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Antibiotic Therapy, Endotoxin Concentration in Cerebrospinal Fluid, and Brain Edema in Experimental *Escherichia coli* Meningitis in Rabbits

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We investigated the effect of cefotaxime and chloramphenicol on endotoxin concentrations in cerebrospinal fluid (CSF) and on the development of brain edema in rabbits with *Escherichia coli* meningitis. Both antibiotics were similarly effective in reducing bacterial titers. Cefotaxime, but not chloramphenicol, induced a marked increase of endotoxin in CSF, from $\log_{10}$ 1.5 ± 0.8 to $\log_{10}$ 2.8 ± 0.7 ng/ml ($P < .01$). This result was associated with an increase in brain water content (405 ± 12 g of water/100 g of dry weight compared with 389 ± 8 g in untreated controls; $P < .01$), whereas in animals treated with chloramphenicol, brain water content was identical to controls. The cefotaxime-induced increase in endotoxin concentration and brain edema were both neutralized by polymyxin B, which binds to the lipid A moiety of endotoxin, or by a monoclonal antibody to lipid A. These results indicate that treating gram-negative bacillary meningitis with selected antibiotics induces increased endotoxin concentrations in CSF that are associated with brain edema.

Bacterial meningitis remains a serious disease associated with high morbidity and mortality [1]. The infecting organism appears to be a major determinant of the prognosis [2], which is particularly grave in gram-negative bacillary meningitis [3, 4]. Liberation of harmful bacterial products as a consequence of treatment is a possible explanation for an adverse outcome despite effective antimicrobial therapy. In experimental meningitis, fragments of the pneumococcal cell wall can induce profound inflammation [5, 6], increased intracranial pressure, and brain edema [7]. Furthermore, in vitro and in vivo experiments have demonstrated that some antibiotics liberate large amounts of endotoxin from gram-negative bacteria [8–10]. Endotoxin has been clearly linked to many of the systemic and local complications of gram-negative infections [11]. In gram-negative bacterial meningitis, endotoxin is consistently found in CSF at the time of diagnosis [12]. During therapy, endotoxin concentrations in the CSF decline progressively over a period of days, but the possibility of an initial rise of endotoxin, after instituting antibiotics, has not been excluded [12].

Endotoxin release from bacteria into CSF during treatment of gram-negative bacillary meningitis may induce harmful effects on the brain. The present studies were designed to determine whether antibiotic treatment induces an increase of endotoxin in CSF and whether this increase is associated with harmful pathophysiological effects in a model of *Escherichia coli* meningitis. Results demonstrate that endotoxin concentrations increased with one antibiotic but not a second antibiotic and that this increase was associated with the development of brain edema. This effect was aborted by polymyxin B, which binds to the active moiety of endotoxin (lipid A), and by monoclonal antibodies to lipid A.

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Materials and Methods

Infecting organism. A K1-positive, serum-resistant strain of *E. coli*, originally isolated from a neonate with meningitis, was used. The organism was stored on glass beads at −70 C, grown overnight in tryptic-soy broth, and then washed and diluted in saline to the desired concentration. MIC/MBCs

of the organism were 0.125/0.125 μg/ml for cefotaxime and 8/>64 μg/ml for chloramphenicol [13].

Model of experimental meningitis. We used a rabbit model of experimental meningitis, which was originally described by Dacey and Sande [14]. In brief, New Zealand white rabbits, 2–3 kg, were anesthetized iv with 30 mg of pentobarbital/kg (Carter-Glogau Laboratories, Glendale, Ariz). A dental acrylic helmet was attached to the skull by using four screws; the helmet allowed the animals to be placed in stereotactic frames constructed to facilitate puncture of the cisterna magna. Three days after attachment of the helmet, the animals were again anesthetized with pentobarbital, and 5–7 × 10^5 cfu of logarithmically growing *E. coli* suspended in 0.3 ml of saline were injected into the cisterna magna by using a spinal needle (3.5 inch, 25 gauge; Becton, Dickinson and Co., Rutherford, NJ). Sixteen hours later, long-lasting anesthesia was induced by iv infusion of 2 g of urethane/kg (Sigma, St. Louis). The animals were placed in the stereotactic frames, the cisterna was punctured to collect the first sample of 0.3 ml of CSF, and treatment was started through an iv line placed in a peripheral ear vein. On individual days, groups of six rabbits were examined. The animals were usually assigned to at least three different treatment or control regimens.

