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Antimicrobial Therapy of Experimental Group B Streptococcal Infection in Mice

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Group B beta-hemolytic streptococci (GB-BHS) frequently cause severe infection in newborns. Previous in vitro studies showed accelerated killing of GB-BHS by ampicillin plus gentamicin as compared with ampicillin alone. To extend the in vitro observations, mice were infected experimentally with GB-BHS and treated with gentamicin plus ampicillin or ampicillin alone. Untreated mice died within 10 to 48 h. Compared with treatment with ampicillin alone, ampicillin-and-gentamicin therapy resulted in improved survival when the antibiotics were given in established infection or as a single dose at the time of infection. Ampicillin and gentamicin accelerated the clearing of bacteremia as compared with treatment with ampicillin alone. In view of these findings, the therapy of GB-BHS infection in newborns and other patients should be reconsidered in that a combination of ampicillin or penicillin G plus gentamicin might be superior to the use of ampicillin or penicillin G alone.

Group B beta-hemolytic streptococcus (GB-BHS) is a frequent and serious cause of infection in infancy (4, 6), despite susceptibility of GB-BHS to penicillin and ampicillin (5, 13). Penicillin or ampicillin is recommended for the treatment of GB-BHS infection of infants (12). Since these agents act by similar mechanisms, the bactericidal effect of one of these agents, ampicillin, against this organism was measured. The rate of killing of GB-BHS by ampicillin was slower than that observed for group A beta-hemolytic streptococci (13); whereas less than 4 h is required for a 99.99% reduction in bacterial colony counts of group A beta-hemolytic streptococci, up to 48 h is required for the same reduction in GB-BHS. Although GB-BHS are resistant to gentamicin as a single drug, killing was accelerated by the combination of ampicillin with gentamicin (13). To examine the relevance of these in vitro findings, we produced experimental GB-BHS infection in weanling mice. The influence of ampicillin alone and in combination with gentamicin on survival in mice and on the kinetics of bacteremia was studied.

MATERIALS AND METHODS

Organism. GB-BHS reference strain Ia was chosen for these studies because infections with this strain are common and often fatal in newborns (3). GB-BHS reference strain Ia, SS-615, was supplied by R. R. Facklam, Center for Disease Control, Atlanta, Ga. The organism was passaged monthly on 5% blood agar and kept at 4°C. Streptococci were grown in tryptose phosphate broth. The minimal inhibitory concentration (MIC) of ampicillin for the organism was 0.06 μg/ml, and the minimal bactericidal concentration (MBC) was 2.0 μg/ml. The MIC of gentamicin for the organism was 8 μg/ml, and the MBC was 16.0 μg/ml.

Antibiotics. Sodium ampicillin (Omnipen N, Wyeth Laboratories Inc., Philadelphia, Pa.) was diluted in normal saline immediately before each administration. Gentamicin (Garamycin, Shering Corp., Kenilworth, N.J.) was diluted in normal saline and stored at 4°C until injection. The total ampicillin dose was 125 mg/kg; the total gentamicin dose was 25 mg/kg.

Antibiotic assay. Gentamicin and/or ampicillin concentrations in serum were measured 1 h after subcutaneous injection by the agar well diffusion technique (8). For ampicillin assay, *Bacillus subtilis* ATCC 6633 was used; for gentamicin assay, *Staphylococcus epidermidis* ATCC 27626 was used. To assay ampicillin in the presence of gentamicin, 4% sodium chloride was incorporated in the agar. Antibiotic standards of ampicillin (5, 10, 20, and 40 μg/ml) or gentamicin (1.25, 2.5, 5, 10, and 20 μg/ml) diluted in NIH Swiss mouse serum, were included on each assay plate.

Therapeutic evaluations. Female NIH Swiss mice, 16 to 19 g, were obtained from Scientific Small Animal Laboratories (Arlington Heights, Ill.). Mice were injected intraperitoneally with approximately 10^7 colony-forming units (CFU) of GB-BHS in 0.1 ml of an undiluted overnight culture. Inoculation of 10 CFU streptococci per mouse resulted in a mortality of 50%. All mice injected with 10^7 CFU died 10 to 48 h after infection. In one series of experiments, the total dose of antibiotics was divided into three portions and injected 4, 10, and 16 h after infection.

Deaths due to streptococcal infection were tabulated for the following 9 days. In some experiments, mice received the total daily antibiotic dose simultaneously with streptococcal infection. Antibiotics were injected subcutaneously as 0.1 ml of ampicillin or 0.1 ml of ampicillin and 0.1 ml of gentamicin in separate sites.

