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Effect of Methylprednisolone on Entry of Ampicillin and Gentamicin into Cerebrospinal Fluid in Experimental Pneumococcal and *Escherichia coli* Meningitis

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The influence of methylprednisolone on the passage of ampicillin and gentamicin into and activity within cerebrospinal fluid was examined in two models of experimental meningitis. Steroid pretreatment reduced the concentrations of these drugs in purulent cerebrospinal fluid of rabbits with experimental pneumococcal and *Escherichia coli* meningitis ($P < 0.05$). However, the resultant mean concentrations of these antibiotics in cerebrospinal fluid still exceeded the minimal bactericidal concentrations of the infecting organisms. The rate of bactericidal effect in vivo was unaffected by steroid therapy in each model. Methylprednisolone did not have deleterious effects on the course of treated experimental meningitis under these short-term (24-h) experiments.

The use of corticosteroids as adjunctive therapy in cases of bacterial meningitis remains controversial. Most clinical studies have shown either no benefit or an equivocal response when steroids were administered for this indication (4). These studies either were retrospective (13) or employed relatively low dosages of these agents (4, 5, 13). Several potential benefits and disadvantages might result from the use of steroids in bacterial meningitis. The benefits are: (i) steroids lower intracranial pressure, elevation of which may be life-threatening in bacterial meningitis (8, 10, 23); (ii) they alleviate cerebral edema, which itself may increase intracranial pressure (6, 14); (iii) as shown in studies on experimental meningitis in our laboratory, these agents return the elevated resistance to the outflow of cerebrospinal fluid (CSF) towards normal, whereas rapidly bactericidal antibiotics alone are without this effect (18); and (iv) steroids can reduce inflammatory changes in CSF (7, 9). The inflammation within the subarachnoid space itself may be detrimental, since leukocytes (probably a membrane component of these cells) directly induce cytotoxic cerebral edema in in vitro preparations (3, 11). However, steroids may be detrimental, because a reduction in CSF inflammation may lead to reduced host defense mechanisms within the CSF or retard the entry of antibiotics into the site of infection. It is this last possibility on which the present experiments were focused. They were designed to determine whether corticosteroid administration (in doses that reduce meningeal inflammation) reduces the entry of antibiotics into purulent CSF and whether corticosteroids inhibit the activity of antibiotics within the CSF in vivo. These problems were approached via use of two classes of antibiotics and two models of experimental meningitis.

(This study was presented in part elsewhere [W. M. Scheld and J. P. Brodeur, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 21st, Chicago, Ill., abstr. no. 451, 1981].)

MATERIALS AND METHODS

Bacterial strains. Both test organisms, *Streptococcus pneumoniae* type III and *Escherichia coli* K_1 antigen positive, were recent clinical isolates from the CSF of patients at the University of Virginia School of Medicine, Charlottesville, Va., and have been extensively characterized in experimental models of meningitis in this laboratory (15, 17). Microtiter broth dilution tests (Dynatech Laboratories, Inc., Alexandria, Va.) were performed with a final inoculum of 10^5 colony-forming units (CFU) for determination of minimal inhibitory concentrations and minimal bactericidal concentrations (MBCs). Both organisms were subcultured from overnight (18-h) cultures and incubated in brain heart infusion broth (Difco Laboratories, Detroit, Mich.), and 0.5% defibrinated sheep blood was added for the pneumococcal studies. The minimal inhibitory concentration was defined as the lowest dilution of antibiotic preventing visible growth after 24 h at 37°C. All clear wells at that time were subcultured on drug-free medium for determination of the MBC, which was defined as the lowest dilution that achieved complete sterility of the wells after a further 24 h of incubation at 37°C.

Rabbit model. New Zealand White rabbits weighing 2 to 3 kg each were prepared with modifications of techniques described previously (2, 20). A dental acrylic helmet was attached to the skull of the animal to facilitate its rigid immobilization within a stereotaxic frame. A 25-gauge Quincke spinal needle, 3.5 inches (≈9 cm) long, was introduced percutaneously and atraumatically into the cisterna magna by a geared electrode introducer. These preparations were used both for initial bacterial inoculation and for CSF sampling later during the course of treatment.