Treatment regimens. Antibiotic therapy consisted of a 3-hr constant infusion, beginning 16 hr after infection, with cefotaxime or chloramphenicol. Infusions were started with a bolus injection equivalent to the amount of drug infused per hour. Antibiotics were administered in doses that would achieve the high CSF concentrations necessary for a rapidly bactericidal action [13, 15]: cefotaxime was infused at a dosage of 100 mg/kg per hr and resulted in CSF steady-state concentrations of ~10–15 μg/ml [13]; chloramphenicol was administered at a dosage of 60 mg/kg per hr and resulted in CSF concentrations of ~12 μg/ml [13, 16].

Neutralization of endotoxin in CSF. We examined the neutralization of endotoxin in CSF by polymyxin B, which binds nonspecifically to the lipid A moiety of endotoxin [17], and by a monoclonal antibody directed against lipid A. Starting 16 hr after infection, polymyxin B was infused for 3 hr at a dosage of 5,000 U/kg per hr, either simultaneously with antibiotic treatment (cefotaxime) or alone. This dosage was chosen because it produced CSF concentrations (4.0 ± 1.0 mg/liter) that were effective in reducing the endotoxin activity without reducing the CSF bacterial titers.

The monoclonal antibody was produced by fusion of an Epstein-Barr virus–transformed peripheral monocyte with a human fusion partner. Details of the generation and characterization are described elsewhere [17a]. The IgM antibody was found to bind to lipid A of all 15 strains of *E. coli* and *Salmonella* tested, including the organism used in this study. The antibody was administered either alone or simultaneously with antibiotic treatment (cefotaxime). Approximately 100 μg of antibody was injected twice intracisternally in 0.3 ml of PBS, 16 and 17.5 hr after infection. This quantity of antibody was chosen because it produced concentrations in CSF that, on the basis of in vitro results, would be sufficient to neutralize the amount of endotoxin released by the *E. coli.*

CSF examination. Bacterial titers in CSF were determined by overnight incubation of serial dilutions on blood agar plates. CSF endotoxin concentrations were determined in twofold serial dilutions of CSF in pyrogen-free water [8]. A commercially available Limulus amebocyte lysate test (Pyrotell®; Associates of Cape Cod, Woods Hole, Mass) was used to determine the presence of detectable endotoxin, and the manufacturer's guidelines were observed. The lower limit of detectability of endotoxin was 3 pg/ml. In the monoclonal antibody preparation, the Limulus amebocyte lysate test indicated a minimal endotoxin content of 0.12 ng/ml. No endotoxin activity was detected in the antibiotics tested.

Brain water content (edema). At the end of the experiment, 19 hr after infection, animals were killed by an iv overdose of pentobarbital. As described in detail earlier [18], the brain was immediately removed and dissected on filter paper. One hemisphere was weighed and then dried to stable dry weight in a vacuum oven at 105 C. The other hemisphere was dissected into gray and subcortical white matter, and these fractions were also weighed and dried. Brain water content was expressed as grams of water per 100 g of dry weight. Using these techniques, we found that homologous hemispheres differed by an average of 3.5 g of water/100 g of dry weight [18].

Statistics. Results are expressed as mean ± SD. Paired comparisons of groups were performed by using the two-tailed Student's *t* test, and multiple comparisons between groups were performed with the Newman-Keuls test [19]. Correlation coefficients

Table 1. Bacterial titers and endotoxin concentration in CSF associated with brain edema in rabbits with *E. coli* meningitis.

