Reduced bacterial adhesion to hydrocephalus shunt catheters mediated by cerebrospinal fluid proteins

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Abstract

Background—Prosthetic infections are a major problem, requiring complex and lengthy management. The role of blood proteins in the pathogenesis of implant infection has been investigated, but research into the role of CSF proteins in shunt infections has not been undertaken, even though a high CSF protein has been assumed to increase the risk of such infections.

Methods—New shunt catheters were exposed to either CSF or individual protein solutions, and the numbers of radio-labelled staphylococci that adhered to them were compared with controls that had been exposed to saline only.

Results—A significant reduction in bacteria adhering to the test catheter was found in each instance. Furthermore, the CSF with the highest protein content, from a patient with intraventricular haemorrhage, had the greatest inhibitory effect on bacterial adhesion. The effect of the solutions on the hydrophobicity of the silicone rubber was also investigated. The silicone rubber was more hydrophilic, and bacterial adhesion was less, with solutions containing a higher protein content, and these findings were in keeping with the current theories on the mechanism of bacterial adhesion to polymers.

Conclusions—A high CSF protein content does not predispose to the development of shunt infections.

(J Neurol Neurosurg Psychiatry 1996;60:671–675)

Keywords: bacterial adhesion; staphylococci; silicone rubber; cerebrospinal fluid; hydrocephalus shunts

Infection is a frequent complication of shunt operations, with a reported incidence varying from 3-3% to 23% per operation in most hands, although very low rates (0-4%) have been reported by Choux and colleagues by the use of a meticulous surgical technique. Certain groups, particularly infants with posthaemorrhagic hydrocephalus, have been reported by some to have a higher incidence of shunt infection than others. These children often have raised CSF protein concentrations, and it has been assumed that this has increased the risk of shunt infection. However, most papers about shunt infections do not mention the preoperative CSF protein concentration, and the association between the two has not been studied.

There has also been concern that a high CSF protein content might impair shunt performance. However, recent work has shown that protein had a minimal effect on CSF viscosity, and did not impair the flow through shunts, and a high CSF protein content did not increase the risk of shunt complications in a prospective clinical study. Moreover, in one study the valve opening and closing pressures were lower when tested with solutions containing protein (up to 9 g/l) than when tested with saline. The role of CSF proteins in the pathogenesis of shunt infections remains to be determined, particularly as most shunt infections involve the lumen only, and not the outer surface.

A model for the study of catheter colonisation has been described, and used to study other aspects of shunt infection. Artificial CSF was originally used to perfuse the system, but the rate of colonisation was so low that this was abandoned in preference to conventional bacterial culture media. Furthermore, a perfusion model would not be suitable for experiments on a patient's CSF, as a sufficient volume would not be available. It was therefore decided to study the effect of CSF proteins on bacterial adhesion to shunt catheters, as this is the first stage in the pathogenesis of shunt infections. In vivo work has shown that infection is more likely with a greater inoculum of bacteria. Therefore, reducing the number of bacteria that adhere to shunts should lessen the risk of an infection developing.

We elected to study CSF from patients being shunted for congenital hydrocephalus, posthaemorrhagic hydrocephalus, and obstructive hydrocephalus due to tumour, together with CSF from a patient being treated for a shunt infection, to determine whether the patients' diagnoses would influence the results. Solutions of albumin and γ-globulin were included as the major CSF proteins. Fibrinogen and fibronectin have both been prominent in the work on blood proteins, and so were also included. Fibrinogen is absent from normal CSF, but was studied as it has been found in CSF after cerebral haemorrhage, and shunts are exposed to blood during insertion. Fibronectin is a glycoprotein that is involved in cell to cell adhesion, and is present in CSF in small amounts.

Hydrophobic interaction is thought to play a major part in the mechanism of bacterial adhesion to polymers, and so the effect of the solutions on the hydrophobicity of silicone rubber was also studied.
Materials and methods

SOLUTIONS
Sufficient CSF was obtained from four individual patients for use throughout the experiment. This was obtained at the time of shunt insertion, or from ventricular drains. Table 1 gives further details of the patients and the CSF composition. The total protein was assayed by spectrophotometry, and the albumin content was measured by electroph-immunoassay. The γ-globulin content was determined by agarose gel electrophoresis and scanning the pherogram with an EDC Scanner (Helena Laboratories, Beaumont, Texas, USA). Assays for CSF fibrinogen and fibronectin were not available.

