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Comparison of Gentamicin and Kanamycin Alone and in Combination with Ampicillin in Experimental *Escherichia coli* Bacteremia and Meningitis

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ABSTRACT. The conventional antimicrobial therapy of gram-negative infection in the newborn is the combination of ampicillin and an aminoglycoside, usually gentamicin or kanamycin. Although gentamicin and kanamycin have been used interchangeably, efficacies of the two drugs have not been carefully compared. In addition, the contribution of ampicillin to the outcome of neonatal gram-negative meningitis is controversial. We evaluated the activity of gentamicin and kanamycin alone and in combinations with ampicillin *in vitro* and *in vivo* against a K_1 *Escherichia coli* strain. *In vitro*, the *E. coli* strain was relatively sensitive to ampicillin, gentamicin, and kanamycin, with the minimal inhibitory and minimal bactericidal concentrations of 2 and 4, 2 and 2, and 4 and 8 μg/ml, respectively. Checkerboard determinations of minimal inhibitory and minimal bactericidal concentrations of drug combinations exhibited an indifferent response for both ampicillin + gentamicin and ampicillin + kanamycin. However, *in vivo* studies using an experimental *E. coli* bacteremia and meningitis model in newbown rats suggested that gentamicin was more effective than kanamycin. This was shown by more rapid bacterial clearance from the blood, a decreased incidence of meningitis in bacteremic animals, and improved survival. Furthermore, the addition of ampicillin improved the outcome of kanamycin, but not gentamicin, suggesting that the contribution of ampicillin may vary depending on the type of aminoglycoside used. These findings suggest that kanamycin is less effective than gentamicin *in vivo* against *E. coli* and should be used in combination with ampicillin to achieve an outcome comparable to that of gentamicin in this model of *E. coli* infection. (*Pediatr Res* 19: 1152–1155, 1985)

Abbreviations

CFU, colony forming units
CSF, cerebrospinal fluid
MBC, minimal bactericidal concentration
MH, Mueller-Hinton broth
MIC, minimal inhibitory concentration

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The most distressing aspect of neonatal gram-negative infection is lack of significant improvements in the mortality and morbidity attributable to recent advances in antimicrobial chemotherapy. A recent multicenter study of neonatal gram-negative meningitis clearly showed that moxalactam, a potent new β-lactam antibiotic, was not more effective than a combination of ampicillin and an aminoglycoside (1). Thus, the aminoglycosides still remain the mainstay in the treatment of neonatal gram-negative infection. However, whether all aminoglycoside antibiotics will have similar activities *in vivo* against susceptible gram-negative bacilli is not known. Moreover, the contribution of ampicillin to the final outcome of neonatal gram-negative meningitis is controversial. In one retrospective study, satisfactory responses to antimicrobial therapy was observed only when the infecting organism was ampicillin-sensitive (2). In a prospective study, however, a favorable effect of ampicillin-sensitivity of the gram-negative isolates was not observed (3).

The present study was therefore performed to compare the activity of the two most extensively used aminoglycosides in the newborn, gentamicin and kanamycin alone and in combinatin with ampicillin *in vitro* and *in vivo* against *Escherichia coli.*

MATERIALS AND METHODS

Organism. A serum-resistant *E. coli* K1 strain (C5) isolated from the CSF of a newborn infant with meningitis (11) was used in *in vitro* and *in vivo* studies.

In vitro studies. MIC and MBC were measured in MH broth (Difco Laboratories, Detroit, MI) by a macrobroth dilution method (4). Antimicrobial agents tested were ampicillin trihydrate (Bristol Laboratories, Syracuse, NY), gentamicin sulfate (Schering Corporation, Kenilworth, NJ) and kanamycin sulfate (Bristol). All antibiotic solutions were diluted serially 2-fold from 256 to 0.5 μg/ml in MH broth. Late logarithmic-phase cultures of the C5 strain containing approximately 1×10^6 CFU/ml were prepared as described previously (5). Equal volumes (0.5 ml) of antibiotic and bacterial dilutions were mixed and incubated at 37° C for 24 h. The MIC was defined as the lowest antibiotic concentration exhibiting no visual turbidity. From each tube, 10 μl was transferred to a quadrant of sheep blood agar plate and incubated at 37° C for 24 h to determine the MBC, defined as the lowest concentration of antibiotics which resulted in ≥ 99.9% killing of the original inoculum.