Preparation of inocula and production of meningitis. Two models of experimental meningitis were employed in these studies. *S. pneumoniae* was grown overnight in 10 ml of brain heart infusion broth supplemented with 0.5% defibrinated sheep blood at 37°C in a 10% CO_2 incubator. After removal of erythrocytes by centrifugation (1,000 rpm for 5 min), the bacteria were centrifuged at 3,000 rpm for 15 min and washed twice in 0.9% NaCl. The inoculum used was 10^7 CFU in a volume of 0.25 ml after removal of 0.2 ml of normal CSF. A similar procedure was followed for the *E. coli* strain (C94 K_1 antigen positive), except the final inoculum was 10^5 CFU in 0.15 ml. All animals developed meningitis as evidenced by fever (>40°C), neurological signs, CSF pleocytosis (400 to >10,000 leukocytes per mm^3; ≥95% polymorphonuclear leukocytes), and CSF bacterial counts of $\log_{10}$ 4.0 to >8.0 CFU/ml. All untreated control animals in each group died within 72 h of inoculation.

General experimental design. Methylprednisolone (Solu-Medrol, The Upjohn Co., Kalamazoo, Mich.) was administered at a dose of 30 mg/kg of body weight intramuscularly 8, 12, and 16 h after intracisternal inoculation of bacteria. This dose was chosen because it has been shown previously to reduce inflammation maximally within the subarachnoid space in rabbits with experimental meningitis (9). This reduction in inflammation is maximal after two doses (i.e., by 16 h) and maintained for ≥24 h after the third dose (9), which encompasses the time frame of our studies. Identical volumes of saline were injected at the same time points in control animals with meningitis induced by identical methods. Immediately after the last injection of methylprednisolone or saline, antibiotic therapy was begun by continuous intravenous infusion through a catheter inserted in the femoral vein via a Sage infusion pump (model 352) and continued for 8 h. Ampicillin and gentamicin were given in doses of 30 and 2.5 mg/kg per h, respectively, to rabbits with pneumococcal and *E. coli* meningitis, respectively. A loading dose, equivalent to 20% of the total 8-h dosage, was given as a bolus by rapid (2- to 3-min) intravenous injection immediately before the infusion to rapidly attain steady-state serum antibiotic concentrations. Serial blood (3 ml) and CSF (0.25 ml) samples were taken from an indwelling femoral arterial catheter and spinal needle, respectively, before treatment and after 4 and 8 h of therapy. Each CSF sample was assayed quantitatively for bacterial numbers in tryptic soy agar (Difco) pour plates (containing 0.5% sheep blood for pneumococci) after appropriate serial 10-fold dilutions in saline. The remaining CSF and serum from blood samples were kept at −70°C until antibiotic assays were performed (within 2 weeks). This period of storage did not affect the assay results.

Antibiotic assays. The concentrations of ampicillin and gentamicin in serum and in CSF were determined by agar well diffusion techniques. Antibiotic medium no. 11 (Difco) containing 0.9 ml of *Bacillus subtilis* spore suspension (Difco) per 100 ml was used for ampicillin determinations. Gentamicin concentrations were assayed with a multidrug-resistant strain of *Staphylococcus epidermidis* (ATCC 27626) by the technique of Alcid and Seligman (1). All specimens and standards were tested in triplicate. Serum standards were diluted in pooled rabbit serum. CSF standards were diluted in saline after zone sizes were found to be equivalent to those obtained after dilution in normal rabbit CSF, infected rabbit CSF, or 0.9% NaCl.

Data analysis. The percent penetration of drug into CSF was defined by the formula: percent penetration = (CSF concentration/serum concentration) × 100%. Statistical analysis of drug concentrations, percent penetration, and change in CSF bacterial numbers during therapy was performed on unpaired data by using Student's *t* test (two-tailed). In addition, covariance and regression line analyses were performed to assess differences between experimental groups in each model.

RESULTS

The effect of methylprednisolone on the entry of ampicillin and gentamicin into CSF in experimental meningitis is shown in Table 1. The values observed after 4 and 8 h of infusion did not differ significantly. Therefore, the results in each experimental group were pooled for analysis (Table 1). Three intramuscular injections of methylprednisolone did not significantly alter the mean steady-state serum levels of either antibiotic during continuous intravenous infusion (Table 1). The mean serum concentrations of ampicillin and gentamicin were approximately 40 and 10 μg/ml, respectively.

