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Delayed Bactericidal Activity of β-Lactam Antibiotics Against *Listeria monocytogenes*: Antagonism of Chloramphenicol and Rifampin

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Penicillins are considered to be the drugs of choice for the treatment of listeric meningitis, and relapse of infection is rare when treatment is given in appropriate doses for at least 14 days. Despite this, in vitro studies by others have shown that penicillins are bacteriostatic against *Listeria* spp. We have shown that thienamycin, penicillin G, and ampicillin are the most active β-lactam antibiotics against *Listeria* spp. Of 10 strains tested, 9 were killed by ≤8 μg of β-lactam antibiotics (≥99.9% killing) when subcultures were performed after 48, rather than 24, h of incubation. In contrast, chloramphenicol, erythromycin, doxycycline, and rifampin were bacteriostatic after 48 h of incubation. In time-kill curves, these last drugs antagonized the bactericidal action of penicillins. In view of the inefficiency of opsonization in the cerebrospinal fluid, these antagonistic combinations should probably be avoided in documented or suspected listeric meningitis.

Listeria monocytogenes is a frequent cause of bacteremia and central nervous system infections in adults (2), particularly in immunodeficient patients (1, 5, 12). Penicillin G (8) and ampicillin (7) are considered the drugs of choice for the treatment of listeriosis. Treatment with these agents is usually successful (17, 28), but there are several case reports describing failure with these regimens, notably relapse of central nervous system infections (12, 18, 22, 27). A critical review of these cases reveals, in our opinion, that patients in most of these supposed penicillin failures received inappropriately low doses or short courses of penicillins or else received potentially antagonistic antibiotic combinations. Two recent reviews have also noted the adverse outcome of patients with listeric meningitis who were treated with a penicillin combined with chloramphenicol (2, 23). In this study, we examined the in vitro bactericidal behavior of penicillins against *Listeria monocytogenes* and the interactions of penicillins with chloramphenicol, rifampin, doxycycline, and erythromycin.

(This paper was presented in part previously [D. L. Winslow, E. Dieckman, J. Damme, and W. J. Holloway, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami Beach, Fla., abstr. no. 224, 1982].)

MATERIALS AND METHODS

Bacterial strains. Ten clinical isolates of *L. monocytogenes* were tested. All were isolated from blood or cerebrospinal fluid of patients from several New England, mid-Atlantic, south Atlantic, and south central states. A variety of serotypes were represented.

Media and antibiotics. Trypticase soy broth (BBL Microbiology Systems) was used for all studies. Antibiotics were obtained from their manufacturers and were diluted from frozen stock solutions shortly before use.

Antibiotic susceptibility testing and antibiotic interaction. A standard method was used for broth dilution susceptibility testing (16). Stationary-phase cultures obtained from overnight growth of organisms in broth were used for the determination of minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) and in time-kill curves. For determining MICs and MBCs, an inoculum of approximately 10^5 CFU/ml was used. Gentle shaking was used both at the time of inoculation and at the time of subculturing. MICs were determined by visual inspection after 18 to 24 h of incubation. MBCs were defined as the lowest concentrations of antibiotics producing ≥99.9% killing of the original inoculum (≤10 colonies on a subculture of 100 μl) and were determined by subculturing after 24 and 48 h of incubation.

Time-kill curves were performed in duplicate with previously described techniques (9). An inoculum size of approximately 5×10^5 CFU/ml was used. Broth was sampled at 0, 4, 24, and 48 h for colony counts.

Ampicillin was the β-lactam antibiotic used in studies of antibiotic interactions. The ampicillin concentration chosen was twice the 48-h MBC for ampicillin (usually 2 μg/ml). The concentrations of chloramphenicol, rifampin, doxycycline, and erythromycin used were based on the relative MICs of individual antibiotics for each isolate with the concentration of ampicillin chosen as the reference. (For example, if the MIC of ampicillin for an isolate was 0.25 μg/ml and the MIC of chloramphenicol for the same isolate was 2 μg/ml, then when 2 μg of ampicillin per ml was used in time-kill curves, the concentration of chloramphenicol was 16 μg/ml). Antagonism was defined as a decrease in killing by the combination of $\geq 1 \times \log_{10}$ as compared to the more active single agent.

