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Clearance of Bacteria from Cerebrospinal Fluid to Blood in Experimental Meningitis

W. MICHAEL SCHELD, TAE-SUNG PARK, RALPH G. DACEY, H. RICHARD WINN, JOHN A. JANE, AND MERLE A. SANDE*

Departments of Medicine and Neurosurgery, University of Virginia School of Medicine, Charlottesville, Virginia 22908

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The occurrence and importance of secondary bacteremia in the pathogenesis of and response to therapy in meningitis is uncertain. *Streptococcus pneumoniae* type III was injected into the cerebrospinal fluid of the cisterna magna in anesthetized, curarized dogs, and sequential simultaneous samples were obtained from the superior sagittal sinus, cisterna magna, and peripheral blood. The results show that: (i) bacteria are rapidly transported from the cerebrospinal fluid to blood but only after active multiplication within the cerebrospinal fluid, and (ii) entrance into the blood from the cerebrospinal fluid occurs before the height of the febrile response or cerebrospinal fluid pleocytosis.

Bacteremia has been demonstrated in 40 to 96% of patients with meningitis upon admission to the hospital (1, 3, 5, 7, 12). The source of the bacteremia remains unclear, but may reflect a primary hematogenous seeding of the central nervous system, a seeding of the bloodstream from a central nervous system source, or a simultaneous spread from an infected source to both central nervous system and blood. The relative contribution of these mechanisms is not known.

Studies of bacterial concentrations in the cerebrospinal fluid (CSF) of children with meningitis (4, 5) have suggested that secondary bacteremia is common and dependent on bacterial multiplication within the CSF. Bacteremia was particularly frequent in those patients with higher bacterial titers within the CSF (5, 6). In previous experiments, bacteremia was documented in 40% of dogs at 24 h after intracisternal inoculation of pneumococci (11); however, the early time course and requirement for bacterial multiplication were not studied. Other studies in the infant rat model of meningitis (8, 10) have suggested that the bacteremia is primary and of central importance in the pathogenesis of meningitis.

The purposes of the present study were: (i) to sequentially analyze the occurrence of bacteremia in two sites, the superior sagittal sinus and peripheral blood, after intracisternal inoculation; and (ii) to relate these findings to the clinical course of the disease.

MATERIALS AND METHODS

An isolate of *Streptococcus pneumoniae* type III, previously characterized in this laboratory (13) and with demonstrable virulence for experimental animals, was incubated in Trypticase soy broth (Difco) plus 0.5% defibrinated sheep blood for 16 h at 35°C with 10% CO_2. After the erythrocytes were removed by centrifugation at 1,000 rpm for 5 min, the supernatant was collected and centrifuged at 3,000 rpm for 15 min. The pellet was washed twice in 0.9% NaCl, centrifuged, and suspended in 2 ml of 0.9% NaCl. This assured a consistent inoculum of 10^7 colony-forming units (CFU) per ml.

Animal model. Nine mongrel dogs (25 to 35 kg) were anesthetized with intravenous sodium pentobarbital (50 mg/kg), and their tracheas were intubated with a sterile cuffed endotracheal tube. A rectal probe was inserted to monitor body temperature continuously. The mean temperature at the time of inoculation was 39.5 ± 0.4°C; this increased to 41.6 ± 0.3°C at 6 to 8 h and was maintained in this range until sacrifice. Under sterile conditions, the femoral artery and vein were exposed and cannulated. The venous cannula was used for administration of drugs and Ringer lactate solution (30 to 50 cm^3/h). The arterial cannula was connected to a transducer to allow continuous recording of blood pressure. Blood pressures remained normal (approximately 140/90) until sacrifice but declined prior to death in those animals that died of meningitis before sacrifice. The animals were paralyzed with 0.1 mg of pancuronium per ml intravenously and mechanically ventilated. End tidal CO_2 was constantly recorded, and arterial blood gases were periodically obtained under sterile conditions from the arterial line and measured for PCO_2, PO_2, and pH. Arterial blood for culture was also withdrawn from this cannula. Respirations were adjusted to maintain PaO_2 greater than 100 mmHg (13.3 kPa) and $PaCO_2$ between 35 and 45 mmHg (4.7 to 6.0 kPa). Pentobarbital and pancuronium were supplemented as necessary.

The dogs were then placed in a prone position with the head held in slight flexion. Under sterile condi-

tions, a partial left occipital craniectomy was performed using a high-speed air drill to expose the left transverse sinus. The transverse sinus in dogs lies totally encased in bone.

The sinus was then cannulated with a heparinized no. 20 intracath. Heparin does not inhibit the growth of pneumococci under these experimental conditions. Removal of only the calvarium over the sinus, leaving the bony tentorium intact, prevented placement of the catheter into the brain or subarachnoid space. The catheter was advanced proximally so that its tip lay at the torcula. A no. 18 spinal needle was then introduced percutaneously into the cisterna magna.

