindamycin HCl and rifampin. These were dissolved in chloroform to yield a solution of 0.2% weight/volume of each agent. Shunt catheters were immersed in this solution for 1 hour at 18°C, washed in ethanol, and air dried. They were then packaged and autoclaved at 121°C for 15 minutes.

Test Organisms

Fourteen strains of S. epidermidis, two of S. hominis, one of S. haemolyticus, and five of S. aureus were speciated (API Staph; bioMérieux, Basingstoke, England). All were clinical isolates and all were susceptible to clindamycin HCl and rifampin on routine testing. Strains were stored at −20°C in cryoprotectant and before use were subcultured two times successively onto 7% horse blood agar (Oxoid Ltd., Basingstoke, England); they were then inoculated into brain-heart infusion (BHI, Oxoid Ltd.) and incubated overnight at 37°C.

In Vitro Test Model

Processed catheters and unprocessed controls were inserted aseptically into a modular multichamber test model (Fig. 1). The catheters were fixed in a vertical position to avoid interference from sedimneted rather than actively adherent bacteria. The shunts were constantly perfused with BHI at a rate of 20 ml/hour (a typical CSF production rate) and maintained at 37°C with 100% humidity at ambient PO2/PCO2. Medical grade silicone catheters (Silastic) were donated by Dow Corning, Maidenhead, England.

Bacterial Challenge Protocol

The initial target period for protection against bacterial colonization was set empirically at 28 days. At Day 1, catheters were challenged by injection of 1 ml of 10⁶ colony-forming units (cfu)/ml of...
which can be operated simultaneously and independently.

BHI culture and clamped for 1 hour. All connectors and connecting tubing between the test catheters and perfusion reservoirs were then changed aseptically to avoid spurious results from colonization of proximal tubing. The perfusion fluid in the reservoirs was monitored for retrograde contamination from the inoculum. The catheters were perfused continuously for 14 days with daily microbiological monitoring consisting of culture and microscopic examination of catheter effluents. If there was no evidence of colonization, the challenge was repeated followed by another 14 days of perfusion and monitoring. If no colonization was then evident, a third challenge was administered and the catheters were perfused and monitored for another 14 days, resulting in a 28-day challenge and perfusion period with a 14-day perfusion and monitoring period. The catheters were then removed aseptically from the module, their luminal contents were flushed and cultured, and segments were fixed for scanning electron microscopy.

Duration of Protective Activity

A second series of experiments was conducted with five strains of S. epidermidis and four strains of S. aureus, and the 14-day challenge was repeated until colonization occurred.

Conditioning Film

A third series of experiments was conducted using the same strains as in the second series; however, in this case a conditioning film was applied to the processed and control catheters before injection of the bacteria. The conditioning film was produced by immediately diluting freshly drawn human blood 1:10 in warm physiological saline to conserve clotting factors and injecting 1 ml of this solution into the test catheters. After clamping for 1 hour, perfusion was begun and the bacterial challenge was administered using the same 2-week challenge protocol until colonization occurred, at which time samples were fixed in preparation for scanning electron microscopy. Isolates from failed catheters were respeciated and their minimum inhibitory concentrations of clindamycin and rifampin were determined by E-test (AB Biodisk, Solna, Sweden).

Results

The 28-Day Challenge

The results of the 28-day challenge are shown in Table 1. All strains colonized unprocessed catheters within 48 hours of the first challenge (Fig. 2). The viable counts in the effluent fell from 10^8 at inoculation to between 10^4 and 10^5 at 24 hours and rose again to 10^8 at 48 hours. Previous experiments have shown that control catheters processed as described here but without antibiotic drugs are colonized at the same rate as the completely unprocessed controls used in this study. The same 17 strains of coagulase-negative staphylococci and five strains of S. aureus failed to colonize processed catheters even after three challenges over the target period of 28 days.

Duration of Protective Activity

The results of the duration of protective activity are shown in Table 2. Again, all strains colonized unprocessed control catheters within 48 hours. The processed catheters became colonized at a mean of 9 days (range 7–10 days) after the fifth challenge, which was administered 56 days after the initiation of constant perfusion. The viable cell counts fell from 10^8 at inoculation to between zero and less than 10^3 at 24 hours and remained at zero until the day

