The Association Between Meningitis and Dural Puncture in Bacteremic Rats

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Clinicians have long been concerned that performance of spinal anesthesia during a period of bacteremia may result in the subsequent development of meningitis. In order to determine whether such an association exists, percutaneous dural puncture was performed in 40 animals during a period of Escherichia coli bacteremia. Twenty-four hours later, spinal fluid was obtained for final analysis by surgically draining the cisterna magna. Twelve animals had E. coli recovered from the surgically drained spinal fluid. Only animals with a circulating bacterial count of at least 50 CFU/ml developed meningitis. Microscopic examination of the brains and spinal cords of animals with infected cerebrospinal fluid showed evidence of central nervous system infection. Bacteremic animals not undergoing percutaneous dural puncture always had sterile spinal fluid (n = 40). Cisternal puncture in the absence of bacteremia did not result in infection (n = 30). Treatment with a single dose of gentamicin before the dural puncture eliminated the risk of meningitis after dural puncture in 30 bacteremic animals. These results demonstrate that dural puncture is associated with the development of meningitis in rats, provided the animals are bacteremic at the time of the puncture. However, antibiotic treatment before the dural puncture appears to eliminate this risk. (Key words: Anesthetic techniques, spinal bacteremia. Complications: meningitis.)

CLINICIANS have long suspected that performance of spinal anesthesia during a period of bacteremia could result in the subsequent development of meningitis. This impression is based largely on anecdotal reports of central nervous system infection following regional anesthesia.1–3 However, three separate clinical studies have provided differing conclusions regarding the association between diagnostic dural puncture performed during a period of bacteremia and meningitis.4–6

In order to determine whether such an association exists, we performed dural puncture in chronically bacteremic (Escherichia coli) rats, 24 h after which the spinal fluid was surgically drained and analyzed for evidence of infection.

Methods and Materials

This study was approved by the Animal Care Committee at the Oregon Health Sciences University. A well-described rodent model of chronic bacteremia was used in the present study.7 This model involves the creation of a subcutaneous abscess cavity infected by E. coli in order to produce a low-grade bacteremia. Male Sprague-Dawley rats were anesthetized by intramuscular injection of ketamine (50 mg/kg) and Xylazine (5 mg/kg). Next a subcutaneous cavity was formed by placing a sterile gauze sponge below the skin through a flank incision. After 3 days, the sterile subcutaneous cavity was inoculated percutaneously, using local anesthesia, with 10⁸ colony-forming units (CFU) of E. coli and Bacteroides fragilis. B. fragilis was included in the inoculum to aid in the maturation of the abscess cavity; it does not enter the circulation during periods of E. coli bacteremia.7

Inoculation of the abscess was repeated every other day throughout the period of bacteremia. Control animals were inoculated with sterile culture media, without bacteria. The E. coli bacteria used in the present study was obtained from the American Type Culture Collection (25922). An 18-h soy–tryptase broth culture was freshly prepared prior to each inoculation. All bacteriologic cultures and analyses performed during this study were conducted by certified technologists in the medical microbiology laboratories at Oregon Health Sciences University.

Twenty-four hours after the last inoculation, the animals were anesthetized, as before, and blood cultures were obtained from the femoral vessels to confirm E. coli bacteremia. Blood for culture (0.1 ml) was inoculated into a set of two culture bottles containing soy–tryptase broth with sodium polyanethanol sulfonate and cultured under standard conditions for as long as 7 days. Laboratory personnel examined both bottles daily for evidence of bacterial growth and performed subcultures routinely at 48 h or when signs of growth appeared. E. coli grow rapidly

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under these conditions, and the organisms were identified by colony morphology, gram stain characteristics, and standard biochemical tests. The numbers of bacteria were determined by counting CFU in serial dilutions using MacConkey–blood agar plates.

Using these techniques, approximately 50% of the inoculated animals had blood cultures positive for *E. coli* at the time of the initial percutaneous cisternal puncture, and only these animals were included in preparing the bacteremic study group (A, B, and D shown in table 1). The remainder of the animals had sterile blood cultures with the exception of two animals, in whom multiple organisms, consistent with contaminants, were cultured; samples from these animals were not included in the study. Blood cultures from two control animals were also contaminated, and samples from these animals were not included in the study results.

Fifteen minutes after obtaining the blood cultures, the anesthetized animals were placed in a neurosurgical head holder, and percutaneous cisternal puncture was performed under sterile conditions after shaving and draping the occiput and washing the skin with antiseptic solution. A 25-G needle (1 inch) with a glass capillary tube attached to the metal hub was advanced percutaneously through the cisternal membranes. Dural puncture was confirmed by the presence of a small amount (5–10 μl) of spinal fluid appearing in the attached capillary tube. In all study groups, this initial fluid was sterile following culture for 7 days on MacConkey–blood agar plates (see below). The appearance of blood-stained fluid was noted in four bacteremic and two nonbacteremic animals. Samples from these animals were included in the study analysis because blood-tinted spinal fluid is not infrequently obtained during spinal anesthesia. However, clotted blood was obtained in one bacteremic and two nonbacteremic animals, and these samples were excluded from the study.

