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Treatment of Experimental *Salmonella typhimurium* Infection with Mecillinam and Ampicillin

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The activities of mecillinam and ampicillin, alone and in combination, were evaluated in mice infected with the LT-2 strain of *Salmonella typhimurium*. The minimal inhibitory concentrations of mecillinam and ampicillin for this strain were, respectively, 6.2 and 0.4 μg/ml of culture medium. In vitro synergy was demonstrated. CF-1 mice inoculated intraperitoneally with 10^4 colony-forming units of the LT-2 strain were used in the therapeutic assessments. Treatment of subgroups with graded doses of the respective penicillins or their combination was initiated 24 h after inoculation and repeated at 6-h intervals for 5 consecutive days. Animals were observed during 21 days for mortality or sacrificed for quantitative cultures of spleen homogenates at the end of the treatment. Ampicillin in doses of ≥0.03 mg and mecillinam in doses of ≥0.5 mg reduced mortality rates from 77% in the saline-treated controls to a range of 0 to 47% ($P < 0.05$). The same doses of antibiotics also extended the median times to death and lowered significantly the means of splenic bacterial counts. When both drugs were combined in doses that were partially effective or subinhibitory alone, no synergistic effects were observed. These results showed that mecillinam and ampicillin given alone were effective in treating *S. typhimurium* infection but that combinations of the two drugs were not synergistic in controlling the course of infections.

The new 6β-amidinopenicillanic acid derivative mecillinam has antibacterial activity against most *Enterobacteriaceae*, including the *Salmonellae* (10, 16). Although mecillinam, like other beta-lactam antibiotics, acts to inhibit cell wall synthesis, it differs from other beta-lactam antibiotics by binding selectively to penicillin-binding protein 2 and by causing bacteria to assume enlarged spherical shapes that are osmotically stable (14, 15). Lysis of bacteria then occurs, and studies with adenyl cyclase-deficient mutants of *Escherichia coli* suggest that cyclic adenosine monophosphate plays a role in mecillinam-induced lysis (1). Tybring and Melchior (17) and Neu (11) showed that the combination of mecillinam and ampicillin produced synergistic inhibition in vitro of some strains of *Salmonellae*. Furthermore, Grunberg et al. (5) demonstrated synergistic action of mecillinam and other beta-lactam antibiotics against experimental infections in mice with *Salmonella* species.

Mecillinam has been used to treat patients with typhoid fever in two studies, one of which reported satisfactory results (4) and the other of which reported treatment failures (8). Because the murine infection with *Salmonella typhimurium* is similar to human typhoid fever with respect to the production of bacteremia and a sometimes fatal accumulation of organisms in the reticuloendothelial cells, we examined the activity of mecillinam in experimental *S. typhimurium* infections by comparing this activity with that of ampicillin. We have also examined combinations of the two drugs for possible synergistic effects in vivo.

MATERIALS AND METHODS

In vitro testing of antibiotic susceptibility. *S. typhimurium* LT-2 was used throughout this study. This strain is virulent in mice (3), resembling in this respect other *Salmonella* strains that have been employed in experimental mouse typhoid (7). Ampicillin trihydrate (Bristol Laboratories, Syracuse, N.Y.) was dissolved in 5% sodium carbonate, and mecillinam (Ro 10-90, 70/000, Hoffmann-LaRoche, Nutley, N. J.) was dissolved in 0.9% NaCl. These solutions in various concentrations were added to NIH broth supplemented with 0.6% NaCl to bring the osmolality to 438 mosmol/liter. Tubes containing 1 ml of broth were inoculated with 10^5 bacteria and were read for turbidity after 24 and 48 h of incubation at 35°C. Checkerboard titrations of ampicillin and mecillinam in combination were carried out by using twofold dilutions of each antibiotic. Additive, synergistic, or antagonistic effects of the drugs were identified via use of isobolograms (12).

Assessments of in vivo activities. All mice used in these experiments were CF-1 females (Carworth Farms, New City, N.Y.), approximately 8 weeks old, weight 18 to 20 g. For these mice, an intraperitoneal

inoculum of 10^3 colony-forming units (CFU) of the LT-2 strain was the 50% lethal dose (observation period, 21 days) (3). In assessments of multiplication in vivo, spleens were removed aseptically 6 h after the last antibiotic dose, washed twice with sterile water, homogenized in 1 ml of 0.9% NaCl by using glass mortars fitted with Teflon pestles, and diluted 10-fold in 0.9% NaCl; 0.1-ml volumes of the concentrated and diluted homogenates were streaked onto nutrient agar plates. Colony counts were made after 24 h of incubation at 35°C.

