Influence of Antibiotic Dose, Dosing Interval, and Duration of Therapy on Outcome in Experimental Pneumococcal Meningitis in Rabbits

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We examined the influence of several pharmacokinetic parameters on cure rates in rabbits with experimental pneumococcal meningitis. When the duration of treatment was kept constant, cure rates improved as the individual dose of ampicillin was increased. On the other hand, when four doses of ampicillin at 60 mg/kg of body weight, producing peak concentrations in cerebrospinal fluid (CSF) of approximately 40 times the MBC, were administered at intervals of 24 instead of 4 h and the duration of therapy was thus prolonged from 12 to 72 h, cure rates also increased (85 versus 25%; P < 0.01). These high cure rates were achieved even though bacterial titers in CSF 24 h after the first dose had reached levels similar to those present at the beginning of therapy. Cure in these animals was explained by the fact that the second ampicillin dose reduced bacterial titers in CSF significantly more than did the first dose (5.2 versus 2.5 log10 CFU/ml; P < 0.02). The clinical relevance of these observations remains to be determined.

Since the introduction of penicillin in the therapy of bacterial diseases 40 years ago, mortality of bacterial meningitis has not been significantly reduced (25), despite the development of many new antibiotics. A recent study found a mortality rate for pneumococcal meningitis of 26% (19), a figure almost identical to those found in studies performed several decades earlier (9, 22).

The pharmacodynamic parameters that determine efficacy in the therapy of bacterial meningitis have not been completely elucidated. In earlier studies using a model of experimental pneumococcal meningitis, we defined some of the factors that influence the therapeutic response to ampicillin (16, 23, 26). First, we found that only antibiotic doses that produce concentrations in cerebrospinal fluid (CSF) 10- to 100-fold above the in vitro MIC show a maximal bactericidal activity in CSF (23). Second, our studies showed that with such high doses, antibiotic concentrations in CSF may fall below the MIC for several hours without loss of efficacy (16, 26). A persistent inhibitory effect of low residual amounts of the antibiotic in CSF seems responsible for this apparent in vivo postantibiotic effect, since the injection of penicillinase into the subarachnoid space in these animals was followed by immediate regrowth of the bacteria (26).

In this study, we examined more closely the influences of the amount of the single dose, the dosing interval, and the duration of treatment on the outcome of experimental pneumococcal meningitis in rabbits. Three different doses of ampicillin, producing peak concentrations in CSF approximately 1, 10, and 40 times the MBC, were administered as bolus injections. Ten treatment groups treated with one of the three doses, given at various dosing intervals for four or eight doses, were examined. The longest treatment regimen (4 days) with high single doses produced the highest cure rates in these experiments. A detailed analysis of bacterial growth characteristics showed that the second antibiotic dose was significantly more bactericidal than the first dose after a long (24 h) but not after a short (4 h) dosing interval.

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MATERIALS AND METHODS

Infecting organism. A type 1 encapsulated strain of Streptococcus pneumoniae, originally isolated from a patient with fatal meningitis, was used in all experiments. Stock cultures were maintained in the vapor phase of a liquid nitrogen vessel. The inoculum was thawed and diluted in 0.9% saline to a final concentration of approximately 2 × 105 CFU/ml. The titer of the inoculum was confirmed by quantitative cultures on Columbia blood agar plates (Oxoid Ltd., London, England) with 4% sheep blood, incubated at 37°C in a 5% CO2 atmosphere. The in vitro MIC and MBC of ampicillin for the strain, as determined by a microdilution method in brain heart infusion (BBL Microbiology Systems, Cockeysville, Md.), were 0.015 and 0.03 μg/ml. Use of inoculum sizes from 102 to 106 CFU/ml did not alter the MIC or MBC.

Model of meningitis. Meningitis was induced in male chinchilla rabbits weighing 2 to 3 kg according to the method originally described by Dacey and Sande (6). One to two days before the experiments, a dental acrylic helmet was attached to the skull of each rabbit by four screws during a short intravenous pentobarbital anesthesia (15 mg/kg of body weight; Nembutal; Abbott Laboratories, Cham, Switzerland). This helmet allows the anesthetized animal to be secured in a stereotactic frame constructed for atraumatic puncture of the cisterna magna. For induction of meningitis, anesthetized animals were placed in the frame, and 0.5 ml of the inoculum was injected directly into the cisterna magna with a 25-gauge, 3.5-in. (ca. 8.9-cm) spinal needle (Becton-Dickinson and Co., St. Augustine, Spain). Bacterial titers and leukocyte (WBC) counts in CSF were determined in all

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animals before institution of therapy and at indicated time points by sampling of 0.3 ml of CSF from the cisterna magna of lightly anesthetized rabbits. The time of death relative to the induction of meningitis was recorded in all animals that died. In surviving animals, cure was documented by determination of bacterial titers in CSF and WBC counts 24 h and 5 days after the last ampicillin dose.