Twenty-four hours after the initial cisternal puncture, the animals were anesthetized again, and under sterile conditions, spinal fluid was obtained by surgically exposing and draining the cisterna magna. Four samples of surgically drained spinal fluid from two bacteremic and three nonbacteremic rats were grossly contaminated with blood as a result of the surgical drainage procedure, and these animals were not included in the study. Although these surgically contaminated samples were not included in the study results, none of the samples grew *E. coli*.

The surgically drained spinal fluid was then cultured on MacConkey–blood agar plates under standard conditions and checked for bacterial growth daily for 7 days. Bacteria were identified as *E. coli* by colony morphology, gram stain characteristics, and standard biochemical tests. In order to determine the sensitivity of our assay, five animals had varying amounts of *E. coli* (10–10³ CFU) administered by injecting the bacteria through the intrathecally placed needle at the time of the initial percutaneous dural puncture. Twenty-four hours later, *E. coli* could be cultured from the surgically drained spinal fluid of animals receiving as few as 10 CFU.

In addition to *E. coli*, surgically drained spinal fluid from one bacteremic and two control animals grew multiple organisms consistent with contaminants. These animals were excluded from the study.

In order to test the effect of antibiotic treatment, 30 bacteremic animals received a single intraperitoneal injection of gentamicin (1.5 mg/kg) immediately after femoral blood culture sampling. Five additional gentamicin-treated animals had 10³ CFU of *E. coli* injected intrathecally in 10 μl of saline through the intrathecally placed needle at the time of percutaneous dural puncture. The remainder of the experiment was performed as before. The *E. coli* organism used in the present study was found susceptible to the antibiotic effect of gentamicin based on in vitro testing.

Some spinal fluid samples from each experimental group were pooled and analyzed for white cell count, protein content, and glucose concentration, using stan-

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Bacteremia,*</th>
<th>Gentamicin†</th>
<th>Dural Puncture</th>
<th>Cerebrospinal Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>40</td>
<td>40 ± 22 (5–100)</td>
<td>No</td>
<td>Yes</td>
<td>12/40§</td>
</tr>
<tr>
<td>B</td>
<td>40</td>
<td>48 ± 25 (2–100)</td>
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<td>0/40</td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>0 (0)</td>
<td>No</td>
<td>Yes</td>
<td>0/30</td>
</tr>
<tr>
<td>D</td>
<td>30</td>
<td>49 ± 35 (5–110)</td>
<td>Yes</td>
<td>Yes</td>
<td>0/30</td>
</tr>
</tbody>
</table>

n = Number of rats in each group.
* Data expressed as mean ± SD (range in parentheses).
† Gentamicin administered prior to dural puncture (see Materials and Methods).
‡ Data expressed as the number of animals with *E. coli* cultured from spinal fluid per total number of animals in that group.
§ P < 0.05 compared to other groups.
† Not statistically different compared to the bacteremic group undergoing cisternal puncture.
dard analytical techniques. Finally, at the time of surgical spinal fluid drainage, the site of the initial cisternal puncture was carefully examined for gross purulent exudate and the animals perfused transcardially with fixative. The brains and spinal cords then were removed for histologic examination for evidence of infection.

Data were analyzed using Fisher’s Exact Test, and P < 0.05 was considered statistically significant. The number of animals in each experimental group shown in table 1 is the number remaining after exclusion of contaminated samples (vida supra).

Results

Percutaneous cisternal dural puncture was performed in 40 animals during a period of E. coli bacteremia (table 1). After 24 h, spinal fluid was obtained for final analysis by surgically draining the cisterna magna. As shown in table 1 (group A), at this time 12 animals had E. coli recovered from the surgically drained spinal fluid. Only animals with a circulating bacterial count of at least 50 CFU/ml prior to the cisternal puncture developed meningitis. The glucose concentration decreased in infected surgically drained spinal fluid, whereas the white cell count and protein increased compared to those of control animals (data not shown). The presence of central nervous system infection in the animals with E. coli recovered in cerebrospinal fluid was confirmed by gross and microscopic examination of the brain and spinal cord. The dura of infected animals was tense, and purulent exudate was present on gross dissection. Microscopic examination revealed the presence of gram-negative rods as well as an intense neutrophilic exudate involving the substance of the brain, the leptomeninges, the subarachnoid space, and meningeal blood vessels. There was no evidence of gross purulence at the site of the initial percutaneous cisternal puncture. Four bacteremic animals had blood-tinged spinal fluid detected at the time of the initial percutaneous cisternal puncture, and none of these animals had E. coli cultured from the surgically drained fluid. As shown in table 1, bacteremic animals not undergoing percutaneous cisternal dural puncture (group B) always had sterile spinal fluid, and dural puncture in the absence of bacteremia did not result in infection (group C). Taken together, these results suggest that dural puncture is associated with the development of meningitis in rats, provided that the animals are bacteremic at the time of the puncture.