In the therapeutic studies, treatment with mecillinam or ampicillin was started 24 h after intraperitoneal inoculation of 10^4 CFU of *S. typhimurium* suspended in 0.2 ml of 0.9% NaCl. Various doses of these antibiotics in 0.1 ml of 0.9% NaCl were injected subcutaneously every 6 h for 5 days. In each dosage group of each experiment, 10 mice were used for observation of mortality, and 5 to 8 mice were used for bacterial enumeration in the spleen. The incubation period of 24 h was selected from results showing that a single large dose of ampicillin (4 mg) given 24 h after inoculation did not protect mice from death, whereas the same dose given 1 h after inoculation resulted in the survival of nearly all mice. Previous studies with ampicillin and other antimicrobial drugs in this model had shown that treatment for 5 days affected the course of infection without eliminating all bacteria from the spleen (3). Mortality rates during 21 days of observation and the mean $\log_{10}$ CFU per spleen at the end of antibiotic treatment were used as measurements of drug effect when compared with saline-treated control mice.

RESULTS

In vitro results. The minimal inhibitory concentrations of mecillinam and ampicillin were 6.2 and 0.4 μg/ml, respectively, as shown in Fig. 1. These drugs had synergistic activity against the LT-2 strain.

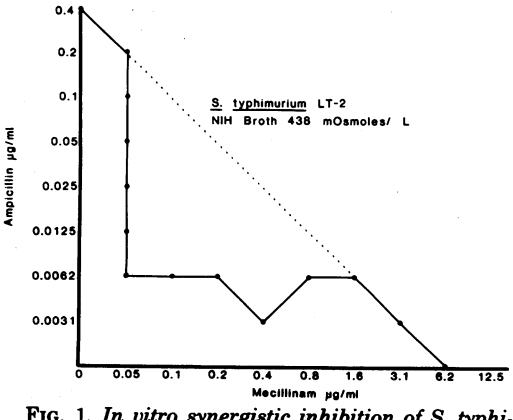

FIG. 1. *In vitro synergistic inhibition of S. typhimurium LT-2 by mecillinam and ampicillin. Each data point is the minimum concentration of each drug alone or minimum concentrations of the two drugs together that inhibited growth. The dotted line shows an indifferent isobol.*

In vivo effects of mecillinam and ampicillin given alone. The efficacies of mecillinam and ampicillin were studied in three consecutive experiments. The mortality among saline-treated control mice was 77% during the 21-day period of observation. As shown in Table 1, mortality was reduced significantly by mecillinam in doses of 0.5 mg or higher and by ampicillin in doses of 0.03 mg or higher ($P < 0.05$ by chi-square analysis). With doses of those drugs that effectively decreased mortality rates, there was a prolongation of survival time among the mice that died. The median days to death in treated mice ranged from 10 to 15.5, as compared with 8 days in controls. The spleens of mice 6 h after the last dose of drug contained significantly fewer organisms than spleens of control mice; means of $\log_{10}$ CFU were 3.90 to 4.95 in the groups receiving mecillinam in doses of 0.5 mg or higher or ampicillin in doses of 0.03 mg or higher as compared with 5.97 in the saline controls ($P < 0.05$ by Student's t test).

In vivo effects of mecillinam and ampicillin in combination. The results of these studies, shown in Fig. 2, show that the drugs produced no more than additive effects when administered in combination. The mortality rates in the grids of combined therapy were