Bacterial titers in CSF were determined by plating 10-fold dilutions on Columbia blood agar with 4% sheep blood incubated for 24 h at 37°C in a 5% CO2 atmosphere. Ampicillin in CSF was inactivated by adding 3,000 U (in 10 μl) of penicillinase (Ferva, Heidelberg, Federal Republic of Germany) to the ex vivo CSF before determination of bacterial counts. The lower limit of detectability was log10 1.3 CFU/ml. To calculate the mean bacterial titers in CSF of an experimental group, a value of log10 0.9 was arbitrarily chosen in the animals with negative CSF cultures. WBC counts were determined in a Sysmex Microcendif counter (CC-170-M; TDA, Kobe, Japan).

Ampicillin concentrations in CSF. Concentrations of ampicillin in CSF were determined in all animals 30 min after intravenous injection of the drug (peak concentration) and at indicated time points during therapy. An agar well diffusion method with Sarcina lutea ATCC 9341 as the indicator organism and antibiotic medium no. 1 (Oxoid) was used. Standard drug solutions were prepared in 0.9% NaCl, and diameters of zones of inhibition were quantitated by using a Quantimet 720 P (Melboures, Royston, Hertfordshire, England).

Treatment regimens. In all experiments, treatment with ampicillin (Penbritin; Beecham Laboratories, Bern, Switzerland) was instituted 18 h after infection. Animals received intravenous bolus injections with one of three doses: 1.4, 15, and 60 mg/kg. These doses were chosen to produce respective peak (30 min after injection) ampicillin concentrations in CSF of approximately 1, 10, and 40 times the in vitro MBC. To examine cure rates, treatment with either of the three doses was continued for a total of four doses. By using dosing intervals of 4, 12, or 24 h, this procedure resulted in a total duration of therapy of 12, 36, and 72 h, respectively. One additional group of rabbits received eight doses of ampicillin at 12-h intervals for examination of the effect of a prolongation of the treatment (from 36 to 72 h) with a constant dose and dosing interval.

In the experiments designed to investigate cure rates after various doses and dosing intervals, groups of approximately 15 animals were examined at the same time. The animals were randomly assigned to one of the treatment regimens or to the untreated control group. Two factors contributed to differences in the number of animals in the treatment groups (Table 1): some animals died as a result of experimental procedures (anesthesia or spinal tap), and we included twice as many animals in two important treatment groups (four doses of 60 mg/kg every 4 h and every 24 h).

When bacterial titers in CSF were examined during a single dosing interval, two groups of five rabbits each were examined. In the last set of experiments, in which bacterial titers were examined in subsequent dosing intervals, different groups of infected rabbits were used for each of the four dosing intervals, since puncture of the cisterna magna could not be repeated more than five or six times in one animal. Data from different treatment groups at overlapping time points were not significantly different in these experiments and were pooled for presentation of results.

RESULTS

Influence of dose, dosing interval, and duration of treatment on cure rates. In the first series of experiments, the relative importance of the amount of each ampicillin dose, the dosing interval, and the duration of therapy for cure were examined in 95 rabbits with meningitis. Single doses produced peak concentrations in CSF of approximately 1, 10, and 40 times the MBC (Table 1). None of the untreated control animals and none of the rabbits treated with ampicillin at 1.4 mg/kg, producing peak concentrations in CSF equivalent to the MBC, survived. In the low-dose treatment group, however, survival time was influenced by the dosing interval. Untreated control animals all died between 24 and 48 h after induction of infection. In animals receiving ampicillin every 24 h, survival time was virtually the same (24 to 72 h); i.e., these animals died before receiving the full course of therapy. The longest survival time was observed in animals treated every 12 h (24 to 120 h; median, 96 h; P < 0.05 compared with control values by the Mann-Whitney rank sum test), whereas treatment every 4 h produced an intermediate survival time (72 to 96 h). Thus, with low doses of ampicillin, very long dosing intervals produced short survival times because animals died before the next dose. Very short dosing intervals had the disadvantage of a short total duration of therapy, during which animals were not cured and died shortly after termination of therapy.

