Mode of action of an antimicrobial biomaterial for use in hydrocephalus shunts

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Objectives: Infection remains a major complication of shunting for hydrocephalus. The causative bacteria, mainly staphylococci, gain access to the shunt at operation and colonize the shunt tubing. This leads to biofilm development requiring shunt removal. As there is no clear evidence to support antibiotic prophylaxis, we have developed an impregnated antimicrobial shunt material. This study aimed to elucidate its mode of action in terms of bacterial adherence and killing kinetics.

Methods: Plain and impregnated material, with and without plasma protein conditioning film, were exposed to Staphylococcus epidermidis. Bacterial adherence was determined by scanning electron microscopy, chemiluminescence and culture. Time taken to kill 100% of bacteria \( t_{K100} \) was determined by serial chemiluminescence, culture and the use of differential viability microscopy.

Results: The antimicrobial material did not reduce bacterial adherence. However, 100% of attached bacteria were killed in 48–52 h, even in the presence of a conditioning film.

Conclusions: Impregnated antimicrobial material is likely to reduce shunt infection rates significantly without the risks and side effects of systemic antibiotics.

Keywords: hydrocephalus shunt infections, prevention, catheters

Introduction

Since the introduction of shunting for the control of hydrocephalus over 40 years ago, infection has remained a major complication. Although rates have fallen, the average is 11% \(^1\) with a much higher rate in infants under 6 months of age (15%–25%) \(^2\) than in older children and adults (3%–5%). Most cases of hydrocephalus are diagnosed in infancy, and the major cause is currently periventricular haemorrhage due to prematurity. Shunts are valved catheters made from silicone elastomer, and are designed to control flow rate and therefore CSF pressure. They are totally inserted, draining CSF from the cerebral ventricles to the peritoneal cavity. Most infections are caused by coagulase-negative staphylococci derived mainly from the patient’s skin flora during surgery. \(^3\) Except for secondary infections due to skin erosion or wound breakdown, infection from other sources is extremely rare. Bacteria colonize the inner surfaces of the shunt tubing, leading to biofilm development. \(^4,5\) Infections are therefore difficult to treat without shunt removal and are associated with secondary complications including diminished mental acuity, \(^6,7\) as well as consuming health and social resources. In one study, prophylactic antibiotics were found to be used by ~85% of UK surgeons, although most used a cephalosporin administered intravenously, and effective CSF concentrations could not be expected to be achieved. \(^8\)

In the same study, a rigorous analysis of the literature revealed no clear evidence of their efficacy in reducing the infection rate. \(^8\) A more recent analysis has reached the same conclusion. \(^9\)

In view of this difficulty in bringing about a reduction in the high infection rate, we developed a process whereby a shunt catheter could be impregnated with antimicrobials after manufacture. \(^10\) We later showed that such catheters could resist bacterial colonization for up to 50 days in a stringent simulation model, even in the presence of a plasma protein conditioning film and under flow conditions. \(^11\) We then set out to determine how the antimicrobial catheters were able to exert their effect, and whether they inhibited bacterial adherence or killed adhered bacteria, and if so, on what time scale.

Materials and methods

Biomaterials and impregnation

Medical-grade barium-filled silicone elastomer sheet identical to the material used in shunt catheters and processed as described previously. \(^8\)
was donated by Codman and Shurtleff Inc. (Raynham, MA, USA). Briefly, the process consisted of expansion of the silicone matrix with chloroform in which the antimicrobial(s), in this case rifampicin and clindamycin hydrochloride, were dissolved, both at 2% w/v. The chloroform was then removed by evaporation leaving molecules of the antimicrobials distributed throughout the whole of the silicone, which was then sterilized by autoclaving. Discs of the processed sheet and unprocessed controls, 4.8 mm diameter and 0.45 mm thick, were cut aseptically. For application of the conditioning film, one series of discs was exposed to 50% fresh human plasma (National Blood Service, Sheffield, UK) at 37°C, with rocking for 1 h, then rinsed in PBS.