The MICs and MBCs of combinations of ampicillin and gentamicin (or kanamycin) were measured in MH broth. Test tubes in a checkerboard fashion received 0.5 ml of ampicillin and/or gentamicin (or kanamycin) and 0.5 ml of bacterial dilutions. Each antibiotic was tested in doubling concentrations of 0.125 through 64 μg/ml. After 24 h incubation at 37° C, MIC

and MBC end points were read as above for each antibiotic alone and in various combinations. The results were expressed as the fractional inhibitory concentration index, which was calculated by the following formula (6, 7):

$$\frac{\text{MIC of drug "A" in combination with drug "B"}}{\text{MIC of drug "A"}} + \frac{\text{MIC of drug "b" in combination with drug "A"}}{\text{MIC of drug "B"}}$$

When the fractional inhibitory concentration index is <0.5, the combination is considered to have a synergistic effect; when the index is >2, the combination is considered antagonistic; when the index is between 0.5–2, the combination is indifferent. Similarly, the fractional bactericidal concentration (FBC) index was calculated.

In vivo studies. Outbred, pathogen-free Sprague-Dawley pregnant rats with timed conception were purchased from Charles River Breeding Laboratories, Wilmington, MA, and gave birth in our vivarium 5–7 days after arrival. *E. coli* bacteremia and meningitis were induced in 5-day-old rats by the method detailed previously (5, 8, 11).

A total of 93 newborn rats from 10 litters was used. At 5 days of age, all members of each litter were inoculated intraperitoneally with 100 CFU of the C5 strain. As determined previously (5, 8, 11), this inoculum produces nonlethal bacteremia (with or without meningitis) in 100% of animals within 18 h of inoculation. Eighteen hours after inoculation and daily thereafter for 4 days, quantitative cultures of blood and CSF were obtained as previously described (5, 8, 11). Immediately after first blood and CSF specimens were obtained, each litter was randomly divided into four treatment groups: 1) gentamicin 5 mg/kg twice daily (900 and 1900 h), 2) kanamycin 15 mg/kg twice daily, 3) ampicillin 50 mg/kg + gentamicin 5 mg/kg twice daily, or 4) ampicillin 50 mg/kg + kanamycin 15 mg/kg twice daily. All drugs were administered subcutaneously. As we have shown previously (11, 12), at the doses used in the present study, the mean serum concentrations (± SD) at 1–2 after subcutaneous administration were 41.6 ± 19.1 μg/ml for ampicillin, 9.9 ± 4.9 μg/ml for gentamicin and 24.5 ± 10.5 μg/ml for kanamycin. A control rat from each litter received only saline (0.05 ml). Animals that died during therapy were removed and postmortem blood specimens were obtained by cardiac puncture. Blood cultures obtained from animals that died > 1 h before daily culture were excluded from quantitative bacteriologic analysis. CSF specimens could not be obtained in dead animals. Therapeutic efficacy was determined by comparing rates of bacterial clearance in blood, incidence of meningitis developing during therapy, and mortality rates among the five groups.

Most animals had at least one blood sample drawn at 1–2 h after subcutaneous administration of antibiotics on therapy day 3 for determination of bactericidal titers. Bactericidal titers were determined by a microtiter technique (9) with serial 2-fold dilutions of serum in MH broth and an inoculum of approximately 10^5 CFU/ml of the C5 strain. The serum bactericidal titers were defined as the highest dilution of serum which resulted in ≥ 99.9% killng.

Statistical methods. The χ^2 test with Yates' correction or Student's *t* test were used where indicated (10). *P* values ≤ 0.05 were considered significant.

Table 1. *Comparison of bacterial counts* in blood and CSF 18 h after inoculation and before therapy among the five treatment groups*

Antibiotics	No. of animals	Blood	CSF
Gentamicin	21	6.02 ± 1.38 (21)†	4.76 ± 2.40 (4)†
Kanamycin	20	6.25 ± 1.52 (20)	4.68 ± 2.01 (5)
Ampicillin + gentamicin	22	6.02 ± 1.85 (22)	4.66 ± 1.43 (6)
Ampicillin + kanamycin	20	6.34 ± 1.17 (20)	5.09 ± 1.22 (6)
Saline	10	5.42 ± 2.27 (10)	5.52 ± 2.37 (3)

* Expressed as mean ± SD (log 10 CFU/ml).