Increasing the single dose improved the survival rate (Table 1). In addition, with the higher doses, a prolongation of the dosing interval and thereby of the total time of treatment also had a positive influence on survival rates; survival rates increased with the highest dose administered (60 mg/kg) from 25% after a treatment duration of 12 h to 85% after treatment for 72 h (P < 0.01 by Fisher's exact test) (Table 1). The importance of the total duration of therapy was also emphasized by the equally high cure rate in animals treated with eight doses every 12 h (Table 1). Thus, the amount of the single dose as well as the duration of therapy determined the cure rate, while, surprisingly, prolongation of the dosing interval to 24 h had no negative influence.

Bacterial dynamics in CSF in relation to dosing interval. In separate experiments, bacterial killing and growth dynamics were analyzed in detail in rabbits receiving the highest

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### TABLE 1. Peak concentrations in CSF and cure rates in rabbits with pneumococcal meningitis treated with ampicillin at various doses and dosing intervals

<table>
<thead>
<tr>
<th>Ampicillin dose (mg/kg)</th>
<th>Peak concn in CSF</th>
<th>Cure rate (no. cured/total no. examined [%]) after given dosing regimen*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μg/ml (mean ± SD)</td>
<td>Multiples of MBC</td>
<td>4 times every 4 h</td>
</tr>
<tr>
<td>1.4</td>
<td>0.04 ± 0.02</td>
<td>1</td>
<td>0/9 (0)</td>
</tr>
<tr>
<td>15</td>
<td>0.23 ± 0.2</td>
<td>8</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td>60</td>
<td>1.31 ± 0.9</td>
<td>43</td>
<td>3/12 (25)</td>
</tr>
</tbody>
</table>

* Cure was defined as sterile CSF cultures 5 days after the last ampicillin dose. ND, Not done.
ampicillin dose. When bacterial titers in CSF were determined in five rabbits for 24 h after one injection of ampicillin, a rapid decline in titers by about $3 \log_{10}$ CFU/ml was observed after 4 h (Fig. 1). This was followed by a plateau phase with titers of approximately $3 \log_{10}$ CFU/ml, which persisted up to approximately 8 h after the dose. Subsequently, a rapid increase to final titers of $7.5 \pm 0.3$ CFU/ml was observed (Fig. 1). Ampicillin concentrations in CSF rapidly declined from a peak of $1.3 \pm 0.9 \mu g/ml$ 30 min after injection and reached $0.03 \pm 0.03 \mu g/ml$ 8 h and $0.03 \pm 0.02 \mu g/ml$ 12 h after initiation of therapy.

When four high doses of ampicillin were administered at 4-h intervals to another group of rabbits, the first dose again produced a rapid decline of titers by approximately $3 \log_{10}$ CFU/ml (Fig. 2). The second dose was followed by a slight, insignificant additional reduction in bacterial titers. The third and fourth doses of ampicillin failed to produce a further decrease in bacterial titers in CSF, but titers remained stable for at least 12 h after the last dose (Fig. 2). Comparison of a single versus four 60-mg/kg doses thus showed that the four doses were clearly superior when judged 24 h after institution of therapy (Fig. 1 and 2). Nevertheless, the plateau phase was followed by rapid bacterial regrowth (Fig. 2) in most of the animals. As a consequence, the high doses given at 4-h intervals cured only 25% of the animals (Table 1), most likely because of the short total duration of therapy. Mean trough antibiotic concentrations before the second to fourth doses were relatively stable (between 0.04 and 0.1 $\mu g/ml$, with no tendency to increase).

**Bacterial dynamics in CSF during consecutive 24-h dosing intervals.** Results of the first and second sets of experiments showed that 85% of the animals treated with four 60-mg/kg doses ampicillin every 24 h were cured (Table 1), even though high bacterial titers were observed at the end of the first 24-h dosing interval (Fig. 1). We therefore analyzed bacterial kinetics in animals treated with high doses of ampicillin during all four 24-h dosing intervals.