TABLE 1

<table>
<thead>
<tr>
<th>Challenge Strain (no.)</th>
<th>Catheter</th>
<th>Challenge†</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis</td>
<td>control</td>
<td>+</td>
</tr>
<tr>
<td>S. aureus</td>
<td>control</td>
<td>+</td>
</tr>
<tr>
<td>S. epidermidis (14)</td>
<td>processed</td>
<td>–</td>
</tr>
<tr>
<td>S. hominis (2)</td>
<td>processed</td>
<td>–</td>
</tr>
<tr>
<td>S. haemolyticus (1)</td>
<td>processed</td>
<td>–</td>
</tr>
<tr>
<td>S. aureus (5)</td>
<td>processed</td>
<td>–</td>
</tr>
</tbody>
</table>

* See Bacterial Challenge Protocol for details of procedures. Control catheters were inoculated at each challenge.
† Control shunts were colonized (+) within 48 hours of challenge. No colonization was evident (−) in processed catheters, that is, no growth in culture, including after dismantling, and no bacteria seen on scanning electron microscopy.
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Effect of Conditioning Film

The effects of conditioning film are shown in Table 3. Catheters were challenged with the same strains as those used to establish duration of activity. All strains colonized control catheters within 48 hours of challenge regardless of the presence of conditioning film. Processed catheters with a conditioning film (Fig. 3) showed the same duration of protective activity as those without (S. epidermidis, range 8–10 days, mean 9 days after fifth challenge; S. aureus, range 7–9 days, mean 8 days after fifth challenge). The viable counts showed the same pattern as in the experiments without conditioning film. One catheter (S. epidermidis, fifth challenge) was excluded because of contamination with Bacillus species.

Discussion

The most common cause of hydrocephalus in children is now periventricular hemorrhage following premature birth. The incidence of shunt infection is also highest in this group, and is thought to be a result of the long hospitalization period before shunt placement, allowing colonization of infants with nosocomial strains of staphylococci that have been shown to be more adherent and multiresistant to antimicrobial drugs than those found in older children and adults. Such infants also have significantly higher skin densities of these organisms.

The organisms that cause shunt infections infiltrate the shunt and the CSF at the time of implantation or revision of the device, and although some studies claim that the various prophylactic antimicrobial regimens in use are effective, a recent metaanalysis by a National Working Party has shown that there is no evidence to support this. In addition to the higher risk in the neonatal period, surgical facilities are not always ideal, and the incorporation of antimicrobial agents into the elastomer from which the shunts are made is an attractive option because it aims to prevent colonization even when large numbers of bacteria are allowed to reach the device. Apart from microbiological considerations, the incorporation process and the presence of the antimicrobial agents must not lead to deterioration in mechanical properties, biocompatibility, or shunt function. The incorporation of drug particles does not satisfy these requirements, but the process that we used resulted in the incorporation of the agents at a molecular level, with no significant effect on biocompatibility or shunt function at the concentrations used. Moreover, the antimicrobial agents are distributed throughout the elastomer matrix, and when the shunt is perfused a slow diffusion gradient is established that leads, after an initial peak release (probably from surface-bound drug) to a low-level release that declines very slowly over 12 to 15 months, giving long-lasting activity. However, it is important to note that release of drug does not necessarily equate with protection against infection; our results showed that after a period of between 42 and 56 days the protective activity had ceased. Duration of protective activity in excess of a few days is desirable in view of the possibility of shunt revision or other invasive procedures such as pressure measurement in the immediate postoperative period.

TABLE 2

<table>
<thead>
<tr>
<th>Challenge Strain (no.)</th>
<th>Catheter</th>
<th>Day of Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>control</td>
<td>+</td>
</tr>
<tr>
<td>S. aureus</td>
<td>control</td>
<td>+</td>
</tr>
<tr>
<td>S. epidermidis (5)</td>
<td>processed</td>
<td>-</td>
</tr>
<tr>
<td>S. aureus (4)</td>
<td>processed</td>
<td>-</td>
</tr>
</tbody>
</table>

* Each challenge with 10⁸ cfu/ml of BHI was followed by 14 days of perfusion and monitoring before the next challenge, and the procedure was repeated until the catheter was colonized. Control catheters were inoculated at each challenge. + = colonized within 48 hours of challenge; ++ = colonized within a mean of 9 days (range 7–10 days) of challenge; − = no colonization evident, that is, no growth at all in culture.
ery perfusion with BHI. Such an effect is seen in clinical practice, when apparent eradication of infection in CSF shunts by antimicrobial therapy is sometimes followed weeks later by relapse.\textsuperscript{18} In vivo, the surviving bacteria are exposed to CSF, which is normally poor in nutrients; the use of high-nutrient BHI is intended to speed up the recovery and regrowth of any surviving bacteria. In shunts in which failure did occur after the antibacterial activity had waned after 56 days, bacterial regrowth consistently occurred after 8 to 10 days. Previous experiments have shown that regrowth times are extended when artificial CSF rather than BHI is used, although the antibacterial activity and its duration are unchanged.

