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Activity of Cefazolin Against Dense Populations of Enterobacteria

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The in vitro activity of cefazolin was assessed by continuous turbidimetric monitoring of cultures of gram-negative bacilli and the results were compared with those previously obtained with other beta-lactam agents using the same strains and methods. Cefazolin was found to induce rapid lysis of ampicillin-susceptible and -resistant strains of *Escherichia coli* at a lower concentration than any other beta-lactam agent tested; its stability to beta-lactamase, as judged by regrowth studies, was generally considerably greater than that of other antibiotics of this group. Tested against 103 ampicillin-resistant enterobacteria, cefazolin was found to be more active than cephalothin against *E. coli*, but no systematic increase in susceptibility to cefazolin was seen with other species. A study of cefazolin in an in vitro model which simulates the hydrokinetic features of the urinary bladder showed it to be as active as ampicillin against ampicillin-susceptible *E. coli* and as active as cephalothin against ampicillin-resistant *E. coli*.

Because conventional titrations of cephalosporins against gram-negative bacilli are subject to marked inoculum effects (2, 10), we have favored continuous turbidimetric monitoring as a method of assessing the in vitro activity of these agents (5). This technique reveals both the intrinsic activity of the compounds against large populations of bacteria, such as are likely to be encountered in infected lesions, and their stability to bacterial beta-lactamases.

We have previously employed turbidimetric methods to compare the in vitro activity of seven beta-lactam antibiotics against ampicillin-susceptible and -resistant gram-negative bacilli (5), and to investigate the influence of the unusual growth conditions of the urinary bladder on the performance of the antibiotic (6, 9). The present paper reports the results of similar investigations into the in vitro activity of cefazolin.

MATERIALS AND METHODS

Bacterial strains. Two ampicillin-susceptible strains of *Escherichia coli*, designated ECSA 1 and ECSA 2, and 103 ampicillin-resistant coliform bacilli (46 *E. coli*, 28 *Klebsiella* sp. eight *Proteus mirabilis*, one *Proteus vulgaris*, one *Proteus morganii*, four *Enterobacter* sp. six *Serratia marcescens*, five *Hafnia* sp., three *Providencia*, one *Citrobacter* sp.), all originally isolated from infected urine, were used. The criterion of resistance was absence of a zone of inhibition or a small zone of inhibition ($\leq$14 mm) around a 10-μg ampicillin disk on blood agar plates seeded with a bacterial inoculum sufficient to produce a subconfluent growth. The two ampicillin-susceptible strains gave inhibition zones of >24 mm when tested in this manner.

Growth medium. The complete medium, having an osmolality of about 325 mOsmol/kg, previously described (5) was used. In experiments with *Proteus* sp. and *S. marcescens*, NaCl was omitted from the medium, thereby lowering the osmolality to about 170 mOsmol/kg, as such strains give an altered response to beta-lactam agents at higher osmolalities (4).

Static turbidimetric system. Cultures were grown from small inocula (ca. 10^4 organisms per ml), derived from overnight broth cultures, in the 12-channel bacterial growth monitoring system described by Mackintosh et al. (8). Antibiotic was added at a standard point in the logarithmic growth phase equivalent to 30% maximum opacity (viable count ca. 5×10^7 organisms per ml). In experiments with the two ampicillin-susceptible (ECSA 1 and ECSA 2) and two of the ampicillin-resistant (*Bur* and *Gen*) *E. coli* strains, cultures were exposed to cefazolin at concentrations of 8, 16, 32, 64, and 128 μg/ml. With the remaining strains, a single pulse of cephalosporin was used to achieve a concentration of 250 μg/ml.

Bladder model. A full description of the model and the experimental rationale have been provided in previous reports (6, 9). Briefly, 20 ml of an overnight broth culture (representing an arbitrary residual bladder volume) are diluted with fresh broth at 1 ml per

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min to simulate the ureteric urine flow rate. At preset intervals (1 h in the present experiments), a "micturition" episode empties the bladder of all but the residual volume. In the present experiments, sufficient cefazolin to achieve an initial concentration of 500 μg/ml was added immediately after the fourth hourly micturition, at which point the culture had emerged from the lag phase and was growing at a rate similar to that obtained in the fluctuating equilibrium state (9).

Microscopy. Morphological changes accompanying exposure of organisms to cefazolin were observed microscopically in simple wet preparations.