Treatment group (*n*)	16 Hr postinfection		19 Hr postinfection		
	Titer*	Endotoxin†	Titer*	Endotoxin†	Edema‡
Uninfected (8)	. . .	. . .	. . .	. . .	382 ± 8
Infected controls (16)	4.5 ± 1.1	1.2 ± 0.9	5.1 ± 1.1	1.3 ± 0.7	389 ± 8
Cefotaxime (15)	5.2 ± 1.0§	1.5 ± 0.8§	2.6 ± 2.0	2.8 ± 0.7‖	405 ± 12‖
Chloramphenicol (7)	4.6 ± 0.9	1.0 ± 0.7	2.7 ± 2.0	1.5 ± 0.8	389 ± 9

NOTE. Results are expressed as mean ± SD.
* Log_{10} cfu/ml.
† Log_{10} ng/ml.
‡ Brain edema, g of water/100 g of dry weight.
§ Not significant compared with corresponding values in animals treated with chloramphenicol.
‖ $P < .01$ compared with corresponding values in other groups.

were determined by the least-square linear regression method.

Results

Sixteen hours after intracisternal infection, rabbits showed signs of meningitis, with lethargy, elevated body temperature (>40 C), CSF pleocytosis with a predominance of neutrophils, and positive CSF cultures for *E. coli* (10^3–10^6 cfu/ml; tables 1 and 2). Infection in the subarachnoid space was accompanied by measurable endotoxin concentrations in the CSF, with mean values in the experimental groups ranging from 10 to 100 ng/ml (tables 1 and 2).

If left untreated, animals had slightly higher titers and endotoxin concentrations in CSF after three additional hours (table 1). By this time (19 hr after infection) animals were killed, and brain water content was determined. Brain water content was higher in infected animals than in uninfected controls (hemispheres, 389 ± 8 vs. 382 ± 8 g of water/100 g of dry weight; $P = .08$).

Both antibiotics used in the study, cefotaxime and chloramphenicol, reduced CSF bacterial titers to similar degrees when infused for 3 hr (mean reduction, log_{10} 2.6 and 1.9 cfu/ml, respectively; table 1). The effect of the two drugs on the endotoxin concentration and brain water content at the end of treatment was, however, markedly different (table 1). Treatment with chloramphenicol was associated with a slight increase of borderline significance ($P < .1$) in CSF endotoxin concentrations. Treatment with cefotaxime, however, was followed by a much more pronounced increase of endotoxin in the CSF, with final concentrations ~10 times higher than that obtained with chloramphenicol ($P < .01$). Chloram-

Table 2. Influence of endotoxin neutralization on brain edema in cefotaxime-treated rabbits with *E. coli* meningitis.

Treatment group (*n*)	16 Hr postinfection		19 Hr postinfection		
	Bacterial titer*	Endotoxin†	Bacterial titer*	Endotoxin†	Edema‡
Uninfected (8)	. . .	. . .	. . .	. . .	382 ± 8
Infected controls (16)	4.5 ± 1.1	1.2 ± 0.9	5.1 ± 1.1	1.3 ± 0.7	389 ± 8
Cefotaxime (15)	5.2 ± 1.0	1.5 ± 0.8	2.6 ± 2.0	2.8 ± 0.7§	405 ± 12§
Cefotaxime + polymyxin (12)	5.0 ± 1.1	1.2 ± 0.8	2.5 ± 1.3‖	0.6 ± 1.0§	392 ± 8 §
Cefotaxime +MAb (11)	4.8 ± 1.5	2.0 ± 1.5	1.2 ± 1.5‖	1.6 ± 1.0§	382 ± 8§
Polymyxin (6)	4.9 ± 1.2	1.8 ± 0.9	4.7 ± 1.5	1.3 ± 1.6	395 ± 10
MAb (6)	3.7 ± 2.3	1.3 ± 1.0	4.7 ± 1.8	1.1 ± 1.1	378 ± 3

NOTE. Results are expressed as mean ± SD.
* Log_{10} cfu/ml.
† Log_{10} ng/ml.
‡ Brain edema, g of water/100 g of dry weight.
§ $P < .01$, cefotaxime vs. others.
‖ Not significant.

phenicol did not induce brain edema above that observed in infected, untreated control animals, whereas treatment with cefotaxime was followed by a significant rise in brain water content (hemispheres, 405 ± 12 vs. a mean of 389 in the two other groups; $P < .01$, table 1).