**Determination of bacterial adherence**

Both series of discs and unprocessed controls (with and without plasma) were then exposed for 1 h to a suspension of *Staphylococcus epidermidis*, APIStaph profile 6706113 (BioMérieux, Basingstoke, UK), isolated from a CSF shunt infection. For this purpose, the bacteria were grown to mid log phase on a shaker at 37°C for 5 h, washed once in PBS and adjusted to OD_{490} 0.6 (≈10^7 cfu/mL). After exposure to the bacterial suspension, the discs were gently rinsed in PBS. Three discs of each series were then sonicated (Ultrawave Ltd, Cardiff, UK) for 20 min at 50 Hz and the sonicates examined by chemiluminescence to determine the numbers of adhered bacteria. Briefly, 125 µL of sonicate was added to each of three wells of an opaque 96-well tray (Zeptogen Ltd, Middlesex, UK), and 125 µL of 2% trypytone soya broth (TSB) was added to three additional wells as a blank. One hundred and twenty-five microlitres of lysing agent (Bactolyse, Lumitech Ltd, Nottingham, UK) was then added to all wells and allowed to stand for 10 min at room temperature. The tray was then read in a luminometer (MicroLumat Plus LB96V, Berthold Technologies GmbH, Bad Wildbad, Germany). The assay detects bacterial ATP, present only in living cells. The chemiluminescence assay was calibrated by plate counting of the planktonic culture. Sonicates were also cultured semi-quantitatively.

**Determination of time to kill**

Triplets of discs were rinsed and sonicated at intervals and the sonicates analysed as above to determine the time taken to kill all the attached bacteria (t_{90}). The culture medium (2% TSB; Oxoid, Basingstoke, UK) was changed daily. Use of 2% TSB was adopted after investigation, to ensure that bacteria survived but did not multiply during the experiments, which lasted 3 days. Survival of the bacteria in 2% TSB for the duration of the experiments was confirmed by chemiluminescence and culture. Separate triplets of discs were retrieved at the same times and the attached bacteria examined microscopically in situ after staining with BacLight differential fluorescence viability stain (Molecular Probes Europe BV, Leiden, The Netherlands). Further discs were removed after 1 h exposure to the bacterial suspension, fixed in cold acetone and examined by scanning electron microscopy (SEM).

**Results**

The adherence of *S. epidermidis* to plain and processed silicone after a 1 h exposure, with and without plasma coating, is shown in Figure 1. Whereas, as expected, plasma coating increased bacterial attachment, there was no significant difference in attachment between plain and impregnated silicone. This can also be seen by SEM in Figure 2. Figure 3 shows the viability of adhered bacteria to impregnated silicone, with and without plasma coating, and the time taken for the number of detectable viable bacteria to fall to zero, as measured by chemiluminescence. The results were confirmed by culture, as shown in Figure 4. Control (plain) catheters showed no reduction in viability of attached bacteria over this period. The shapes of the graphs for culture and for chemiluminescence show some discrepancy around the 24–48 h point, probably due to clumping of the sonicated bacteria from the biomaterial. The mean time to kill all adhered *S. epidermidis* (t_{90}) was 50 h (48–52 h). This was confirmed by in situ differential fluorescence of attached bacteria (Figure 5). Further perfusion after this time showed no survivors. Plain, unimpregnated material showed no reduction in viability of attached bacteria over this period.

**Discussion**

Almost all infections in cerebrospinal fluid shunts occur at the time of insertion (or revision) and have been shown to be caused mainly by bacteria entering the incision from the patient’s skin during the procedure.3 The shunt and/or the ventricular CSF are then contaminated, resulting in colonization of the shunt lumen. Skin preparation is known to afford only temporary clearance of surface skin flora, and re-colonization occurs after about 15–20 min,12 resulting in contamination of the incision, drapes and instruments. Whereas this might be unimportant in, for instance, a laparotomy, in implant surgery it becomes a critical event. In tubular implants such as catheters, the inner surface offers an environment for bacterial colonization5–7 that is protected from the immune system. In cerebrospinal fluid shunts, it is this surface on which the vast majority of shunt infections begin, and any attempt at prevention must focus on this. Although antibiotic prophylaxis is widely used, there is little or no evidence of benefit. Most trials are under-powered or poorly designed, often with the use of historical controls, and the meta-analyses that have been conducted have themselves been criticized.8 Also, the antimicrobials most commonly used for this purpose, cephalosporins, do not give effective concentrations in the CSF in the absence of inflammation. An important additional point is that the current figures for incidence of shunt infection are largely drawn from centres where antibiotic prophylaxis is practised. We have therefore developed a process whereby shunt catheters can be impregnated with antimicrobials post-manufacture in an attempt to reduce the incidence of bacterial colonization. The process results in
The antimicrobials being distributed throughout the polymer matrix, and it is not merely a coating. Criteria for choice of antimicrobials included consideration of spectrum of antibacterial action, safety in clinical use, stability and physico-chemical characteristics, as well as rigorous pre-clinical testing. A combination of rifampicin and clindamycin hydrochloride emerged as being most suited to the purpose. As coatings can easily be obliterated by protein conditioning films, testing included this factor. Results showed that the conditioning film had no effect on antimicrobial activity or on the time taken to kill the adhered bacteria. This point is important in view of the intended clinical application. CSF proteins are deposited on the shunt material after implantation. Many recipients of CSF shunts are either premature infants with post-haemorrhagic hydrocephalus, or adults with subarachnoid haemorrhage or post-traumatic hydrocephalus. All these patients can be expected to have a raised CSF protein concentration and to deposit a conditioning film rapidly on the inner surface of the shunt. Premature infants with hydrocephalus also constitute the highest risk group for infection. Pre-clinical testing

