Antibiotic Prevention of Experimental Staphylococcal Meningitis

PETER KARVOUNIS, M.D., MATHEW SMITH, M.D., FRANK MAZZAPICA, B.S., GERALD FALK, B.S. AND HARRY H. LEVEEN, M.D.
Department of Surgery, Brooklyn Veterans Administration Hospital, and State University of New York, Downstate Medical Center, Brooklyn, New York

Postoperative infection in the neurosurgical patient is a serious complication often resulting in fatal meningitis or brain abscess. Further major surgery is often required. The incidence of infections in neurosurgical cases is not much different from that in general surgery. Coagulase-positive staphylococci are the most common infecting organisms. Burke\(^1\) has shown that antibiotics prevent soft tissue infections providing the drug is given within 3 hours of the bacterial inoculation. If there is a longer delay, the antibiotic loses its ability to prevent infection and merely modifies the subsequent course.

These data indicate that if antibiotics are to prevent an infection, they must be available to kill the bacteria before a biochemical lesion has been established. Thus, there is a valuable period during which prevention of infection is possible. However, the established duration of this period in soft tissue infections may be exceeded by the appreciable time necessary to establish effective bactericidal concentrations in the cerebrospinal fluid.

The statement that antibiotics do not penetrate the blood-brain barrier is only a half truth since factors of time and concentration are usually neglected. Radioactively-tagged penicillin does enter the cerebrospinal fluid slowly, but high concentrations in the cerebrospinal fluid are not achieved because the penicillin is actively removed from the fluid by a mechanism analogous to secretion.\(^4\)

High blood concentrations do elevate cerebrospinal fluid levels. Trauma and inflammatory changes alter the permeability of vessels and favor the passage of antibiotics from the blood to the cerebrospinal fluid.

Since even small traces of antibiotics influence the growth of bacteria, the efficacy of antibiotics in the prevention of infection requires therapeutic trial under optimal conditions. The ideal proof would be protection against otherwise fatal meningitis. To simulate the clinical problem, we have induced fatal meningitis experimentally by the intrathecal injection of coagulase-positive staphylococci, and have studied the effect of a suitable antibiotic on these animals.

Materials and Methods

A frozen tube of hemolytic *Staph. aureus*, coagulase-positive, phage type 75, 53, 54, 47, previously isolated from a patient with a wound infection, was thawed at room temperature. This organism was subcultured overnight in trypticase soy broth and the subcultured strain used to inoculate two bottles, each containing 100 ml of this media. These were incubated at 37° C for 16 hours and then centrifuged for 15 minutes at 3,000 rpm. After the supernatant fluid was decanted, the sedimented bacteria were pooled and re-suspended in 40 ml of sterile isotonic saline. Previously-plotted growth curves of this organism indicated that a 100-ml 16-hour broth culture yielded concentrations of approximately 100,000,000 viable bacteria per ml. The saline bacteria suspension was estimated to contain 500,000,000 viable organisms per ml. This estimate was checked by further serial dilutions of aliquots of the saline bacteria suspension. Dilutions of 1:1,000,000 and 1:10,000,000 were plated out and counted in duplicate. The average concentration of viable organisms was calculated at 585,000,000 per ml.

Twenty-four adult male albino rabbits weighing approximately 2½ kg each were used in the experiment. The rabbit was chosen because it is particularly easy to perform a lumbar puncture with it. Preliminary work on rabbits had shown that a subcutaneous inoculum of 250,000 organisms produced a small abscess at the injection site.
Four animals were given 75 mg/kg Nafcillin intramuscularly 24 hours prior to a 1 ml inoculum of the saline suspension of bacteria injected into the lumbar subarachnoid space. Four other animals received the same dose of intramuscular Nafcillin, but at the same time as the bacterial inoculations. Two other groups of four rabbits were similarly treated with the exception that 125 mg/kg of Nafcillin were given intravenously. A fifth group of four rabbits received 125 mg/kg Nafcillin intramuscularly just before spinal injection of 585,000,000 staphylococci.

A control group of four animals were given the same intrathecal bacterial inoculation (1 ml) but received no antibiotics.

Nafcillin was injected intramuscularly twice daily for 1 week at the initial dose level in all surviving animals except those in the control group. Surviving animals were sacrificed 2 weeks after the initiation of the experiment. All dead animals were autopsied and specimens of the meninges, brain, and blood were obtained for culture and histological study.

Results

All four animals that did not receive Nafcillin died within 48 hours, three within 24 hours. None of the 12 animals receiving Nafcillin simultaneously with the bacterial inoculum survived more than 72 hours. Six of these animals succumbed within 24 hours and five within 48 hours. Neither the route of antibiotic administration nor the size of the dose had any statistically significant effect on survival time. All of the animals that died during the course of the experiment showed symptoms as well as gross pus in all parts of the neuraxis. Even the meninges of the brain showed evidence of inflammation. Cultures of tissues in all these cases were bacteriologically positive for Staph. aureus. Microscopic examination of the tissue confirmed the presence of a suppurative meningitis. The blood cultures of these animals were uniformly negative.

All eight animals that were given Nafcillin 24 hours prior to the bacterial inoculation into the spinal subarachnoid space survived and were completely asymptomatic for the 2-week experimental period. These animals were sacrificed after 2 weeks. The gross and microscopic appearances of the brains and spinal cords were normal. Cultures of the cord, meninges, and brain were sterile.

Although we did not make quantitative measurements of drug concentration in the cerebrospinal fluid, we assume that the achievement of effective levels across the blood brain barrier explain the uniform survival in groups that received Nafcillin prior to the bacterial inoculation. From 3 to 5 times the recommended dosage of Nafcillin was used in all animals.

Discussion

Wright has reported that 5.7% of the intracranial procedures at the Massachusetts General Hospital from 1952 to 1963 developed wound sepsis. Most of these infections were caused by coagulase-producing staphylococci. In Cairn's series at Oxford, 8.5% of the patients became infected and all died as a result of this sepsis. Generally speaking, the mortality following neurosurgical infections has been high. A conservative estimate based on the study of published material indicates that 20% of infected cases succumb as a direct result of infection. Up to the present time, the systemic use of antibiotics postoperatively has not been effective. The prophylactic (preoperative) use of antibiotics deserves more serious consideration.

It is essential that all coagulase-producing staphylococci be susceptible to the antibiotic used. Unfortunately, penicillin levels are not easily achieved in the cerebrospinal fluid. Experimental studies with radioactively-tagged penicillin confirm the fact that there is diminished entry into the cerebrospinal fluid and demonstrate that there is active removal from the cerebrospinal fluid comparable to that observed in the renal tubules. Probenecid effectively raised the level of penicillin in the cerebrospinal fluid but reduced it in the brain tissue. The extra-cellular position of penicillin is the only concern in wound prophylaxis. Moreover, high plasm levels exceed the ability of the transport system to remove penicillin, and this can result in effective penicillin levels in the cerebrospinal fluid.

In our experiments, Nafcillin was chosen as an effective penicillinase-resistant penicillin. Sustained high blood levels may not be achieved after oral administration; therefore, the antibiotic must be administered by the parenteral route.

Our study confirms that animals can be protected from otherwise fatal staphylococcal infections of the central nervous system if an