† Numbers in parentheses indicate the number of animals positive by culture.

RESULTS

In vitro findings. The MICs and MBCs of the C5 strain were 2 and 4 μg/ml for ampicillin, 2 and 2 μg/ml for gentamicin, and 4 and 8 μg/ml for kanamycin. The fractional inhibitory/fractional bactericidal concentrations were 1.28/0.85 for ampicillin + gentamicin and 0.93/0.86 for ampicillin + kanamycin, indicating that the addition of ampicillin to gentamicin or kanamycin exerted an indifferent effect.

In vivo findings. E. coli bacteremia was present in 93 (100%) and meningitis (positive CSF culture) in 24 animals (26%) 18 h after infection and before beginning therapy. At this time, the prevalence of meningitis and the bacterial counts in blood and CSF were not significantly different among the five treatment groups (Table 1). A total of 23 animals died within 10 h of beginning therapy and these animals were not available for subsequent determination of bacterial counts in blood and CSF.

Table 2 compares the serum bactericidal titers and bacterial clearance from blood (expressed as the change in log 10 CFU/ml of blood) among the five treatment groups. As noted, the number of animals available for these observations decreased

Table 2. *Comparison of serum bactericidal titers* and bacterial clearance† in blood among the five treatment groups*

Therapy regimen	Serum bactericidal titers	Δ Bacterial counts after treatment day completed		
		1	2	3
Gentamicin	≤5.65 ± 1.60	−4.36 ± 2.37 (5/15)‡	−4.44 ± 2.50 (5/15)	−4.70 ± 2.42 (3/15)
Kanamycin	≤5.02 ± 1.71	−3.30 ± 2.75 (10/17)	−3.93 ± 2.96 (6/14)	−4.72 ± 2.92 (2/11)
Ampicillin + gentamicin	12.64 ± 3.34	−5.19 ± 2.02 (2/17)	−5.28 ± 2.02 (2/17)	−5.48 ± 1.87 (1/17)
Ampicillin + kanamycin	9.18 ± 4.72	−5.65 ± 2.23§ (3/14)	−6.17 ± 1.25§ (1/13)	−6.21 ± 1.29§ (0/13)
Saline	<4	+3.08 ± 1.41 (6/6)	NA‖	NA

* Expressed as geometric mean ± SEM of reciprocals of serum bactericidal titers.

† Expressed as the change in log 10 CFU/ml of blood.

‡ Numbers in parentheses indicate the number of animals positive for culture after completion of treatment day/the number of animals positive for culture before therapy and available for subsequent determinations of bacterial counts.

§ Significantly greater than that of kanamycin alone ($p < 0.02$, < 0.01, and < 0.05, respectively, after 1, 2, and 3 days of completed therapy).

‖ Deniotes no survivors available.

Table 3. *Development of meningitis in surviving animals free of meningitis at the beginning of therapy*

Therapeutic regimen	Development of meningitis during 4 days of therapy (%)
Ampicillin*	11/23 (48)
Gentamicin	3/15 (20)
Kanamycin	5/12 (42)
Ampicillin + gentamicin	2/13 (15)
Ampicillin + kanamycin	1/10 (10)
Saline (control)	3/3 (100)

* Ampicillin data were derived from our previous experiments (Ref. 11).

Table 4. *Comparison of mortality among the five treatment groups*

Therapy regimen (no. of animals)	Mortality		
	Early*	Late†	Overall (%)
Ampicillin (41)‡			26 (63)
Gentamicin (21)	6	0	6 (29)
Kanamycin (20)	3	8	11 (55)
Ampicillin + gentamicin (22)	5	0	5 (23)
Ampicillin + kanamycin (20)	6	1	7 (35)
Saline (10)	4	6	10 (100)

* Early death—animals dying during the 1st day of therapy.

† Late death—animals dying after the 1st day of therapy.