Intravenous infusion of polymyxin B for 3 hr produced a bacteriostatic effect on *E. coli*, and CSF endotoxin concentrations and brain water content were similar to untreated, infected controls (table 2). When polymyxin B was infused simultaneously with cefotaxime, the cefotaxime-associated increase of endotoxin in the CSF and the associated possible brain edema were eliminated (table 2).

A similar effect was observed with a monoclonal IgM antibody to lipid A. The antibody had no effect on CSF bacterial counts; titers rose from $\log_{10}$ 3.7 ± 2.3 to 4.7 ± 1.8 cfu/ml between 16 and 19 hr after infection. During the same period, the measurable endotoxin concentration in CSF decreased, and the final brain water content was reduced (hemispheres, 378 ± 3 g of water/100 g of dry weight; table 2). Injecting the antibody intracisternally during cefotaxime treatment of infected animals reduced both the measurable endotoxin concentration and the brain water content, with the latter reaching values comparable to those in uninfected controls (table 2).

The association of endotoxin release and the development of brain edema was supported by the positive correlation found between CSF endotoxin concentrations 19 hr after infection and the degree of brain edema ($r = .28$, $n = 73$; $P = .03$). Brain edema predominantly developed in the subcortical white matter. Water content was clearly highest in the white matter of animals treated with cefotaxime alone (267 ± 20 g of water/100 g of dry weight, compared with 235 ± 8 g of water/100 g of dry weight in uninfected controls; $P < .01$), which corresponds to an increase of 14%. Differences in gray matter water content were less pronounced. Nevertheless, animals treated with cefotaxime alone also had the highest water content in gray matter (445 ± 18 g of water/100 g of dry weight compared with 430 ± 7 g of water/100 g of dry weight in controls; $P < .06$).

Discussion

This study provides important new information about the pathophysiology of bacterial meningitis and has potential relevance for the therapy of gram-negative bacillary infection. The results suggest that treating *E. coli* meningitis with some, but not all, antibiotics is associated with a marked increase of CSF endotoxin concentrations early after beginning therapy. The endotoxin induces a significant increase in brain edema; this effect can be blocked by selective binding of the lipid A component of endotoxin.

A major focus in the antimicrobial treatment of bacterial meningitis and other serious infections has been the need to achieve a rapid bactericidal action [20–22]. Almost from the beginning of the antibiotic era, however, there has been concern that rapid lysis of gram-negative organisms could be detrimental to the host [23]. Recent work [8–10] has provided evidence for this concern. Antibiotics in vitro, as well as in a rabbit model of gram-negative sepsis, induced marked release of endotoxin. The present study extends these findings to a model of gram-negative bacillary meningitis and correlates the changes in endotoxin concentrations in the CSF with a pathophysiological parameter. The two antibiotics studied differed substantially in their effect on endotoxin concentrations in the CSF. Chloramphenicol, an inhibitor of bacterial protein synthesis, induced only a small increase of endotoxin in our model of meningitis and in the rabbit model of gram-negative sepsis [10]. However, a cell wall–active cephalosporin, cefotaxime in our study and moxalactam in the sepsis model [10], induced a large increase in endotoxin concentration.

Endotoxins are responsible for many of the pathophysiological effects of gram-negative infections (reviewed by Morrison and Ulevitch [11] and by Ryan [24]). The lipopolysaccharide molecules interact with humoral and cellular systems and lead to fever, septic shock, disseminated intravascular coagulation, adult respiratory distress syndrome, and other complications of gram-negative infections. The effect of endotoxin on the brain in gram-negative meningitis has not been previously studied.