RESULTS

The MICs and MBCs for all antibiotics are shown in Table 1. All isolates tested showed tolerance to the lethal effect of all antibiotics when subcultures were performed after 24 h of incubation as defined by a difference of ≥32-fold between MICs and MBCs. A significant decrease in the MBC was seen only with the β-lactam antibiotics when subculturing was deferred until after 48 h of incubation. After 48 h of incubation, MBCs for all three β-lactam antibiotics against 9 of 10 isolates were ≤ 8 μg/ml. If MBCs were defined by 99% killing, MBCs of all 10 isolates would have been ≤ 1 μg/ml for all three β-lactam agents. In a preliminary experiment comparing stationary-phase and log-phase cultures of *L. monocytogenes*, we could not demonstrate a significant effect of growth phase on tolerance.

Examples of typical time-kill curves for a single isolate of *L. monocytogenes* are shown in Fig. 1 and 2. The delayed bactericidal activity of ampicillin against all 10 isolates at 48 h was antagonized by the addition of chloramphenicol, rifampin, doxycycline, or erythromycin. (The time-kill curves seen with ampicillin-doxycycline and ampicillin-erythromycin were essentially identical to those seen with ampicillin-chloramphenicol and ampicillin-rifampin.)

DISCUSSION

Bactericidal antibiotics are believed by many authorities to be desirable in the treatment of meningitis (19) owing to the inefficiency of opsonization within the cerebrospinal fluid (3, 4). Paradoxically, penicillin and ampicillin are almost always efficacious in the treatment of listeric meningitis (17, 28), despite their bacteriostatic action in vitro (6, 14) and lack of demonstrable activity against intracellular bacteria. We believe that our data showing the delayed bactericidal activity of β-lactam antibiotics against *L. monocytogenes* at least partially explain their efficacy in the treatment of listeric meningitis. We speculate that the limited tolerance of *L. monocytogenes* to the lethal effect of β-lactam antibiotics resembles the tolerance of *Staphylococcus aureus* (20), which is of marginal clinical significance (J. J. Rahal, Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., Symposium on Staphylococcal Infections, 1980), more closely than it resembles the tolerance of the enterococci (10, 15), in which combination therapy is generally necessary for cure of endocarditis. Previous investigators have noted that the addition of an aminoglycoside to a penicillin accelerates the killing to *Listeria* spp. in vitro, with killing of ≥99.9% generally noted by 24 h (6, 14). Although synergistic therapy resulted in improved survival in a rabbit model of listeric meningitis (21), the clinical superiority of combination therapy over penicillin or ampicillin alone has not been demonstrated, and many clinicians have reservations about the toxicity of aminoglycosides and their poor penetration into the central nervous sys-

TABLE 1. Comparative in vitro activity of antibiotics against 10 *L. monocytogenes* strains

Antibiotic	Concn (μg/ml) for antibacterial activity				
	MIC range	MIC_{90}[a]	MBC range after incubation:		MBC_{90}[a] after 48 h of incubation
			24 h	48 h	
N-Formimidoyl thienamycin	≤0.03–0.12	0.12	2–>128	≤0.25–128	8
Penicillin G	0.06–0.5	0.25	16–128	0.5–64	8
Ampicillin	0.12–0.5	0.25	4–>128	<0.25–8	2
Rifampin	0.06	0.06	>128	32–>128	>128
Erythromycin	0.06–0.25	0.12	>128	>128	>128
Doxycycline	0.12–0.25	0.12	32–>128	32–>128	>128
Chloramphenicol	2–8	4	>128	>128	>128

[a] MIC_{90} and MBC_{90}, Concentrations of antibiotics at which 90% of isolates were inhibited or killed, respectively (as defined in the text).

tem. We speculate that if there is any beneficial effect in adding aminoglycosides to a penicillin in cases of human listeriosis, it is probably present only in the first 24 to 48 h of therapy.