In contrast to the untreated bacteremic animals, none of the antibiotic treated animals had E. coli present in the surgically drained spinal fluid (table 1, group D). The degree of bacteremia at the time of dural puncture was similar in treated and untreated groups (table 1, groups A and D). Finally, control experiments showed that gentamicin treatment did not interfere with the recovery and growth of E. coli (10^8 CFU) intentionally injected intrathecally at the time of spinal fluid drainage (n = 5; data not shown).

Discussion

Clinicians have long suspected that an association exists between the performance of a lumbar puncture during a period of bacteremia and later development of meningitis. Anecdotal cases of meningitis after spinal anesthesia during a presumed period of bacteremia have been reported.1,2 However, modern spinal anesthesia has a well-documented history of safety, and infectious complications are extremely rare.3,4 Furthermore, several retrospective clinical studies have provided differing results regarding the risk of dural puncture during a period of bacteremia.5-6

The uncertainty surrounding the risk of dural puncture may be due in part to the fact that the development of meningitis requires many steps, only one of which involves the integrity of the meninges. Exactly how bacteria cross from the blood stream into the spinal fluid is not known. Survival of bacteria in the blood stream and spinal fluid, as well as the integrity of the immunologic system and the virulence and number of organisms involved, are all important factors in the development of meningitis. However, under the conditions of the present study, dural puncture was associated with the development of meningitis in rats, provided that the animals were bacteremic at the time of the puncture. A circulating bacterial count of 50 CFU/ml at the time of dural puncture was required to produce meningitis. Treatment with a single dose of gentamicin prior to the dural puncture appeared to eliminate the risk of meningitis. The dose of gentamicin administered in this study was similar to the clinically used dose of this antibiotic, and the absorption of gentamicin is rapid after intraperitoneal administration.10 Gentamicin may reduce the risk of infection after dural puncture by decreasing the bacterial count in the circulation to less than the required level, because this drug enters the spinal fluid poorly. Alternatively, gentamicin may enter the spinal fluid directly through the dural tear and inhibit bacterial growth within the spinal fluid.

Our results are consistent with the results of an early study by Petersdorf et al.,11 in which they found an association between meningitis and cisternal puncture performed during bacteremia in dogs. However, the dogs in that study were given lethal doses of intravenous Pneumococcus, and the acute bacteremia produced far exceeded clinically relevant levels. In contrast, the level of bacteremia produced in the present study was similar to clinically observed levels.12 In one study, patients with infective endocarditis were found to have a circulating bacterial count of 2–100 CFU/ml, and half of the patients had a
bacterial count of greater than 30 CFU/ml. This level is similar to the circulating bacterial count of 50 CFU/ml that was required to produce meningitis in our model.

Although animal models of disease permit careful control of experimental variables, clinical conditions cannot be duplicated exactly. The present experimental model differs in several ways from conditions encountered clinically. Although E. coli commonly causes bacteremia in surgical and obstetric patients, it is an uncommon cause of meningitis. In addition, the relative size of the dural tear produced by a 26-G needle in the present study is clearly greater in rats compared to that in humans. The cisternal site of dural puncture is not normally used in clinical anesthesia. Lastly, spinal anesthesia involves the injection of a local anesthetic, and these drugs have been reported to be bacteriostatic. These experimental differences render the clinical relevance of the animal data in the present study difficult to interpret. Despite these differences, our data suggest that dural puncture may be a risk factor for the development of meningitis.

However, several additional clinical factors may decrease the risk of infection after dural puncture. Treatment of febrile obstetric and surgical patients with antibiotics is common, and our data suggest that antibiotic treatment reduces the risk of dural puncture. Furthermore, the overall incidence of bacteremia, even in febrile surgical and obstetric patients is low, and the common organisms responsible are not virulent mediators of meningitis.

Our results do not permit conclusions to be drawn regarding the risk of meningitis after epidural anesthesia in bacteremic patients. In addition, our study did not address the risk of dural puncture in immunocompromised patients or virally infected patients.

In summary, these results demonstrate that dural puncture is associated with the development of meningitis in rats, provided the animals are bacteremic at the time of the puncture. However, antibiotic treatment prior to the dural puncture appears to eliminate this risk.

References