Quantitative blood cultures. Mice were infected as described above and injected 1 h later with 125 mg of ampicillin per kg or the same dose of ampicillin and 25 mg of gentamicin per kg, or were left untreated. Groups of 20 animals were exsanguinated by cardiac puncture at each of four time points: 1 h after infection (at the time of antibiotic injection) and hourly for the next 3 h. Undiluted blood (0.1 ml) and 10-fold dilutions of blood were plated on blood agar, and CFU per milliliter of blood were counted after overnight incubation.

RESULTS

Survival. The efficacy of ampicillin was compared with that of ampicillin and gentamicin in established GB-BHS infection. Table 1 shows data from three replicate experiments in which mice were infected and treated 4, 10, and 16 h later with ampicillin alone (125 mg/kg divided in three doses) or the same dose of ampicillin plus gentamicin (25 mg/kg divided in three doses). One hour after the first dose of antibiotics, serum levels of ampicillin averaged 18 μg/ml and those of gentamicin averaged 5 μg/ml. Ninety-five animals treated with ampicillin had a survival of 53% at the end of 9 days, whereas 95 animals treated with ampicillin and gentamicin had a survival of 73% ($\chi^2 = 9.06$, $P < 0.01$). Of animals given the same dose of gentamicin alone, none survived.

We attempted to determine whether simultaneous treatment with ampicillin or ampicillin and gentamicin would protect mice from lethal GB-BHS infection. Animals were given either ampicillin alone (125 mg/kg) or ampicillin and gentamicin (125 and 25 mg/kg, respectively) as a single dose at the time of infection. One hour after the antibiotic dose, serum levels of ampicillin averaged 30 μg/ml and those of gentamicin averaged 8 μg/ml. Table 1 shows the data from three replicate experiments. The group of mice treated with ampicillin had a survival of 43% 9 days after inoculation, whereas 98% of the group receiving ampicillin and gentamicin survived ($\chi^2 = 54$, $P < 0.01$). In all experiments, untreated mice had no survivors at 48 h after infection with GB-BHS. Animals treated with gentamicin alone had a 15% survival.

Kinetics of streptococcal bacteremia. The improved survival of mice receiving combined antibiotics compared with those treated with ampicillin alone was investigated by studies of the kinetics of clearing streptococcal bacteremia. One hour after infection, ampicillin (125 mg/kg) or ampicillin (125 mg/kg) and gentamicin (25 mg/kg) were given as a single dose. No deaths occurred in mice receiving antibiotics during the sampling period.

Figure 1a illustrates the incidence of bacteremia among the three groups. Bacteremia was present in 100% of the untreated animals at all times. With ampicillin treatment, 90 to 95% of mice remained bacteremic 1, 2, and 3 h after treatment. In contrast, 52% of mice treated with ampicillin and gentamicin were bacteremic 2 h after treatment, and only 33% were bacteremic 3 h after treatment ($\chi^2 = 12.7$, $P < 0.01$). Figure 1b illustrates the comparative level of bacteremia among the three groups. At each time, the concentration of bacteria in blood was less in mice receiving ampicillin plus gentamicin than in those receiving ampicillin

TABLE 1. *Cumulative survival of GB-BHS-infected mice*

Treatment (total dose in mg/kg)	No. of mice in group	No. of survivors on day after inoculation:						Percent survivors on day 9
		1	2	3	5	7	9	
Three-dose regimen[a]								
None	20	0						0
Ampicillin (125)	95	93	85	78	59	53	51	53
Gentamicin (25)	20	20	4	0				0
Ampicillin (125) + gentamicin (25)	95	90	90	82	73	72	69	73
Single-dose regimen[b]								
None	20	2	0					0
Ampicillin (125)	117	117	97	80	67	54	51	43
Gentamicin (25)	40	32	23	12	9	6	6	15
Ampicillin (125) + gentamicin (25)	117	117	117	116	115	115	115	98

[a] Treatment given in three divided doses 4, 10, and 16 h after infection.
[b] Treatment given at the time of infection.