Owing to the bacteriostatic activity of chloramphenicol against *Listeria* spp., the marked in vitro antagonism of the bactericidal activity of ampicillin is not unexpected. This antagonism may explain some of the reported failures of β-lactam antibiotics in the treatment of listeric meningitis (12, 22, 27). In addition, a widely quoted study which purported to show the superiority of ampicillin over penicillin G in treating listeric meningitis (11) was flawed by the fact that 9 of 12 penicillin-treated patients received chloramphenicol concomitantly, whereas only 2 of 12 ampicillin-treated patients received chloramphenicol. Since the early institution of appropriate therapy of listeric meningitis appears to influence the outcome (28), we believe that chloramphenicol should be avoided in the empiric treatment of meningitis in immunocompromised patients for whom the likelihood of listeric meningitis is high. The antagonistic effect of the addition of doxycycline or erythromycin or ampicillin may be analogous to the ampicillin-chloramphenicol interaction.

Because of its activity against intraleukocytic bacteria (13), rifampin would seem to be an attractive agent with which to treat listeric infections. When checkerboard techniques based on MICs are used to determine synergy, ampicillin-rifampin combinations are generally additive and occasionally synergistic against *Listeria* spp. (24). Since the bactericidal activity of both ampicillin and rifampin is minimal at 24 h, we cannot explain why one investigator (24) was

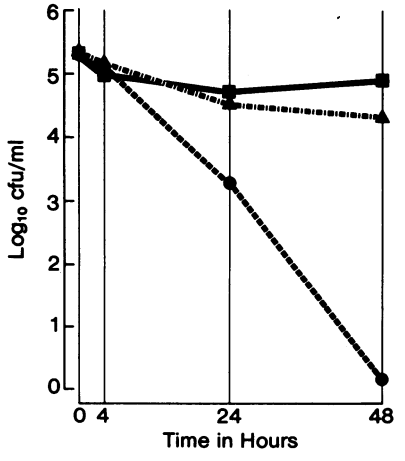

FIG. 1. Antibiotic interactions in time-kill curves. Symbols: ●, ampicillin (4 μg/ml); ■, chloramphenicol (16 μg/ml); ▲, ampicillin (4 μg/ml) plus chloramphenicol (16 μg/ml).

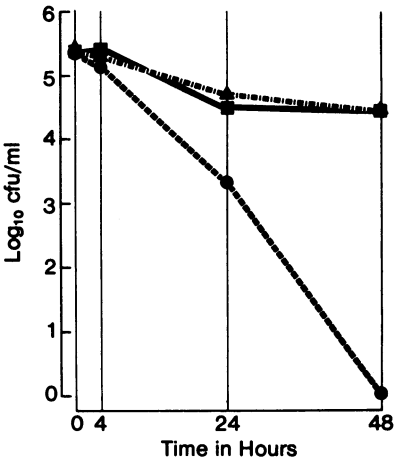

FIG. 2. Antibiotic interactions in time-kill curves. Symbols: ●, ampicillin (4 μg/ml); ■, rifampin (1 μg/ml); ▲, ampicillin (4 μg/ml) plus rifampin (1 μg/ml).

able to demonstrate bactericidal synergy with these drugs in combination. In an earlier study involving a modified checkerboard and 24-h MBCs, we found that the combination of ampicillin and rifampin generally had bacteriostatic activity (D. L. Winslow and G. A. Pankey, Abstr. 8th Int. Symp. Probl. Listeriosis, 1981, in press). The antagonism of rifampin observed in this study against the bactericidal activity of ampicillin in killing curves at 48 h of incubation was also not entirely unexpected since a similar phenomenon has recently been observed with vancomycin-rifampin combinations against *S. aureus* (26). The antagonism of rifampin against the bactericidal activity of antibiotics is not limited to cell-wall-active agents; we have observed rifampin to be antagonistic against the bactericidal action of trimethoprim against *Listeria* spp. (Winslow and Pankey, in press). In a mouse model of listeric infection, ampicillin and rifampin in combination appeared superior to ampicillin alone (25), but the mice were not immunosuppressed and probably did not have meningitis. In a rabbit model of listeric meningitis, rifampin was less rapidly bactericidal than ampicillin, and the combination of penicillin plus rifampin was no more active than penicillin alone (21). Because of the inefficient opsonization of bacteria within the cerebrospinal fluid (19), the behavior of *Listeria* spp. as an intracellular pathogen in meningitis may not be important, and our in vitro observations may have clinical significance. Since there are no in vivo studies to show unequivocally any benefit from the use of rifampin alone or in combination with other agents in listeric meningitis, we believe that the use of rifampin (as well as chloramphenicol, doxycycline, and erythromycin) should

probably be avoided in treating humans with listeriosis.

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