Brain edema is a common and potentially fatal complication of bacterial meningitis. In experimental pneumococcal meningitis, we have previously documented a consistent rise in the brain water content as a consequence of the infection in the subarachnoidal space [18]. Treatment with antibiotics or corticosteroids reverses this increase in brain water content [18]. Similar changes in brain water content that are caused by live pneumococci can also be induced by injecting bacterial cell wall products [7]. The present study demonstrates that the apparent liberation

of endotoxin by antibiotic treatment of *E. coli* meningitis is associated with a dramatic increase in brain water content. This development of brain edema takes place predominantly in the subcortical white matter, a localization that is indicative of vasogenic brain edema [25]. Endotoxin damages endothelial cells directly [26, 27] or by stimulating granulocytes [28]. Morphological alterations of the endothelium of the blood-brain barrier (opening of the tight junctions between the endothelial cells) and increased permeability to proteins and fluid are consistent findings in meningitis [29]. In the case of gram-negative meningitis, it is thus likely that endotoxin damages the endothelium of the cerebral capillaries (blood-brain barrier); this damage subsequently leads to vascular leakage and the development of vasogenic brain edema.

The molecular structure of the endotoxin responsible for the majority of its harmful effects is the lipid A portion of the core region [12, 24]. It is well established that inactivation of lipid A prevents many of the endotoxin-mediated biologic effects in vitro and in vivo. Polymyxin B, a cyclic polypeptide antibiotic that binds to lipid A [17], has been used successfully to this end [30–33], although not all functions of endotoxin can be neutralized by polymyxin B [34]. In a recent study of experimental meningitis, preincubating polymyxin B of *Haemophilus influenzae* type b lipopolysaccaride reduced the potential of the lipopolysaccharide to induce an inflammation in the CSF [35]. In the present study, polymyxin B prevented the increase, in the CSF, of measurable endotoxin induced by cefotaxime and also reduced the associated increase in brain edema. This finding supports the essential role of endotoxin in the development of brain edema associated with cefotaxime treatment, because polymyxin B alone had no effect.

Antibodies to the glycolipid core region of endotoxin have attracted major attention as tools in the prevention or treatment of the septic complications associated with gram-negative bacteremia [36, 37]. Because the core region of lipopolysaccarides of different gram-negative bacteria is relatively stable immunologically, antibodies to the core region of one strain are protective against the endotoxin of different strains [38, 39]. More recently, monoclonal antibodies against the core region of endotoxin have been developed; these antibodies also show cross-reactivity [40]. In this study we used a monoclonal IgM antibody to lipid A with cross-reactivity to lipid A of several *E. coli* and *Salmonella* strains (data not shown). Injecting this antibody into the CSF protected against a cefotaxime-associated increase in endotoxin and brain edema. This finding strongly supports the concept that the lipid A portion of endotoxin was responsible for the development of brain edema in our model of *E. coli* meningitis. It is interesting that the monoclonal antibody did not differ from polymyxin B in its capacity to neutralize measurable endotoxin in the CSF, but was clearly more effective in reducing brain water content. This could imply an additional nonspecific effect of the IgM macromolecule beyond its protective action against lipid A. We also found a slight reduction of brain edema with another IgM antibody not directed against endotoxin (data not shown), and these macromolecules may have some hypertonic effects on brain water content.

Our study examined neither the mechanisms responsible for the increase, in CSF, of endotoxin concentrations associated with cefotaxime treatment nor the molecular basis of the effect of polymyxin B or the monoclonal antibody. Moreover, the clinical relevance of our findings is not fully established. Nevertheless, it appears likely that brain edema in bacterial meningitis contributes to morbidity and mortality and that a potentiation by endotoxin may be harmful [25, 41]. Interestingly, >50% of the brain herniations in the series of bacterial meningitis reported by Dodge and Swartz [41] occurred >2 hr after lumbar puncture, at a time when therapy presumably had been initiated. Conceivably, endotoxin-induced brain edema may have played a role in some of these cases. Further studies will be necessary to elucidate the mechanisms responsible for both the increase and inactivation of endotoxin documented in this study and to explore the value of therapeutic strategies that block the release of potentially deleterious bacterial products or inactivate their effect on the host.

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