‡ Ampicillin data were derived from our previous experiments (Ref. 11).

with death in each group. As shown in Table 2, the addition of ampicillin to gentamicin or kanamycin significantly increased the serum bactericidal titers for both drugs ($p < 0.01$). However, the contribution of ampicillin to the efficacy of gentamicin and kanamycin *in vivo* was inconsistent. While the addition of ampicillin significantly ($p < 0.05$) increased the bacterial clearance *versus* kanamycin alone at 1, 2, and 3 days of completed therapy, ampicillin did not add any significant enhancement to the bacterial clearance by gentamicin (Table 2).

A total of 24 animals developed meningitis defined as positive CSF culture 18 h after inoculation and before therapy (Table 1). Most animals with meningitis, however, died within 1 day of therapy (16/24 or 67%) and bacterial clearance from CSF could not be properly assessed. Instead, the five treatment groups were compared for their efficacy to prevent the development of meningitis (Table 3). Among the 82 bacteremic animals without meningitis before therapy, 65 animals survived beyond 1 day of therapy and were available for this comparison. All survivors in the control group developed meningitis. This complication occurred more often in animals receiving kanamycin alone (42%) than in animals receiving gentamicin (20%), ampicillin + gentamicin (15%), and ampicillin + kanamycin (10%), but the differences were not statistically significant.

Table 4 summarizes the mortality among the five treatment groups. Overall, antimicrobial therapy significantly decreased the mortality rates (35% of 83 animals receiving antibiotics *versus* 100% of 10 animals receiving saline, $p < 0.001$). Although the differences among the four antimicrobial therapy groups were not significant, the mortality with kanamycin therapy was approximately 2-fold greater than that of the other antimicrobial regimens. Of note, almost all deaths in the gentamicin, ampicillin + gentamicin, and ampicillin + kanamycin groups (17/18 or 94%) occurred early during the first day of therapy, whereas most deaths in the kanamycin group (8/11 or 73%) occurred after 1 day of therapy.

DISCUSSION

The conventional antimicrobial therapy of gram-negative infection in a newborn is a combination of ampicillin and an aminoglycoside, usually gentamicin or kanamycin. Although both gentamicin and kanamycin have a high degree of activity *in vitro* against gram-negative bacilli, comparison of *in vivo* activity of the two drugs has been limited. Holloway and Taylor (13) have suggested a favorable effect of gentamicin over kanamycin *in vivo*, although the differences of the outcome were not statistically significant between the two.

We use an experimental model of *E. coli* bacteremia and meningitis in newborn rats. As shown previously (5, 8, 11), this model bears several important similarities to *E. coli* infections in human infants: age dependency, susceptibility to the K1-possessing strains, hematogenous infection of the meninges without the need for direct bacterial inoculation into the CSF and high mortality. *In vitro*, the *E. coli* strain was equally sensitive to both gentamicin and kanamycin as well as to ampicillin, and the addition of ampicillin to gentamicin or kanamycin exerted an indifferent response. The *in vivo* findings, however, suggested that gentamicin was more effective than kanamycin in eradicating the *E. coli* from blood, preventing the development of meningitis in bacteremic animals and decreasing mortality. As we have shown previously, the activity of ampicillin alone against the C5 strain is very limited *in vivo* with the mortality of 63 and 48% of bacteremic animals developing meningitis during ampicillin therapy (11). Thus, the efficacy observed *in vivo* with the combination of ampicillin and kanamycin was far greater than that expected with either drug alone, suggesting that the addition of ampicillin improved the outcome of kanamycin. In contrast, the efficacy of ampicillin + gentamicin combination was similar to that of gentamicin. These findings suggest that the contribution of ampicillin varies depending on the type of aminoglycoside used.

One intriguing finding was the time of death occurring among animals of the five treatment groups. As shown in Table 4, most deaths (94%) in the gentamicin, ampicillin + gentamicin, and ampicillin + kanamycin groups occurred early before the completion of 24 h therapy. In contrast, relatively few animals in the kanamycin group (27%) died within 24 h of therapy. This difference in rates of early death may reflect in part the *in vivo* effect of antibiotics upon the release of toxic bacterial products such as endotoxin from *E. coli*. Since kanamycin appeared less bactericidal *in vivo*, it may produce less bacteriolysis with lesser amounts of endotoxin, resulting in lower incidence of early death, whereas gentamicin is more bactericidal *in vivo* and may release larger amounts of endotoxin, leading to higher incidence of early death. Further studies are needed to elucidate factors responsible for early death in this model of neonatal gram-negative infection.

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