ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1981, p. 786-788
0066-4804/81/050786-03$02.00/0

Vol. 19, No. 5

Effect of Subinhibitory Concentrations of Mecillinam on the Serum Susceptibility of *Escherichia coli* Strains

PETER W. TAYLOR,[1]* HOWARD GAUNT,[1] AND FRANK M. UNGER[2]

Department of Microbiology, The University, Leeds LS2 9JT, United Kingdom,[1] and Sandoz Forschungsinstitut, A-1235 Vienna, Austria[2]

Received 18 August 1980/Accepted 28 January 1981

Four serum-resistant clinical isolates of *Escherichia coli* were grown in the presence of various subinhibitory concentrations of mecillinam or pivmecillinam and then exposed to the bactericidal action of human serum. All strains became more serum susceptible as a result of pregrowth in medium containing mecillinam, but the concentration of antibiotic needed to produce the effect varied according to the strain being used. Production of ovoid or round cells was a prerequisite for sensitization to serum. Growth in the presence of mecillinam did not alter the response to serum of a serum-susceptible *E. coli* strain.

Current screening procedures for the detection of compounds of potential use in the chemotherapy of bacterial infections are usually based on in vitro inhibition of bacterial growth. It appears that compounds exist that are able to modify the virulence properties of bacteria in such a way as to increase their susceptibility to host defense mechanisms. For example, serum-resistant *Escherichia coli* became sensitized to the bactericidal action of human serum when pregrown in the presence of low concentrations of diphenylamine (3). Exposure of serum-resistant, polymyxin B-resistant *Proteus mirabilis* to polymyxin B rendered the cells susceptible to human serum (9). *Staphylococcus aureus* grown in the presence of subinhibitory concentrations of nafcillin showed an increased susceptibility to phagocytes (4). Very recently, Alexander and coworkers found that subinhibitory concentrations of rifampin converted a strain of *E. coli* from serum resistant to serum susceptible (1). Similar compounds might prove useful in the treatment of infectious diseases by virtue of their ability to phenotypically attenuate bacterial pathogens in the host, but this activity would not be detected by traditional screening methodology.

In recent years both clinical observations and studies with experimental animal models have indicated that serum resistance is an important determinant of pathogenicity in a number of infections caused by gram-negative bacteria (11). We have, therefore, instituted a search for compounds that phenotypically convert serum-resistant strains of *E. coli* to serum-susceptible strains, by using a screening procedure based on the Steers-Foltz replicator assay for serum bactericidal activity described by Provonchee and Zinner (7). Results obtained with the 6-β-amidinopenicillanic acids mecillinam and pivmecillinam (5) indicate that subinhibitory concentrations of these compounds are able to influence the serum reactivity of a number of *E. coli* strains; an investigation of this phenomenon forms the basis of the present report.

MATERIALS AND METHODS

Reagents. Normal human serum was obtained from the National Blood Transfusion Service, Seacroft, Leeds, United Kingdom, and stored in small aliquots at −20°C until required. Mecillinam (FL1060) and pivmecillinam (FL1039) were the gifts of L. Tybring, Leo Pharmaceutical Products, Ballerup, Denmark.

Bacteria. Four serum-resistant clinical isolates of *E. coli* were selected for this study (Table 1). Because of a report (6) that pregrowth in the presence of mecillinam may increase resistance to the bactericidal action of serum, a susceptible clinical isolate was also included.

Serum bactericidal assay. A range of serial dilutions of either mecillinam or pivmecillinam was prepared in 10 ml of nutrient broth, and the tubes were inoculated with 0.1 ml of an overnight nutrient broth culture of the test organism. After 18 h of incubation at 37°C, 1 ml from each culture showing growth was transferred to 9 ml of nutrient broth containing the corresponding concentration of antibiotic. The tubes were then incubated at 37°C with agitation until the optical density of the cultures had reached a value of 0.2 to 0.4 (usually 90 to 180 min). Cells were then deposited by centrifugation and resuspended to a concentration of about 10^6 bacteria per ml in 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 8.4). A sample (0.5 ml) of the suspension was added to 1.5 ml of serum, and viable counts were obtained at the beginning of the test and after 1, 2, and 3 h of incubation at 37°C.