Experimental design. Base-line samples were obtained of CSF (0.8 ml) of the cisterna magna, venous blood (1.0 ml) from the sagittal sinus, and arterial blood (1.0 ml) from the femoral artery. Then 1 ml of *S. pneumoniae* suspension (10^7 CFU) was injected intracisternally. Samples were obtained from the cisterna magna (0.2 ml), the sagittal sinus (1 ml), and the femoral artery (1 ml) at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, and 12 h after inoculation (or until death if earlier than 12 h). All samples were quantitatively titrated by twofold dilutions in 0.9% NaCl and plated in Trypticase soy agar pour plates containing 0.5% defibrinated sheep blood. All plates were counted after 18 h of incubation at 35°C with 10% CO_2.

RESULTS

Seven dogs survived the experimental conditions for longer than 8 h and are included for analysis (five survived the full length of the experiment, 12 h). All three locations sampled (cisterna magna, superior sagittal sinus, and femoral artery) were sterile at the time of intracisternal inoculation. After inoculation of $\log_{10}$ 7.0 *S. pneumoniae*, the mean ± standard deviation bacterial titer in the CSF at 15 min was $\log_{10}$ 5.775 ± 0.495 (Fig. 1). This mean titer gradually declined until a nadir was reached at 1.5 h of 4.031 ± 0.638. Between 1.5 and 3 h the titer increased until a plateau of approximately $\log_{10}$ 5.8 (Fig. 1; Table 1) was reached and maintained for the duration of the experiment (12 h). All samples from the superior sagittal sinus were sterile until 1.5 h, when one sample became positive (Fig. 1; Table 1). Between 2 and 7 h after inoculation, titers gradually increased from 10^1 to 10^2 CFU/ml of superior sagittal sinus blood. In individual animals, organisms were not found in the superior sagittal sinus until 0.75 to 5 h (mean 2.1 h) after the bacterial titer began to rise (multiplication) in the CSF. Pneumococci did not appear in the femoral arterial blood until 1.5 to 2 h after the organisms were present in the superior sagittal sinus (Fig. 1; Table 1). The mean ± standard deviation titer gradually increased to 1.014 ± 0.830 between 7 and 12 h after inoculation. Observations were extended for one animal until death at 21 h postinoculation. At that time, the CSF bacterial concentration was $\log_{10}$ 7.301, and $\log_{10}$ 4.654 was found in the femoral arterial blood.

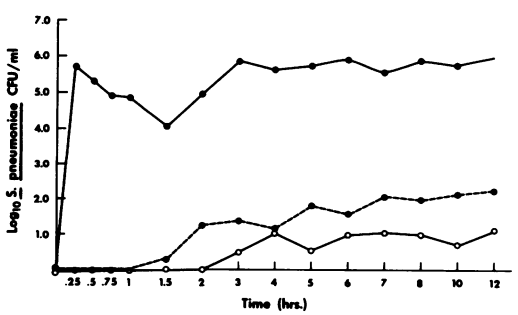

FIG. 1. *Change in bacterial titer with time after intracisternal inoculation. CSF (●——●), superior saggital sinus (●- - -●), femoral artery (○——○). Each point represents the mean of seven animals.*

All of the animals developed clinical signs of meningitis including chills, fever, and CSF pleocytosis. Neutrophils first appeared in the CSF within 30 min of inoculation (Table 1) and gradually increased in number. However, the peak CSF white count (mean ± standard deviation = 1,896 ± 312 cells/mm^3, with greater than 95% polymorphonuclear) and febrile response (mean ± standard deviation, 41.6 ± 0.3°C) did not develop until 6 to 8 h after inoculation, which was significantly later than the appearance of organisms in the superior sagittal sinus and peripheral blood.

DISCUSSION

This study demonstrates that secondary bacteremia occurs uniformly within 2 h after intracisternal inoculation of pneumococci in dogs. A previous study (11) obtained blood cultures only after 24 h when 40% were positive.

In the present study, organisms were detected in the superior sagittal sinus before the peripheral arterial blood, an observation which may be related to several factors. The concentration of organisms would be expected to be greatest in the sagittal sinus blood if bacterial invasion of the bloodstream from the subarachnoid space is via the arachnoid villi. Several investigators (2, 14) have demonstrated that particles as large as erythrocytes (7 μm) are cleared intact by bulk flow through transcellular fenestrae or "pores." However, once organisms gain entry into the sagittal sinus and then into the venous pool, their concentration would be greatly decreased by dilution and might become undetectable (less than 1/ml) in the peripheral blood. Two hours after infection, four of the seven dogs had sagittal sinus bacteremia (mean titers of >10 organisms per ml), but none of the animals had detectable arterial bacteremia. At 4 h all sagittal

TABLE 1. *Culture results and CSF pleocytosis in experimental pneumococcal meningitis*[a]