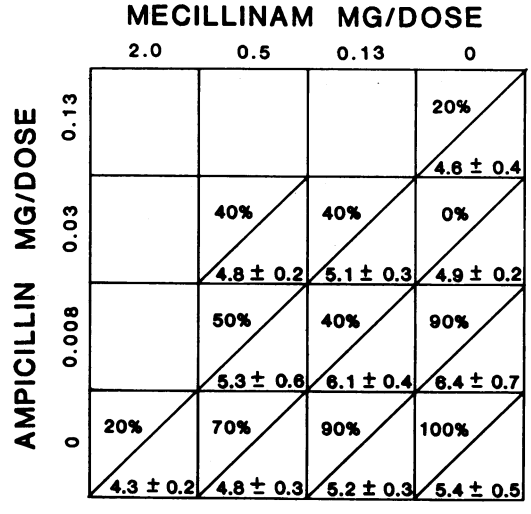

FIG. 2. *In vivo activity of mecillinam and ampicillin. CF-1 mice were infected intraperitoneally with 10^4 S. typhimurium LT-2 and treated with mecillinam or ampicillin alone or in combination for 5 days starting 24 h after inoculation. The upper part of each square shows the mortality rate during 21 days of observation. The lower part of each square shows the mean $\log_{10}$ CFU per spleen (± standard error) at the end of the treatment. Synergy was not observed.*

TABLE 1. *Effects of mecillinam and ampicillin on mortality, time of death, and number of splenic bacteria in mice infected with S. typhimurium LT-2*[a]

Treatment		% Mortality (range)[b]	Median time of death (days) after infection (range)		Log_{10} CFU in the spleen[c]
Saline		77 (40–100)	8	(3–13)	5.97 ± 0.31
Mecillinam (mg/dose)					
2.0	(400)[d]	10 (0–20)[e]	11.5	(7–16)	3.90 ± 0.23[f]
0.5	(100)	47 (20–70)[e]	10	(3–16)	4.95 ± 0.23[f]
0.13	(25)	67 (50–90)	8	(5–18)	5.50 ± 0.34
Ampicillin (mg/dose)					
0.13	(25)	20 (20–20)[e]	11.5	(7–17)	4.44 ± 0.30[f]
0.03	(6)	13 (0–30)[e]	15.5	(7–17)	4.90 ± 0.15[f]
0.008	(1.5)	70 (50–90)	8	(6–11)	6.36 ± 0.48

[a] Drugs were injected subcutaneously every 6 h for 5 days starting 24 h after intraperitoneal inoculation of 10^4 bacteria.

[b] Results were pooled from three experiments in which each treatment group contained 10 mice which were observed for mortality for 21 days after infection. Within parentheses are shown the ranges in mortality in three experiments.

[c] Mean ± standard error of CFU per homogenized spleen in three experiments with a total of 12 to 18 mice in each treatment group. Mice were killed 6 h after the last dose of antibiotic.

[d] Milligrams per kilogram per day is shown within parentheses.

[e] Proportion of dead animals less than in saline control group by chi-square analysis ($P < 0.05$).

[f] Mean number of CFU was significantly less than that of the saline control by Student's t test ($P < 0.05$).

usually lower than the corresponding marginal values but not lower than the marginal mortalities of the next higher doses, indicating additive but not synergistic effects of the drugs on mortality. The mean splenic bacterial counts were never lower than both marginal values.

DISCUSSION

The in vivo studies summarized here show that mecillinam and ampicillin were effective in reducing mortality, extending survival times, and decreasing the bacterial populations of the spleen in mice infected with the LT-2 strain of *S. typhimurium*. The results of the in vitro studies show that these drugs exhibit synergy against this LT-2 strain of *S. typhimurium*, confirming the findings of others that these two antibiotics are synergistically active against many species of *Enterobacteriaceae* (11, 17). In vivo, however, combinations did not exhibit synergistic effects in dosages, ranging from effective to subinhibitory, of the respective drugs. This result differs from that of Grunberg et al. (5). This divergence may be due to the initiation of treatment immediately after inoculation in their study. Thus, the inoculum may have been killed before the infection was firmly established in the host cells. Synergy was also noted by Scheld et al. (13) in experimental meningitis caused by *E. coli*, a model of an extracellular infection. Thus, it appears that bacteria in extracellular locations are more susceptible to the synergistic action of mecillinam and ampicillin. *Salmonellae* in intracellular locations may be insusceptible to this action because of a lower pH than in the extracellular fluid or a slower rate of bacterial multiplication.

Our inability to show mecillinam-ampicillin synergy in experimental *S. typhimurium* infections actually may be in accord with the finding of Lorian and Atkinson (6) that *S. typhimurium* exposed to subinhibitory concentrations of ampicillin or mecillinam was not more susceptible to the bactericidal action of serum and blood than were untreated bacteria. The disparity between our in vitro and in vivo results on synergy may reflect difficulties in standardizing definitions of synergy and deriving therapeutic applications from in vitro studies (29).

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