Third, the possible effect of deposition of conditioning film proteins on the catheter surface must be taken into account.\textsuperscript{13} This could affect adherence of challenge organisms,\textsuperscript{26} but it could also negate the protective effect of the process. Modifications intended to resist bacterial adherence to the biomaterial surface have not, to our knowledge, been tested in the presence of a conditioning film, although this rather than the modified biomaterial is usually the presenting surface in clinical use.\textsuperscript{24} Conditioning film proteins were included here in excess in both tests and controls as seen on scanning electron microscopy (Fig. 2), although no analysis of the conditioning film was performed. There was no difference in the timing or rate of colonization of control catheters, and the protective effect of the process and its duration were also not affected. This aspect might be particularly important because the highest rate of CSF shunt infection occurs in newborn infants, and the majority of cases of hydrocephalus occur in this group after periventricular hemorrhage. Although it was considered unsafe until recently to place shunts in these children while their CSF protein levels remained high due to hemorrhage, this has now been disproved.\textsuperscript{9–11} Processed shunts must therefore be shown to be effective even under conditions of high protein concentrations.

The shunt catheters were tested definitively in vitro using the model described rather than an animal model because the latter is probably unnecessary. Colonization of CSF shunts is initially confined to the lumen in most cases, and this can best be modeled in a controlled environment chamber such as the one used here. The main departure from our earlier studies is the use of BHI, an enriched perfusion medium, instead of CSF or its synthetic equivalent, which we have used previously for other purposes. The BHI was used to shorten the perfusion periods by speeding up bacterial recovery and growth. The use of synthetic CSF results in control catheter colonization becoming apparent after 7 to 10 days of perfusion (unpublished data), as opposed to 1 to 2 days with BHI. External bacterial colonization of shunts related to surgery, which is rare in most centers, is really a postoperative wound infection enhanced by the presence of biomaterial. Although it is possible that the process will also prevent external shunt infections, we did not set out to demonstrate this. Additionally, an in vitro model of device-related infection similar in principle to ours has been validated in vivo.\textsuperscript{7} Simple implantation of processed and control catheter segments into animal tissue would not allow the effects of long-term perfusion to be taken into account and would preclude testing of the lumen. Also, tissue-derived conditioning film would attach to the outer surface of the segments, but not to the lumen.

We are not aware of this process or these agents having been used elsewhere in precisely this way. Hampl, et al.,\textsuperscript{17} have reported the use of a shunt catheter processed in essentially the same way, but using rifampin alone. Apart from concerns about resistance arising from use of this antibiotic agent alone,\textsuperscript{19} we have shown a clear advantage in the use of two agents, and specifically rifampin in combination with clindamycin.\textsuperscript{4} Furthermore, according to the paper by Hampl, et al., they introduced 9% by weight of the agent into the catheters compared to 0.15% (clindamycin) and 0.054% (rifampin) in those used in our study (Johnson & Johnson Professional, Inc., data on file). Ideally, an active agent should be delivered by a diffusion gradient only at a rate sufficient to provide a protective antimicrobial concentration in the Nernst layer\textsuperscript{12,20} at the interface of the biomaterial and the host and beyond this.

Fig. 3. Left: Electron micrograph showing \textit{S. epidermidis} in an unprocessed catheter without conditioning film. Note slime production, represented by strands of dehydrated exopolymer between cells. Original magnification × 1800. Right: Electron micrograph showing \textit{S. aureus} in an unprocessed catheter with conditioning film. Note the irregular conditioning film on the silicone surface with cocci apparently attached to it. Original magnification × 18,000.
be diluted to a concentration too low to present problems of general or local toxicity. We have also shown previously that concentrations approximately 10 times higher than the normal therapeutic range used in hydrocephalus shunts, after impregnation with antimicrobials, can probably be used in high-risk patients with raised CSF pressure and shunt function, and shows no evidence of toxicity. Clinical trials are now in progress.

Acknowledgment

We are grateful to Johnson & Johnson Professional, Inc., Raynham, Massachusetts for permission to use data on file.

Disclosure

Dr. Bayston is the author of U.S. Patent PCT/US88/04102, assigned to Colorado Biomedical, Inc.

References


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TABLE 3

Serial challenge of control and processed catheters, establishing the limit of the protection period and determining the effect of conditioning film

<table>
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<th>Day of Challenge</th>
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