The high sterilization rate after four 60-mg/kg doses of ampicillin every 24 h observed in the previous experiments was reproduced in these additional studies (Fig. 3). Again, we observed a rapid rise of bacterial titers in CSF during the second half of the first dosing interval (Fig. 3). Most striking, the second ampicillin dose reduced bacterial titers in CSF by $5.2 \log_{10}$ CFU/ml, a significantly more pronounced bactericidal action than was seen after the first dose (reduction by $2.5 \log_{10}$ CFU/ml; $P < 0.02$ by Student’s $t$ test). Concordant with this finding, only 1 of 9 animals had sterile CSF cultures after the first dose, whereas 7 of 10 had sterile cultures after the second dose ($P < 0.05$ by Fisher’s exact test). In subsequent dosing intervals, bacterial titers progressively declined; after the fourth dose, pneumococci in the CSF of most animals were eliminated (Fig. 3). The median WBC count in CSF was $1,000/mm^3$ (range, 440 to 14,000) before the first ampicillin dose. It then rose to $12,400/mm^3$ (2,020 to 36,400) 4 h and to $17,000/mm^3$ (2,000 to 24,100) 12 h after institution of therapy. At the beginning of the second dosing
interval, the median WBC count in CSF was 1,700/mm³ (400 to 8,020), which rose to a peak of 4,700/mm³ (1,380 to 26,860) 4 h later. Thus, WBC counts in CSF were clearly lower during the second dosing interval, when the unexpectedly high bactericidal activity of ampicillin was observed. The large variation in WBC counts (and, to a lesser degree, of bacterial titers) in CSF between individual animals and the marked increase in WBC in CSF after institution of antibiotic therapy are commonly observed in studies using this model of meningitis.

**DISCUSSION**

Beta-lactam antibiotics are the standard therapy for bacterial meningitis (11, 24). However, the optimal treatment regimen for these potent antibiotics is not well defined. This study was designed to better define the influence on cure rates of each antibiotic dose in relation to the dosing interval and the total duration of therapy.

As in our previous studies (23, 26), the amount of the individual dose of ampicillin emerged as an important factor influencing the response to therapy. After 4 days of therapy, most of the animals were cured if they were treated with the highest ampicillin dose, resulting in peak concentrations in CSF that were approximately 40 times the MBC. The importance of the peak concentration in CSF can be explained at least in part by our previous findings of a linear correlation between concentrations of beta-lactams in CSF and the bactericidal activities of these drugs in rabbits with meningitis (23). Antibiotic concentrations in CSF in the range of the MBC produced a static effect on meningial pathogens in CSF, whereas concentrations of at least 10 to 30 times the MBC produced a maximal bactericidal activity, which decreased bacterial titers in CSF by approximately 1 log₁₀ CFU/ml per h (23). Since host defense mechanisms in CSF are unable to clear the infection (7), a merely bacteriostatic antibiotic activity is insufficient for cure of meningitis in this model (18). Thus, as shown by the dose-dependent cure rates in this study, only antibiotic doses that result in concentrations in CSF high enough to produce a pronounced bactericidal action are consistently effective in the therapy of bacterial meningitis.

The dose-dependent bactericidal activity of beta-lactam antibiotics in meningitis is unexpected. In vitro, beta-lactam antibiotics do not show a dose dependency at concentrations above the MBC (23, 27), whereas such an effect is well known for aminoglycosides (27). On the basis of studies in experimental models of infections other than meningitis, these differences between the two antibiotic classes translate into the following rules for optimal dosing schedules (5): for beta-lactam antibiotics, the best results are obtained when drug concentrations at the site of infection are constantly above the MIC, whereas very high peak concentrations do not significantly improve their effects (1, 14). For aminoglycosides, on the other hand, the peak concentrations obtained relative to the in vitro susceptibility of the infecting organism is critical for the outcome (12). During therapy with aminoglycosides, concentrations may fall below the MIC for prolonged periods of time without loss of efficacy (5, 9a). This may be explained by the postantibiotic effect of these drugs (5). Interestingly, our studies suggest that in the treatment of meningitis, beta-lactam antibiotics display characteristics that appear similar to those of aminoglycosides in other infections.

A second important variable that influenced cure rates in our study was the total duration of therapy. Even with the highest dose, only animals that were treated for 4 days (four times every 24 h or eight times every 12 h) achieved cure rates of >80%. This is in some contrast to results of our earlier study (26), in which a 12-h treatment regimen was sufficient to sterilize the CSF in >90% of the animals treated with the highest dose. Two differences between the studies may explain this discrepancy. First, in this study cure was defined as sterile CSF 5 days after completion of therapy, whereas in the earlier study CSF cultures were performed 36 h after the last antibiotic dose. Second, we used a different, more virulent strain of *S. pneumoniae* in this study, which
may have been more difficult to eradicate. The discrepant results of the two studies underscore the importance of subtle differences of study design in experimental models and should caution against uncritical extrapolations of experimental findings to clinical situations. Nevertheless, the study presented here emphasizes the need for sufficiently long treatment periods in pneumococcal meningitis.