RESULTS

The original screening procedure utilized *E. coli* strain LP1674 as the test organism; when pregrown in the absence of mecillinam, this strain grew rapidly in serum (Fig. 1). However, pregrowth in the presence of mecillinam at concentrations as low as 0.025 μg/ml led to a marked reduction in the serum resistance of this strain. At 0.1 μg of mecillinam per ml, less than 10% of antibiotic-treated bacteria survived 1 h of incubation in serum (Fig. 1). A similar, although less marked, effect was observed when strain LP1674 was pregrown in medium containing pivmecillinam (Table 2). With both mecillinam and pivmecillinam, a phenotypic conversion to serum susceptibility is suggested by the fact that the 2- and 3-h viable counts were frequently higher than those recorded after 1 h of exposure to antibiotic-free serum. Three other serum-resistant *E. coli* strains, all producing the K1 capsular polysaccharide, were also examined; in all cases, conversion to serum susceptibility occurred after exposure to both mecillinam and pivmecillinam (Table 2). There was, however, marked variation in the concentration of antibiotic needed to produce the effect, which appeared to be dependent on the strain used. Antibiotic-treated bacteria were able to grow in serum heated to 56°C for 30 min, indicating that killing was complement mediated.

In all cases, cells became serum susceptible only after their conversion by the antibiotic to spherical forms. There was, however, no obvious

TABLE 1. *Characteristics of E. coli strains used in this study*

Strain	Serotype	Source	Reference	Reaction to serum
LP1674	O7:K1	Urinary tract infection	10	Resistant
C6	Rough:K1:H7	Neonatal meningitis	2	Promptly susceptible
C8(78)	O7:K1	Neonatal meningitis	2	Resistant
C10	O7:K1	Neonatal meningitis	2	Resistant
C14	K1	Neonatal meningitis	2	Resistant

TABLE 2. *Modification of the response of serum-resistant E. coli strains to the bactericidal action of human serum due to pregrowth in the presence of mecillinam or pivmecillinam*

Strain	Mecillinam (μg/ml)		Pivmecillinam (μg/ml)	
	MIC[a]	Concn producing reduction in serum resistance[b]	MIC[a]	Concn producing reduction in serum resistance[b]
LP1674	12.5	0.025–6.2	50	0.8–25
C8(78)	0.8	0.1–0.4	3.2	0.4–1.6
C10	0.8	0.4	1.6	0.4–0.8
C14	100	0.1–50	100	1.6–50

[a] MIC, Minimal inhibitory concentration, taken as the lowest concentration of antibiotic at which no visible growth occurred following overnight incubation of cells at 37°C (see text).

[b] All strains listed in this table grew rapidly in serum when pregrown in the absence of antibiotics. The range recorded here includes pregrowth concentrations causing a 50% or more reduction in viable count after 1 h of incubation of antibiotic-treated bacteria in human serum.

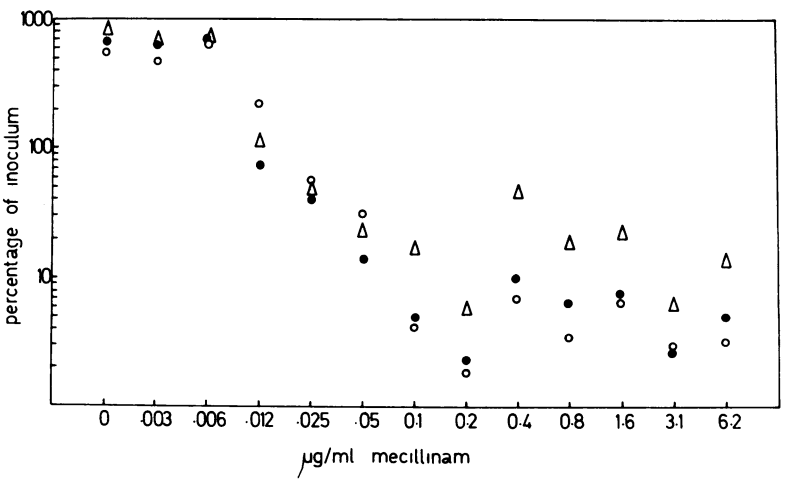

FIG. 1. *The effect of pregrowth in the presence of various concentrations of mecillinam on the serum reactivity of E. coli LP1674. Percentage of inoculum after 1 h* (○), *2 h* (●), *and 3 h* (△) *of incubation in human serum. Each value represents the mean of at least three determinations.*

relationship between reactivity in serum and cell mass, as determined by interference microscopy.

One serum-susceptible strain, *E. coli* C6, was also examined. This strain was rapidly killed by serum, with no surviving cells being found after 1 h of incubation in serum. Pregrowth in the presence of either mecillinam or pivmecillinam had no effect on the survival of this strain in serum.