RESULTS

Morphological response. The concentration ranges over which different morphological changes were induced in *E. coli* strain ECSA 1 after 1 h exposure to cefazolin are shown in Fig. 1.

Turbidimetric studies. (i) Response profiles. Providing osmotic protection is withheld, beta-lactam antibiotics induce lysis of susceptible gram-negative bacilli after a period of time which varies with the antibiotic concentration (5). On continued incubation, regrowth of culture which had initially succumbed to antibiotic action sometimes occurs (apparently due to antibiotic breakdown), even in strains which appear susceptible when tested by conventional techniques (5). From the records obtained in the continuous opacity monitoring device, the time elapsing between the addition of cefazolin and (A) lysis of the culture and (B) regrowth of the culture was measured (Fig. 2). The times are plotted against antibiotic concentration in Fig. 2 to 4, together with similar turbidimetric response profiles (5) of other beta-lactam antibiotics for comparison. The two ampicillin-susceptible *E. coli* strains behaved in a very similar manner and only the results obtained with strain ECSA 1 are shown (Fig. 2). Results obtained with the strains *Bur* and *Gen* are shown in Fig. 3 and 4, respectively.

In terms of lysis (an index of intrinsic activity), cefazolin was found to be the most active agent among those tested against ampicillin-

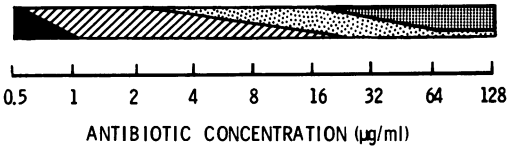

FIG. 1. *Morphological response profile of an ampicillin-susceptible strain of E. coli (ECSA 1) after 1 h exposure to cefazolin. Black, normal; hatching, filaments; light stippling, emergent spheroplasts; dense stippling, lysed cell debris.*

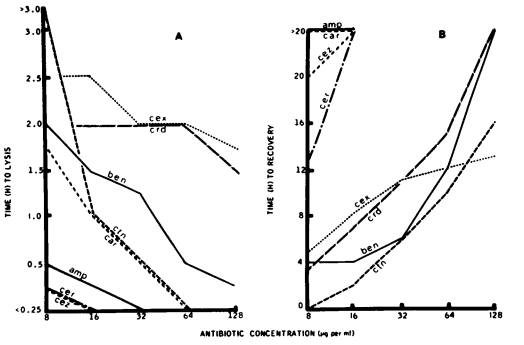

FIG. 2. *Turbidimetric response profiles of an ampicillin-susceptible E. coli strain (ECSA 1) for eight beta-lactam antibiotics, showing the time elapsing after the addition of various antibiotic concentrations before (A) lysis of the culture occurred or (B) regrowth of the culture occurred. Abbreviations: ben, benzylpenicillin; amp, ampicillin; car, carbenicillin; cer, cephaloridine; ctn, cephalothin; cez, cefazolin; cex, cephalexin; crd, cephradine.*

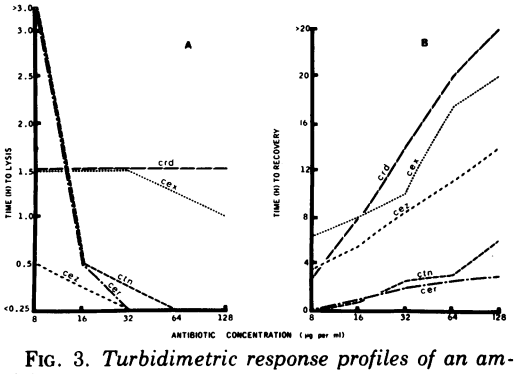

FIG. 3. *Turbidimetric response profiles of an ampicillin-resistant E. coli strain (Bur) for five beta-lactam antibiotics. Explanation and abbreviations as for Fig. 2.*