The most unexpected finding in this study was the effectiveness of the 24-h-dosing-interval, high-dose regimen. Because of the relatively short half-life of ampicillin in rabbits (23), this long dosing interval clearly exceeded the duration of the total inhibitory effect of ampicillin in CSF. As a consequence, bacteria in CSF grew to high titers at the end of the first dosing interval, immediately before the second dose. Nevertheless, a high percentage of these animals were cured after the fourth dose. This result can be explained by the pronounced decrease in bacterial titers after the second antibiotic dose, which was about twice that after the first dose. However, the rapid decline of bacteria after the second dose was limited to animals treated every 24 h, whereas the second dose showed only a minimal effect in rabbits treated every 4 h. The third dose was even followed by a slight increase in bacterial titers in the 4-h dosing schedule. Thus, changes in bacterial titers after the second and subsequent ampicillin doses depended on the dosing interval in our experiments. In in vitro pharmacokinetic models, a similar phenomenon has been observed for aminoglycosides and quinolones against gram-negative organisms (2, 3). Additional experiments must examine whether the influence of the dosing interval on the bactericidal activity of antibiotics is a general phenomenon in meningitis and other infections. It is also important to point out that our results should not be interpreted as indicating that cure is not possible with very frequent doses. Clinical experiences clearly indicate that a large majority of patients are cured with antibiotics given very frequently if therapy is long enough (11).

The improved bactericidal action of the second antibiotic dose after a very long dosing interval is a novel observation, the explanation for which is not obvious. We do not believe that CSF granulocytes were responsible for the observed differences in killing rates for several reasons. First, granulocytes are virtually ineffective in reducing bacterial titers in CSF of rabbits with pneumococcal meningitis (7). Second, the granulocyte count during the first dosing interval was clearly higher than during the second interval, when bacteria were reduced the most. Third, bactericidal rates after the first dose of ampicillin were similar in normal and in neutropenic rabbits (data not shown).

Several factors may influence the susceptibility of bacteria to antibiotics; these include inoculum size (28), growth rate (4, 15), temperature (10), pH of the medium (8, 21), and patterns of previous exposures to antibiotics (13). Bacterial growth rates may very well have influenced the bactericidal effect of the ampicillin doses in our experiments. For instance, bacteria showed no apparent growth before the second and subsequent doses when ampicillin was administered every 4 h, and the antibiotic had a minimal effect on bacterial titers in these animals. On the other hand, pneumococci grew rapidly, with a minimum doubling time of 1.2 h, during the second half of the first 24-h dosing interval. This growth rate is much faster than the doubling time of 2.8 h that we found in a previous study in untreated, febrile rabbits with meningitis caused by a different strain of S. pneumoniae (20). It is possible that this rapid growth rate made the bacteria exquisitely susceptible to the action of the second antibiotic dose in the every-24-h regimen.

Among the factors that may influence bacterial growth in this animal model of pneumococcal meningitis, temperature plays an important role (17, 20). Unfortunately, we measured temperature only before the first treatment dose in this study, at which time all rabbits were highly febrile (>40°C). Thus, it is possible that the animals were less febrile before the second dose and that the pneumococci therefore grew particularly fast. Whether other factors also play a role in determining the bactericidal action of ampicillin in this model is not known. Specifically, no study has been made of whether the pattern of previous antibiotic exposure may alter the antibiotic susceptibility or lysis characteristics of the organism, as has been recently shown in vitro (13).

In summary, our results confirm the importance of large single doses of antibiotics in the therapy of pneumococcal meningitis that produce high antibiotic concentrations in CSF (>10 times the MBC). Furthermore, even with very rapidly bactericidal antibiotics, the duration of therapy is critical. Our results also seem to indicate that the effect of the antibiotic on the pneumococcus in CSF is influenced by the duration of the preceding dosing interval. Whether this finding has any relevance for therapy of patients with pneumococcal meningitis remains to be determined by additional studies.

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LITERATURE CITED