DISCUSSION

In recent years, a number of reports have appeared demonstrating that certain compounds, particularly antibiotics, are able to modify the virulence properties of bacteria at concentrations at which they exert little or no antibacterial activity. In the present report, we have shown that pregrowth of serum-resistant strains of *E. coli* in medium containing subinhibitory concentrations of mecillinam renders them susceptible to the complement-mediated bactericidal action of human serum. Although direct evidence is lacking, it would seem likely that this transition to sensitivity is phenotypic and results from inhibition of biosynthesis of cell surface structures that are thought to protect gram-negative bacteria from attack by activated complement components; this possibility is currently being investigated. The formation of oval or round cells accompanies the effect in each case.

We have screened a large number of β-lactam compounds, including ampicillin and penicillins, for the ability to modify the serum reactivity of gram-negative bacteria, and have so far found this activity to be restricted to mecillinam and some of its analogs (P. W. Taylor and F. M. Unger, unpublished data). It is therefore tempting to speculate that this activity is related in some way to the unique mode of action of mecillinam, which binds exclusively to penicillin binding protein 2 (8). In some cases, the successful outcome of chemotherapy with mecillinam may be due in part to its virulence-modifying properties, which would be expected to assume significance if tissue levels of the antibiotic dropped below the effective minimal inhibitory concentration. In this respect it is interesting to note that it has not yet been possible to isolate abnormal forms in animal experiments or from patients receiving mecillinam (12).

Recently, Lorian and Atkinson (6) examined the serum susceptibility of five species of *Enterobacteriaceae* grown in the presence of subinhibitory concentrations of mecillinam and found an antibiotic-induced increase in susceptibility with only one strain (*E. coli* 14). In most cases the serum bactericidal effect on round cells was less than the effect on control cells grown in the absence of the antibiotic. However, their technique employed bacteria grown on nitrocellulose membranes placed on solid medium in an attempt to prevent damage to the cells from osmotic effects. It will require further work to ascertain whether or not this difference in methodology is sufficient to explain the results obtained. In addition, the bacterial strains used may be of importance; in the present study, the effect was noticeably more marked with some *E. coli* strains than with others.

ACKNOWLEDGMENTS

We thank L. Tybring for gifts of mecillinam and pivmecillinam, R. Bortulussi for strains (C prefixed), and Malcolm Pepper for supplying human serum.

LITERATURE CITED

1. **Alexander, W. J., C. G. Cobbs, and R. Curtiss III.** 1980. Modification of bacterial serum susceptibility by rifampin. Infect. Immun. **28**:923-926.
2. **Björksten, B., R. Bortolussi, L. Gothefors, and P. G. Quie.** 1976. Interaction of *E. coli* strains with human serum: lack of relationship to K1 antigen. J. Pediatr. **89**:892-897.
3. **Feingold, D. S.** 1969. The serum bactericidal reaction. IV. Phenotypic conversion of *Escherichia coli* from serum resistance to serum sensitivity by diphenylamine. J. Infect. Dis. **120**:437-443.
4. **Friedman, H., and G. H. Warren.** 1974. Enhanced susceptibility of penicillin resistant staphylococci to phagocytosis after in vitro incubation with low doses of nafcillin (38177). Proc. Soc. Exp. Biol. Med. **146**:707-711.
5. **Godtfredsen, W. O.** 1977. An introduction to mecillinam. J. Antimicrob. Chemother. **3B**:1-4.
6. **Lorian, V., and B. A. Atkinson.** 1979. Effect of serum and blood on Enterobacteriaceae grown in the presence of subminimal inhibitory concentrations of ampicillin and mecillinam. Rev. Infect. Dis. **1**:797-806.
7. **Provonchee, R. B., and S. H. Zinner.** 1974. Rapid method for determining serum bactericidal activity. Appl. Microbiol **27**:185-186.
8. **Spratt, B. G.** 1977. The mechanism of action of mecillinam. J. Antimicrob. Chemother. **3B**:13-19.
9. **Sud, I. J., and D. S. Feingold.** 1975. Detection of agents that alter the bacterial cell surface. Antimicrob. Agents Chemother. **8**:34-37.
10. **Taylor, P. W.** 1978. The effect of the growth environment on the serum sensitivity of some urinary *Escherichia coli* strains. FEMS Microbiol. Lett **3**:119-122.
11. **Taylor, P. W., C. Hughes, and M. Robinson.** 1979. Plasmids and the serum resistance of enterobacteria, p. 135-143. *In* K. N. Timmis and A. Pühler (ed.), Plasmids of medical, environmental and commercial importance. Elsevier/North Holland Publishing Co., Amsterdam.
12. **Tybring, L.** 1977. Special aspects of laboratory investigations with mecillinam. J. Antimicrob. Chemother. **3B**:23-27.