Time (h)	CSF			Superior saggital sinus		Femoral artery	
	% Positive cultures	Bacterial titer[b]	Leukocyte count (per mm^3)[c]	% Positive cultures	Bacterial titer[b]	% Positive cultures	Bacterial titer[b]
0	0	0	0	0	0	0	0
0.25	100	5.775 ± 0.495	0	0	0	0	0
0.5	100	5.335 ± 0.780	14 ± 12	0	0	0	0
0.75	100	4.911 ± 0.828	32 ± 16	0	0	0	0
1	100	4.905 ± 0.878	92 ± 34	0	0	0	0
1.5	100	4.031 ± 0.638	175 ± 46	14.3	0.301 ± 1.377	0	0
2	100	4.919 ± 0.912	748 ± 222	57.1	1.260 ± 0.956	0	0
3	100	5.890 ± 0.322	ND[d]	100	1.372 ± 0.621	28.6	0.556 ± 1.149
4	100	5.621 ± 0.531	1,330 ± 338	100	1.016 ± 0.531	71.4	1.008 ± 1.260
5	100	5.735 ± 0.166	ND	100	1.862 ± 0.789	100	0.621 ± 0.898
6	100	5.910 ± 0.268	1,896 ± 312	100	1.608 ± 0.647	100	0.992 ± 0.860
8	100	5.862 ± 0.478	ND	100	1.989 ± 0.721	100	1.012 ± 0.721
12	100	5.998 ± 0.620	1,523 ± 259	100	2.301 ± 0.677	100	1.168 ± 0.478

[a] $n = 7$, except for CSF leukocyte count, where $n = 5$.
[b] Mean $\log_{10}$ titer ± standard deviation.
[c] Mean ± standard deviation.
[d] ND, Not done.

sinus samples contained organisms at the same mean titer, and all femoral artery specimens were positive but at bacterial concentrations lower than those found in the sagittal sinus.

In addition to dilution by the peripheral blood, the delay in appearance of organisms in the peripheral blood could be accounted for by a clearance mechanism between the sagittal sinus and the arterial blood. The lung could serve this function, effectively clearing the pneumococci during the first several hours of low-grade bacteremia, but, after saturation, allowing systemic arterial bacteremia. Our data suggest that venous bacteremia of a specific degree ($\geq 10^1$ CFU/ml) and duration (>1.5 h) may be necessary to exceed the clearance mechanisms. A similar time course has been documented after intravenous injection of pneumococci in rabbits (15), an effect partially dependent on the "virulence" of the organism. After h 5 it appears that the filtering capacity of the lung has been exhausted, and mean bacterial titers in the peripheral blood remain approximately 1 log lower for the remainder of the experiment (7 h). This 1-log difference can be explained by dilution alone. Bacteria were not detected in either the sagittal sinus or the femoral artery blood until after active multiplication began in the CSF. Moreover, bacteremia was present, although at a low level, 4 to 6 h before the peak CSF pleocytosis and febrile response.

After intracisternal injection of $\log_{10}$ 7.0 *S. pneumoniae*, the initial titer at 15 min in the CSF was 5.775 ± 0.495 logs, a titer consistent with the dilutional effect of the total CSF volume within the subarachnoid space. The further reduction in viable bacterial counts over the next 90 min is likely due to bactericidal mechanisms operative within the CSF, especially polymorphonuclear pleocytosis and active phagocytosis. Although CSF white counts did not exceed 150 cells/mm^3 until 1.5 h after bacterial injection, active phagocytosis was suggested from Gram stains by demonstration of intraleukocytic bacteria obtained as early as 30 min. After 1.5 h, bacterial titers progressively rose, reflecting active bacterial multiplication within the CSF. Only after multiplication began were organisms detected in the superior sagittal sinus.

After intranasal instillation of *Haemophilus influenzae* in infant rats, the majority of animals develop meningitis (8–10). The bacteremia was considered primary because: (i) bacteremia always preceded the development of meningitis; (ii) titers of bacteremia in blood of $\geq 10^3$ CFU/ml were necessary, but not always sufficient, for the development of meningitis; (iii) meningitis did not occur without concomitant bacteremia; and (iv) bacterial titers in blood exceed those in CSF early in the infection, whereas this ratio is reversed after 3 to 4 days (8, 9). However, secondary bacteremia cannot be excluded since, as bacterial titers and CSF white counts rise in the subarachnoid space, meningitis increases in frequency, and high titers (approximately 10^5 CFU/ml) of bacteria are maintained in the blood, even 7 days after intranasal inoculation. This observation may represent continuous clearance of organisms from CSF to blood. The high titers (approximately 10^5 CFU/ml) of bacteria in the bloodstream in this model are greater than those found in this study (generally $\leq 10^2$

CFU/ml), which may reflect interspecies variation and differences in the time course of the experiments. Dogs are extremely susceptible to overwhelming pneumococcal infection (11), whereas the mortality rate is low in the infant rat model of *H. influenzae* meningitis (≤20%). In addition, bacterial titers exceeding 10^5 CFU/ml were usually not achieved until ≥24 h after inoculation in the latter model (8, 9). In the present study, one dog was followed until death at 21 h postinoculation. The degree of bacteremia in this animal (>10^4 CFU/ml) closely paralleled the other studies.

Studies in children with meningitis (4–6) also suggest the occurrence of secondary bacteremia. The frequency of bacteremia was directly related to the concentration of organisms within the CSF (5, 6), and was almost uniformly present above 10^6 CFU/ml of CSF. This study suggests that secondary bacteremia is a common occurrence in meningitis and is dependent upon active bacterial multiplication within the subarachnoid space.

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