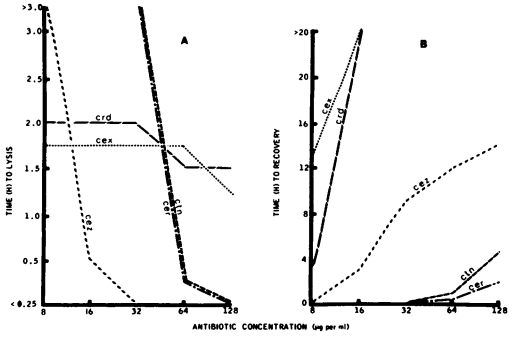

FIG. 4. *Turbidimetric response profiles of an ampicillin-resistant E. coli strain (Gen) for five beta-lactam antibiotics. Explanation and abbreviations as for Fig. 2.*

resistant *E. coli* and only cephaloridine showed an equivalent lytic activity against the ampicillin-susceptible *E. coli* strains. In terms of regrowth (chiefly an index of susceptibility to beta-lactamase), a difference was observed between strains. Against the ampicillin-susceptible strains ECSA 1 and ECSA2, cefazolin was almost as active as ampicillin or carbenicillin; with the ampicillin-resistant strains *Bur* and *Gen*, cefazolin occupied an intermediate position between the poorly active cephalothin and cephaloridine on the one hand, and the more active cephalexin and cephradine on the other.

(ii) Ampicillin-resistant enterobacteria. Exposure to a single dose of 250 μg of cefazolin per ml caused rapid lysis of the cultures in all but six (three *Klebsiella* sp., three *Hafnia* sp.) of the 103 strains tested. The times elapsing before regrowth varied considerably among strains; these times are compared with those previously obtained with the same strains exposed to cephalothin in Fig. 5 (*E. coli*) and 6 (*Klebsiella* and other species). The diagonal line in these figures represents the line of equivalent activity, so that points lying above that line represent strains suppressed for a longer period by cefazolin, and those lying below the line represent strains suppressed for a longer period by cephalothin. Only one *E. coli* strain was suppressed for a longer period by cephalothin than cefazolin. With the *Klebsiella* and other species, no such systematic superiority of cefazolin was observed.

(iii) Bladder model. The ampicillin-susceptible *E. coli* strain (ECSA 1) and four ampicillin-resistant (*Bur*, *Obr*, *Kin*, *Hos*) *E. coli* strains were examined in this system. In all cases, addition of sufficient cefazolin to produce an initial concentration of 500 μg/ml caused immediate lysis of the cultures corresponding to that seen in the static system, but enhanced by the dilution effect. The time taken for the opacity to reattain the level prevailing at the point of antibiotic addition varied with the strain as shown in Table 1. Data previously obtained with the same strains exposed to ampicillin, cephaloridine, and cephalothin are included for comparison (12; D. Greenwood

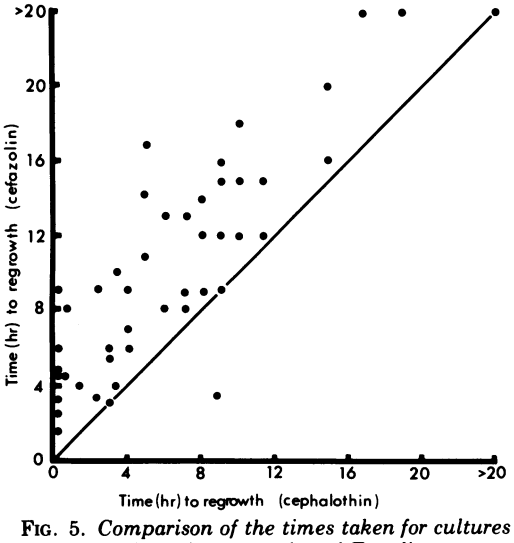

FIG. 5. *Comparison of the times taken for cultures of 46 ampicillin-resistant strains of E. coli to regrow after exposure to 250 μg of cefazolin or cephalothin per ml.*

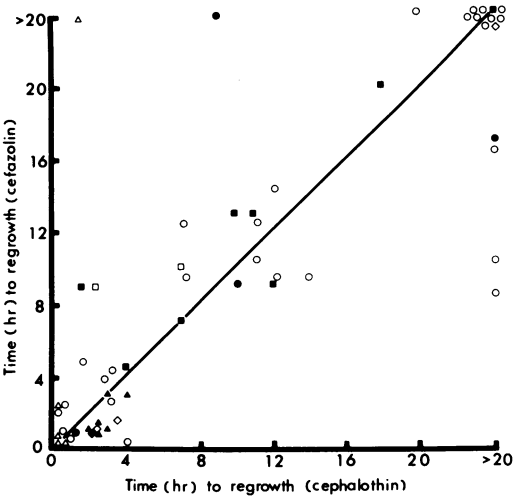

FIG. 6. *Comparison of the times taken for cultures of 57 ampicillin-resistant strains of coliform bacilli to regrow after exposure to 250 μg of cefazolin or cephalothin per ml. Symbols:* ○, *Klebsiella sp.;* ●, *Enterobacter sp.;* ■, *P. mirabilis;* □, *P. vulgaris and P. morganii;* △, *Hafnia sp.;* ▲, *S. marcescens;* ◇, *Providencia;* ◆, *Citrobacter sp.*