Methylprednisolone reduced the mean concentrations of both antibiotics in CSF. The mean concentration of ampicillin in the CSF of nonsteroid-treated animals with pneumococcal meningitis was 5.3 μg/ml, as compared with 2.3 μg/ml in rabbits pretreated with methylprednisolone ($P < 0.01$; Table 1). This latter concentration still exceeded the MBC for the test strain (0.1 μg/ml) by more than 10-fold. A similar but less marked decrease in concentrations of gentamicin was also noted in the CSF of rabbits with experimental *E. coli* meningitis. Methylprednisolone pretreatment reduced the mean gentamicin concentration to 1.6 μg/ml, significantly less ($P < 0.05$) than the mean value of 2.3 μg/ml noted in animals receiving gentamicin alone. Both of these levels exceeded the MBC of the test strain (1.0 μg/ml) by a small margin. These changes in CSF antibiotic concentrations were reflected in the overall percent penetration of drug into CSF (defined as the CSF concentration divided by the serum concentration and multiplied by 100). Methylprednisolone reduced the

TABLE 1. Effect of methylprednisolone on concentrations of ampicillin and gentamicin in serum and CSF of rabbits with experimental meningitis

Organism and drug[a]	No. of animals	Mean ± SD concn (μg/ml) in:		% Penetration (mean ± SD)[b]
		Serum	CSF	
S. pneumoniae				
AP	27	39.5 ± 6.2	5.3 ± 2.0	13.5 ± 3.1
AP + MP	17	42.5 ± 5.1	2.3 ± 0.8[c]	5.4 ± 1.4[c]
E. coli				
GT	26	9.6 ± 3.0	2.3 ± 0.6	24.0 ± 5.2
GT + MP	20	10.4 ± 2.6	1.6 ± 0.5[d]	15.6 ± 0.5[d]

[a] AP, Ampicillin; MP, methylprednisolone; GT, gentamicin.
[b] Calculated by the formula: percent penetration = (CSF concentration/serum concentration) × 100%.
[c] $P < 0.01$.
[d] $P < 0.05$.

mean percent penetration of ampicillin from 13.5 to 5.4%, a difference which was significant ($P < 0.01$). Similar results were obtained with gentamicin, of which the mean percent penetration in rabbits with experimental *E. coli* meningitis was reduced from 24% to approximately 15% by steroid pretreatment, again with a significant decline noted after methylprednisolone ($P < 0.05$; Table 1).

The effect of methylprednisolone on the activity of ampicillin in experimental pneumococcal meningitis is shown in Fig. 1. Steroid pretreatment did not alter the mean numbers of bacteria found in CSF before initiation of antibiotic infusion. The numbers of pneumococci in the CSF increased a mean of 0.8 logs in 8 h in untreated animals, all of which died within 48 h of inoculation. In contrast, the administration of ampicillin, with or without methylprednisolone treatment, produced a rapid decline in numbers of pneumococci in CSF of approximately 3 to 3.5 logs and 5.5 to 6 logs in dimension after 4 and 8 h of therapy, respectively (Fig. 1). There were no differences between groups that received ampicillin with and without methylprednisolone at the time points studied. Therefore, it was concluded that methylprednisolone did not interfere with the action of ampicillin at the dosage employed in this in vivo model.

The activity of gentamicin against *E. coli* meningitis is shown in Fig. 2. There were no differences among the three experimental groups before therapy in mean numbers of bacteria in CSF ($\approx 10^{5.5}$ CFU/ml). The numbers in the untreated controls increased steadily thereafter, with death occurring within 3 days of inoculation. The mean decline in numbers of *E. coli* in CSF of 1.8 logs in animals receiving gentamicin alone and 1.5 logs in those receiving both methylprednisolone and gentamicin was not significantly different (analagous to results observed in animals with experimental pneumococcal meningitis).

DISCUSSION

This study examined the influence of methylprednisolone, in a dosage known to maximally reduce CSF inflammation, on the entry and activity of two antibiotics in CSF in experimental meningitis in rabbits. Two types of experimental meningitis (pneumococcal, *E. coli*) and two antibiotic agents, representing two different classes, were employed. In each case, steroid pretreatment did not alter the mean steady-state serum antibiotic concentration, which closely approximated levels found in humans during standard parenteral regimens. Steroid administration did lower the mean CSF antibiotic concentrations by 30 to 60%, with a corresponding decline in antibiotic penetration into purulent CSF (Table 1). However, methylprednisolone did not significantly influence the bactericidal effect in vivo of ampicillin in experimental pneumococcal meningitis or of gentamicin in *E. coli* meningitis (Fig. 1 and 2).