The following high purity human proteins were purchased from Sigma Chemical Company (Poole, UK) and were made up in sterile phosphate buffered saline (PBS; Unipath, Basingstoke, UK) at pH 7.2 to: albumin, 150 mg/l; γ-globulin, 60–0 mg/l; fibrinogen, 5–0 mg/l¹⁰; and fibronectin, 2-4 mg/l.²¹

The protein solutions were prepared fresh for each experiment, whereas the CSF, being of limited volume, was stored at 4°C and reused. The control solution consisted of sterile PBS only. The degree of hydrophobicity between a solution and solid is measured by the angle of contact between them. The contact angles of the specimens to silicone rubber were measured using a Cahn Dynamic Contact Angle Analyser 312 (Cahn Instruments, Cerritos, California, USA).²⁸

BACTERIA
The bacteria consisted of one Staphylococcus aureus strain and 11 coagulase negative staphylococci. The ability of the bacteria to adhere to polystyrene had previously been measured using a standard laboratory test (Christensen’s test).²³ Two coagulase negative staphylococci were laboratory control organisms, which were weakly and strongly adherent to the polystyrene. The others were all from recent shunt infections. Overall, six bacteria were weakly adherent, two (including S aureus) had medium results, and three were strongly adherent.

RADIOLABELLING OF BACTERIA
Several colonies from a fresh culture of the test organism were inoculated into 5 ml nutrient broth (Lab M, Bury, UK) to which 3-7 MBq of ³H-methyl thymidine (Amersham International, Buckinghamshire, UK) were added. The bacteria were grown at 37°C for 24 hours with agitation. The organisms were extracted by centrifuging at 2800 rpm for 20 minutes at 4°C, washed twice with sterile PBS, and then resuspended in PBS. The concentration of the suspension was then adjusted to give an absorbance of 0.5 ± 0.05 at 350 nm. Tenfold serial dilutions were made, and 20 ml of each dilution were cultured on Columbia agar (Unipath). The plates were incubated overnight at 37°C, the colonies were counted, and the concentrations of the organisms in colony forming U/mm were calculated.

MEASUREMENT OF ADHERENT BACTERIA
Barium impregnated silicone rubber peritoneal catheters were donated by Phoenix Bioengineering Inc, Bridgeport, Pennsylvania, USA. A 3 cm section of catheter was tested with each solution. The catheter was divided into 1 cm lengths and opened longitudinally to increase the available surface area.

The catheter sections were incubated with either CSF, the control, or a protein solution, for 60 minutes at 37°C. They were washed in PBS and were incubated with the bacterial suspension for 60 minutes at 37°C with agitation. The catheter segments were washed in PBS and were then transferred to a scintillation vial. Adherent bacteria were dissolved using 1 ml of Optisolve (Pharmacia Wallac, Milton Keynes, UK) for 30 minutes, and the catheters were removed and discarded.

A 3 ml aliquot of Hi-Safe II scintillation fluid (Pharmacia Wallac) was added to the Optisolve and the activity was measured in a Wallac 1410 Liquid Scintillation Counter. The experiments were repeated three times for each organism against each solution.

Results
The effects of the solutions on bacterial adhesion were expressed as the ratio of the number of bacteria adherent to the test catheter compared with its control. All the CSF specimens and protein solutions inhibited bacterial adhesion, and this did not depend on the bacterial species or the result of Christensen’s adherence test.²⁷ This was analysed using Student’s paired tailed t test, combining the results from all organisms, and the reduction in adherence was significant in each instance (0.05 > P > 0.02 for albumin, γ-globulin, fibrinogen, CSF 3, and CSF 4; 0.02 > P > 0.01 for fibronectin, CSF 1, and CSF 2). Figure 1 (coagulase negative staphylococci) and fig 2 (S aureus) show the degree of inhibition of bacterial adhesion produced by each solution. The CSF had a greater inhibitory effect than the protein solutions, and specimens with a higher protein content had a greater effect than those with less total protein. The protein concentration was compared with the degree of reduction in bacterial adhesion using linear regression, and the effect was statistically significant for the coagulase negative staphylococci (P < 0.001), but not for S aureus (0.5 > P > 0.2). The solutions that caused the greatest reduction in bacterial adhesion also had the greatest effect in making the silicone rubber hydrophilic, as determined by a