Figure 2. SEM images showing adherence of *S. epidermidis* to plain (a and b) and impregnated (c and d) medical-grade silicone, coated with plasma conditioning film, demonstrating that bacteria adhere to both plain and impregnated silicone in similar numbers.

Figure 3. Time taken to kill all adhered bacteria ($t_k_{100}$) measured by chemiluminescence and expressed as RLU. Results were similar with and without conditioning film. The results show means of three experiments for each point. Error bars are not shown as S.E. did not exceed ±3%, with the exception of the 5 h readings where the S.E. was ±9.8%. Background (blank) readings were all ≤200 RLU. Control (plain) catheters showed no reduction in viability of adhered bacteria over this period.

Figure 4. Viable counts of adhered bacteria, which are shown as RLU in Figure 3. The semi-quantitative results show means of three experiments for each point. No bacteria could be cultured after 49 h. Control (plain) catheters showed no reduction in viability of adhered bacteria over this period.
of the impregnated shunt showed that it had a duration of protective activity of ≥50 days, even in the presence of conditioning film. However, no data were available on the mode of action of such antimicrobial biomaterials. Specifically, it was not known whether they reduced bacterial adhesion, or whether they killed bacteria after attachment, and if so how long they took to achieve 100% kill \((t_{K100})\). The results presented show that the impregnated material does not influence bacterial attachment; that it kills 100% of adhered bacteria even in the presence of a conditioning film; and that it takes between 48–52 h to do so. It is important to confirm that all attached bacteria are killed as anything less would constitute clinical failure, with perhaps delayed presentation of shunt infection. The length of time taken to kill all attached bacteria compared with the time taken to kill those in planktonic state is explained by the well-known phenotypic changes that bacteria undergo on attachment to surfaces. The slow kill of attached bacteria suggests another reason for failure of prophylactic antibiotics, as well as the failure of coated devices with a limited in vivo protective activity in other clinical settings.

Hampl et al. have described a rifampicin-impregnated shunt catheter, produced using the same process as that used here, but containing 9% w/w rifampicin. Crystals of the drug were demonstrated on the outer surface. The catheters in our study contained 0.054% and 0.1% rifampicin and clindamycin, respectively. The results shown here demonstrate that such very high concentrations of drug are not necessary to achieve \(t_{K100}\). Moreover, we have shown that concentrations >2% w/w result in deteriorating mechanical properties of the silicone. In addition, the use of rifampicin alone is likely to select for resistant mutants. The dual-drug principle applied in the catheters in our study, in which simultaneous mutations in genes coding for completely different targets (RNA polymerase and ribosomal binding site) are required for survival, is intended to make it extremely unlikely that resistant mutants would appear. The bacterial challenge used in the investigations reported here is high, and this would make detection and selection of rifampicin-resistant mutants more likely, but none was found. The microbial insult to the shunt is confined to the time of surgery, and no bacteria survive in the impregnated catheters. In addition, antimicrobial activity is confined to the Nernst layer at the shunt surface, and is dissipated rapidly beyond this. The antimicrobials are therefore unable to impact on the normal flora sites to select resistant strains. Systemically administered prophylactic antibiotics affect the patient and all the normal flora sites, resulting in side effects and risk of resistance, in the case of shunting without clinical benefit. We therefore consider that the use of silicone shunt catheters impregnated with rifampicin and clindamycin is likely to bring about a significant reduction in shunt infection, with minimal risk of side effects or development of resistance.

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References