These experiments were designed to maximally reduce CSF inflammation before drug administration in an attempt to delineate any possible deleterious effect of steroids on the entry of antibiotics into CSF. If steroids were used as adjunctive therapy in cases of bacterial meningitis in humans, this effect might be less substantial. Although the dosage in humans would be equivalent to that used in this study (30 mg/kg), both steroids and antibiotics would be started together, as opposed to the sequential method of administration employed in this experimental study.

In this study, steroids did reduce antibiotic entry into CSF, but the bactericidal effect at the site of infection was unaffected, as judged by the rate of decline of concentrations of bacteria in CSF during therapy. However, in each experimental model, the resultant antibiotic concentrations in CSF still exceeded the MBC of the test organism. In previous studies from this labora-

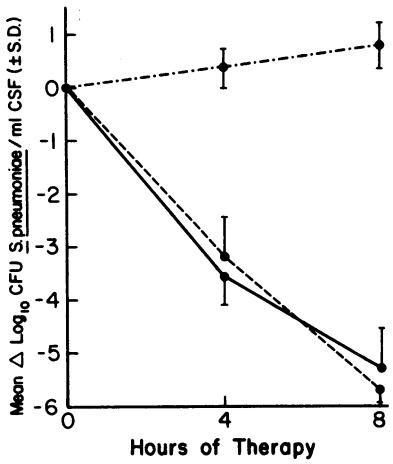

FIG. 1. Mean (± standard deviation) change in *S. pneumoniae* concentration (in $\log_{10}$ CFU/per milliliter) during therapy with untreated controls (●-·-·-●), ampicillin alone (●------●), or ampicillin after methylprednisolone (●——●).

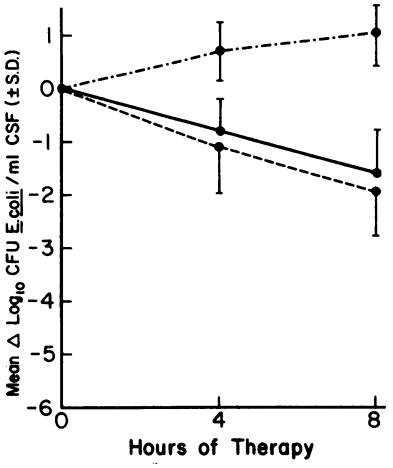

FIG. 2. Mean (± standard deviation) change in CSF *E. coli* concentration (in $\log_{10}$ CFU per milliliter) during therapy with untreated controls (●-·-·-●), gentamicin alone (●------●), or gentamicin after methylprednisolone (●——●).

tory, it was demonstrated that drug concentration(s) in CSF must exceed the MBC of the pathogen by several times to achieve maximal bactericidal activity in vivo (19, 21, 22; W. M. Scheld and M. A. Sande, Clin. Res. 27:355A, 1979; W. M. Scheld and M. A. Sande, J. Clin. Invest., in press). These observations have recently been confirmed by others in studies with experimental meningitis caused by group B streptococci and *E. coli* (16). The clinical implications are potentially important. In cases of pneumococcal meningitis treated with penicillin, the ratio of the concentration of drug in the CSF to the MBC of the infecting organism is large and may not be significantly affected by the administration of steroids. In other types of cases, such as pneumococcal meningitis treated with chloramphenicol (in which the MBCs are higher [12]) or gram-negative bacillary meningitis treated with aminoglycosides, the ratio of the concentration of drug in the CSF to the MBC of the infecting organism is lower and may be adversely influenced if steroids are also given. The effect of steroids on CSF penetration of chloramphenicol was not addressed in this study. Since chloramphenicol is lipophilic and crosses noninflamed meninges well, the effect of steroids on CSF entry of this agent may not be quantitatively similar to that observed with β-lactams or aminoglycosides in this study. In addition, aminoglycosides were chosen for analysis in our experiments because, until recently, these agents were the drugs of choice in gram-negative bacillary meningitis. Owing to favorable recent experience, newer β-lactam antibiotics (e.g., moxalactam and cefotaxime) may supplant the aminoglycosides in these infections; the effect of methylprednisolone on the penetration of these newer agents into purulent CSF should be addressed in future experiments.

Our results suggest that, although steroids may decrease antibiotic entry into purulent CSF, if the resultant CSF drug concentrations still exceed the MBC of the pathogen in vivo (especially if by a wide margin), the bactericidal effect may not be affected. This impression requires further confirmation with other experiments; the influence of steroid therapy on survival in meningitis in both experimental animals and in clinical trials in humans must be performed before any final recommendations on the adjunctive use of these compounds for meningitis can be formulated.

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