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (months)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Total protein (g/l)</th>
<th>Albumin (g/l)</th>
<th>γ-Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF 1</td>
<td>78-2</td>
<td>M</td>
<td>Craniopharyngioma</td>
<td>0.31</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>CSF 2</td>
<td>11-2</td>
<td>F</td>
<td>Congenital hydrocephalus</td>
<td>0.14</td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>CSF 3</td>
<td>7-7</td>
<td>M</td>
<td>Shunt infection</td>
<td>1.42</td>
<td>0.64</td>
<td>0.40</td>
</tr>
<tr>
<td>CSF 4</td>
<td>9-9</td>
<td>F</td>
<td>Intraventricular haemorrhage</td>
<td>2.31</td>
<td>1.35</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Only CSF 3 was from a patient receiving antibiotics (intravenous cefuroxime and gentamicin for three days). The CSF antibiotic concentrations are not known.
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Figure 1 Effect of CSF and protein solutions on the adhesion of coagulate negative staphylococci to silicone rubber. The chart shows the mean and 95% CI for the effect on 11 bacteria tested three times with each specimen. Results are given as the ratio of the number of bacteria adherent to the test catheter compared with its control and expressed as a percentage. All solutions reduced the number of adherent bacteria, but the CSF had a greater effect than the protein solutions, and those with a higher protein content had a greater effect than those with less. Alb = albumin; Glob = γ-globulin; Fg = fibrinogen; Fn = fibronectin.

Figure 2 Effect of CSF and protein solutions on the adhesion of S. aureus to silicone rubber. The chart shows the mean and 95% CI for the three times the bacteria were tested with each specimen. Results are expressed in a similar manner to fig 1. Alb = albumin; Glob = γ-globulin; Fg = fibrinogen; Fn = fibronectin.

Figure 3 Effect of challenging CSF against the coagulate negative staphylococci that caused shunt infections in those patients. The chart shows the mean and 95% CI for the three times each bacterium was tested against the CSF associated with it, and the results are expressed in a similar manner to fig 1. The “shunt infection CSF” was obtained from a ventricular drain three days after shunt removal and the commencement of intravenous cefuroxime and gentamicin. The “intraventricular haemorrhage (IVH) CSF” was obtained via ventricular aspiration from a patient with posthaemorrhagic hydrocephalus. A shunt was inserted three weeks after this specimen was obtained and the patient developed a shunt infection two days later.

Table 2 Contact angles and surface tension of the experimental solutions

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Protein (g/l)</th>
<th>Surface tension (mN/m)</th>
<th>Contact angle (degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>nil</td>
<td>68.5</td>
<td>104.0</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>0.0005</td>
<td>62.1</td>
<td>101.0</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.0024</td>
<td>63.1</td>
<td>101.0</td>
</tr>
<tr>
<td>γ-Globulin</td>
<td>0.06</td>
<td>56.2</td>
<td>75.2</td>
</tr>
<tr>
<td>Tumour hydrocephalus (CSF 1)</td>
<td>0.11</td>
<td>52.6</td>
<td>81.8</td>
</tr>
<tr>
<td>Congenital hydrocephalus (CSF 2)</td>
<td>0.14</td>
<td>50.6</td>
<td>79.2</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.15</td>
<td>56.6</td>
<td>74.8</td>
</tr>
<tr>
<td>Shunt Infection (CSF 3)</td>
<td>1.42</td>
<td>49.0</td>
<td>71.1</td>
</tr>
<tr>
<td>Posthaemorrhagic hydrocephalus (CSF 4)</td>
<td>2.31</td>
<td>49.0</td>
<td>71.1</td>
</tr>
</tbody>
</table>

Discussion

The infection of prosthetic devices causes problems that are not encountered with other infectious diseases. The commonest bacteria involved do not normally produce infection in the absence of foreign materials, and a smaller number of other pathogenic bacteria can initiate an infection when prostheses are present. The infecting organisms are often resistant to antibiotics that would normally overcome them if the foreign material was absent, and there is evidence that some materials can impair host defences. It would therefore seem that a microenvironment is formed in the vicinity of an implant that favours the growth of infecting organisms.

Interest has been focused on whether host proteins aid this process by promoting the adherence of bacteria to implanted polymers, but to date only plasma proteins have been studied. Most workers have reported that pretreating catheters with plasma, or albumin in plasma concentrations, inhibited the adherence of staphylococci to a wide variety of polymers. This effect with albumin is important, as it is the protein that adsorbs most to hydrophobic polymers. Immunoglobulins have not been as widely studied, but have been shown to reduce the adhesion of coagulate negative staphylococci to Teflon catheters. The effect was less pronounced than with albumin, however.

There is agreement that plasma fibrinogen enhances S. aureus adhesion, but opinion differs about its effect on the adhesion of coagulate negative staphylococci. Only one group has reported unequivocal results—a negative correlation. Other groups have found strain dependent effects, with most strains being unaffected, some showing increased adherence and others reduced adhesion.