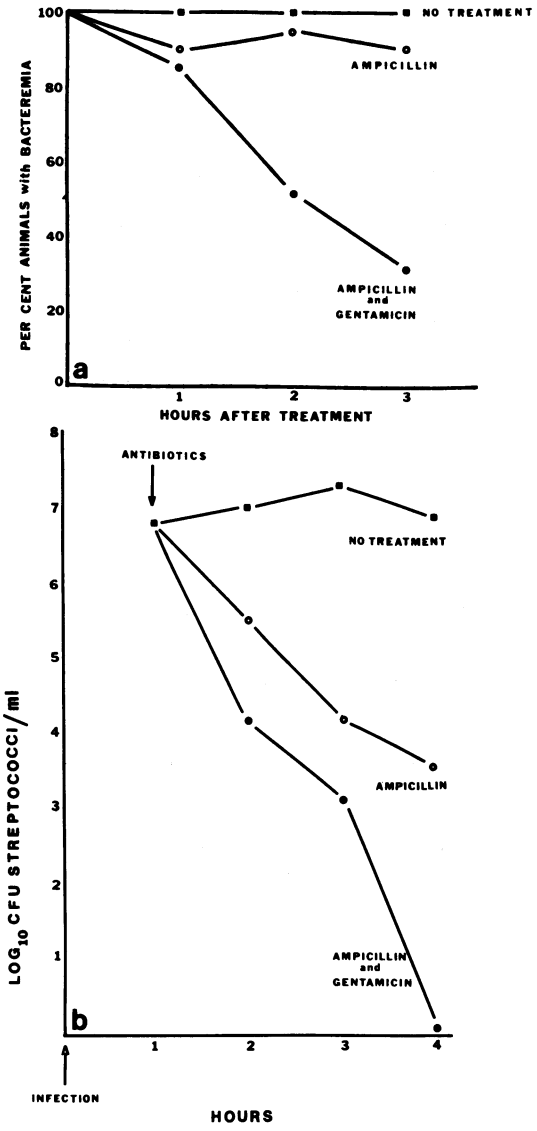

FIG. 1. *Inoculation with 10^7 CFU of streptococci 1 h before treatment with antibiotics. Each point shown is the average of 20 mice. (a) Percentage of animals with bacteremia; (b) quantitation of bacteremia. The variation for each average shown for the last time point was less than 2 $\log_{10}$.*

alone. The average CFU of streptococci per milliliter of blood in all animals in the untreated group varied from 7×10^6 to 2×10^7. In mice treated with ampicillin alone, the average CFU of streptococci per milliliter of blood had decreased to 7×10^3 3 h after therapy. In comparison, 3 h after antibiotic administration, an average of 0.8 CFU per ml of blood was observed in animals that received ampicillin plus gentamicin.

DISCUSSION

The observation that gentamicin accelerates the bactericidal effect of ampicillin for GB-BHS in vitro (13) led to an investigation of the effect of this drug combination in mice after experimental streptococcal infection. Although as few streptococci as 10 CFU produced death in 50% of untreated animals, 10^7 CFU of streptococci were used to establish infection and resulted in bacteremia of approximately 10^6 CFU/ml. The dose of ampicillin and the resultant serum levels of ampicillin were similar to dosages and levels recommended for newborn infants (11). Although these studies were performed with ampicillin, future studies should be carried out to determine whether similar results are observed with penicillin G, as would be expected. The dose of gentamicin was higher than for human use, but was necessary to obtain blood levels similar to the ones recommended in humans (11). Although ampicillin and gentamicin were more effective than ampicillin alone in the treatment regimens reported here as shown in Table 1, a difference between the two groups in a number of other treatment schedules did not occur. A comparable effect of dosing on survival was observed by Andriole (1, 2) and Lumish et al. (10) in experimental pseudomonas infections of normal or neutropenic rodents with carbenicillin and gentamicin or tobramycin.

GB-BHS are susceptible to ampicillin and penicillin with MICs only a fewfold higher than for group A beta-hemolytic streptococci. Nevertheless, the rate of bactericidal effect of ampicillin against GB-BHS is considerably slower than against group A beta-hemolytic streptococci (13). With the increased penicillin concentrations required for effectiveness against a large inoculum size (7) and the slow bactericidal rate of ampicillin alone against GB-BHS (13), it is possible that the presently recommended dosages for penicillin in newborn infants may produce levels in cerebrospinal fluid that are inadequate for eradication of infection. Although GB-BHS are more susceptible to penicillins and less susceptible to gentamicin than are enterococci, the enhanced activity of the combination of the two drugs against GB-BHS is similar to that seen for enterococci (14). Greater activity of the combination is also observed with *Listeria monocytogenes* (9).

In the experiments reported here we have shown that the combination of drugs was more effective than ampicillin alone in the treatment of mice with established infection (Table 1). The combination was also more effective than ampicillin alone in the protection of mice against lethal streptococcal infection (Table 1). Not only was incidence of bacteremia lower

(Fig. 1a), but in the presence of bacteremia the concentration of streptococci was lower (Fig. 1b) in animals given the combination. The differences in ampicillin plus gentamicin compared with ampicillin alone may account for the improved survival observed with the combination. The correlation of these findings with our in vitro observations (13) shows that in experimental infection, the combination of antibiotics produced a more rapid bactericidal effect and beneficially influenced the outcome of infection. Based on our present data and our previous observations in vitro, we suggest that further examination of the benefits of combined therapy with ampicillin or penicillin and gentamicin for patients with GB-BHS infection is indicated.

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