TABLE 1. *Comparison of the times taken for cultures of E. coli in the bladder model to recover to the opacity level prevailing at the time of antibiotic addition after exposure to cefazolin and four other agents*

Strain	Time (h) to recovery after a single dose (500 μg/ml) of:				
	Cefazolin	Cephaloridine	Cephalothin	Cephalexin	Ampicillin[a]
ECSA 1	11	8	6.5	6	10
Bur	8	4.5	7.5	8	NL
Kin	7	6	7	5	NL
Obr	10	6	7	5	ND
Hos	3	2	4	3.5	ND

[a] NL, No lytic effect produced; ND, not done.

and F. O'Grady, Br. J. Exp. Pathol., in press). Cefazolin was found to be as effective as cephalothin against three of the five strains, and substantially more effective against the remaining two. The effectiveness of cefazolin against the ampicillin-susceptible strain was comparable to that of ampicillin itself.

DISCUSSION

The morphological response profile of cefazolin (Fig. 1) shows that this agent was able to induce the characteristic spectrum of morphological changes in gram-negative bacilli over a fairly narrow concentration range. The activity of cefazolin, according to morphological criteria, was found to be at least as great as cephaloridine, the most active of the cephalosporins examined in this way in a previous study (5).

Conventional titrations of cephalosporins against gram-negative bacilli are subject to inoculum effects which are particularly marked in the case of ampicillin-resistant strains, and we have previously argued (4) that, because of this fact, turbidimetric methods may give a better indication of the relative susceptibility of such strains than more conventional methods.

Turbidimetry confirms the high activity of cefazolin against both ampicillin-susceptible and -resistant *E. coli* and shows it to be one of the most intrinsically active beta-lactam agents against this species. With other ampicillin-resistant species, however, cefazolin was not found to be consistently superior to cephalothin, which is itself more active than cephaloridine against ampicillin-resistant enterobacteria (manuscript in preparation). In a comparison of 103 strains of ampicillin-resistant coliform bacteria, using a single pulse of 250 μg of cephalosporin per ml (a concentration readily achieved in the urine) and taking regrowth within 8 h (the common interval between doses in conventional therapeutic regimens) as the criterion of resistance, 30 of 46 strains of *E. coli* (65%), 19 of 28 *Klebsiella* (68%), and 13 of 29 other species (45%) were found to be susceptible to cefazolin, whereas 18 *E. coli* (39%), 17 *Klebsiella* (61%), and 9 other species (31%) were found to be susceptible to cephalothin.

In the urinary bladder, bacteria and antibiotic are subject to a relatively complex hydrokinetic washout effect caused by dilution of the bladder contents by ureteric urine and by periodic micturition. Because beta-lactam antibiotics are rapidly excreted into the urine, the stiuation after a single dose of these agents approximates to the introduction of a single large pulse of antibiotic. In the in vitro model intended to simulate these conditions, cefazolin suppressed the growth of ampicillin-resistant *E. coli* for longer than the usual 8-h interval between doses in only one case (Table 1, *Obr*), but for longer than 6 h in two of the other three cases. Of other beta-lactam agents tested, only cephalothin suppressed growth of ampicillin-resistant *E. coli* in this system for comparable periods. Furthermore, cefazolin is the only cephalosporin so far tested in which the activity against an ampicillin-susceptible strain (ECSA 1) in these complex conditions was as good as ampicillin itself.

In clinical use, the agent has plainly been effective in the treatment of urinary infection (1), but the superiority over comparable agents which is apparent in the model has yet to be demonstrated. It might be expected that the superior performance of cefazolin would be adversely affected in vivo by its high degree of protein binding and low apparent volume of distribution (7). The precise influence of these factors has yet to be established, but no severe detrimental effect has been apparent in the clinical performance of the agent so far (3).

ACKNOWLEDGMENTS

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