Opinion also differs about the influence of fibronectin on bacterial adhesion. Some have reported increased numbers of adherent bacteria, but one group found that the adhesion of S. epidermidis was reduced, and others have reported that this reduction was strain dependent. Others reported that fibronectin had a concentration dependent effect on S. epidermidis adhesion: plasma concentrations reduced adherence, but a 1% solution promoted adhesion. Adhesion of S. aureus was inhibited irrespective of concentration.
Our study has shown that all the protein solutions and CSF specimens studied inhibited the adhesion of staphylococci to silicone rubber. As there is a minimum number of bacteria necessary for the development of an infection, reducing the number of adherent bacteria should reduce the likelihood of a shunt infection developing. Our albumin and globulin results are consistent with the work on blood proteins, and, being the predominant CSF fractions, concur with our CSF results.

Our finding that *S. aureus* adhesion was inhibited by fibrinogen is contrary to all published reports, although the inhibitory effect was less than with the other solutions. This may be due to experimental error, but is unlikely as the finding was consistent over three experiments. Differences between *S. aureus* strains might be responsible; for example, surface proteins (protein-A, clumping factor) are thought to be important in adhesion of *S. aureus* to exogenous proteins, but are absent in some strains. The strain used in this study produced clumping factor, but its protein-A status was not known. Another possible explanation is a difference in technique—in particular, the use of a lower fibrinogen concentration. Solutions containing 35 mg/l and 4 g/l have been used by other groups, whereas 5 mg/l was more appropriate for studies on CSF. This might be too low to promote binding to the relevant receptors.

There have been other differences in technique that might have contributed to the inconsistent results, including different times of exposure to the protein (one to three hours) and bacterial suspensions (15 minutes to two hours), different numbers of washes (up to five), different methods of bacterial extraction (solvents or ultrasound), and ways of counting them (radio-labelling or viable counts). A criticism of our study might be the use of stored CSF and bacteria, but it was not possible to obtain fresh CSF from the same patient for each experiment, and CSF from different patients would not have the same composition. We have no information on the effect that storing CSF might have on the results. However, the bacteria were regularly typed, and a change in their biochemical patterns was not found. One patient in our study was receiving antibiotics for a shunt infection at the time that his CSF was taken. This would not have influenced the results as bacterial adhesion is a physicochemical process, dead bacteria being as adherent as living organisms.

Our study suggests that a high CSF protein content does not increase the risk of shunt infections, and this agrees with both prospective and retrospective clinical studies. Other reasons are therefore needed to account for the high shunt infection rates that some have reported in patients with intraventricular haemorrhage. One explanation is that they have often had invasive procedures (lumbar punctures, ventricular taps, external drains) to reduce their CSF protein concentration, and these are known to increase the shunt infection rate. They are also younger than other hydrocephalic patients, and, as they have a less mature immune system, are more prone to infection. In addition, they have usually been in hospital for the whole of their lives, and thus have a different skin flora to other infants, with possibly a greater incidence of more pathogenic organisms. The fact that they also have higher CSF protein content is thus probably coincidental.

The chief mechanism for the adhesion of coagulase negative staphylococci to polymers is considered to be hydrophobic interaction: hydrophobic bacteria adhering strongly to the most hydrophobic materials. Ludwicka and coworkers found that silicone rubber was the most hydrophobic of the 10 biomedical polymers that they studied, and this suggests that it is the most liable to infection. Our findings support this theory, as the solutions that led to the greatest reduction in bacterial adhesion also gave the least contact angles against silicone rubber (the material becomes the most hydrophilic). There are methods for determining the hydrophobicity of bacteria, but they were not available for this study.

Our study involved bacterial adhesion to silicone rubber only, as this is the principal material used in shunt construction. However, there are some shunt systems that use other materials, including polymers and stainless steel. Work has not been performed on how proteins affect bacterial adhesion to these materials, but groups that have studied the effect of blood on several different polymers have usually found similar results between them.

Research into the mechanisms of bacterial adhesion has led to attempts to reduce it by applying a coating to the catheters. Success in vitro has been reported with heparin, Hydrogel, and bacitracin A, which are all hydrophilic, but technical difficulties in the coating process have made a commercial application impractical. Host proteins have a similar effect, and if this could be exploited the incidence of shunt infection might be reduced.

Conclusions

Cerebrospinal fluid has an inhibitory effect on bacterial adhesion to silicone rubber, which seems to be due to rendering the material more hydrophilic. Thus a high CSF protein concentration itself should not increase the risk of shunt infections, although other factors in hyperproteinorrhagic patients might increase the risk of such infection.

We thank the Danzow family and the Wade Charitable Trust for financial assistance with the project, and the Phoenix Bioengineering Inc for the donation of the shunt catheters.

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