ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1977, p. 26-30

Vol. 11, No. 1
Printed in U.S.A.

Lysis of Enterobacteria by Cefoxitin, Cefuroxime, and Cephalothin

C. S. GOODWIN[1]* AND JOYCE P. HILL
Division of Hospital Infection, Clinical Research Centre, Harrow, Middlesex HA1 3UJ, England

Received for publication 2 June 1976

Cefoxitin, cefuroxime, and cephalothin were added to dense populations of beta-lactamase-producing enterobacteria, and the subsequent turbidity changes were monitored continuously. Viable counts and antibiotic assays were made at intervals after the addition of antibiotic, and the morphological appearances of the organisms were observed. Cephalothin caused lysis of most of the organisms tested, but even at high concentrations, after a few hours the antibiotic was destroyed and the organisms recommenced logarithmic growth. Cefoxitin produced lysis of all the strains of *Escherichia coli* and *Klebsiella* species tested, with supression of regrowth. With cephalothin and cefoxitin the viable counts after the addition of antibiotic correlated with the turbidity measurements. Cefuroxime infrequently caused lysis that suppressed multiplication, and the organisms became long and filamentous while the turbidity readings increased; the viable counts did not correlate with the turbidity measurements. Cefuroxime and cefoxitin were not destroyed by the beta-lactamases of any of the strains of enterobacteria that were studied.

Many enterobacteria produce beta-lactamases, including cephalosporinases; cephaloridine and cephalothin lyse such enterobacteria, but are destroyed after a few hours and the organisms recommence logarithmic growth (3, 6). Cefazolin appears to be more stable to cephalosporinases (3, 5). Cefuroxime is a new semisynthetic cephalosporin that is active against indole-positive *Proteus* species and very active against *Haemophilus influenzae* (9). Cefoxitin is one of the cephamycin antibiotics, which have been shown to be significantly resistant to cephalosporinases (2, 7) and are active against enterobacteria and *Bacteroides* that are normally resistant to cephalothin and cephaloridine (1). Intravenous doses of cefoxitin in humans produce higher serum concentrations than does cephalothin (4). Using continuous turbidimetric monitoring in a biophotometer, we have studied the ability of cefoxitin, cefuroxime, and cephalothin to lyse dense populations of enterobacteria and then suppress the phase of regrowth. Other workers with the biophotometer have related their results to the minimal inhibitory concentrations (MICs) of antibiotics determined with an inoculum of 10^4 organisms/ml (6), but we have related our results to MICs for the denser population of bacteria that we found at the time of addition of the antibiotic. The morphological appearance of organisms after the action of cefoxitin and cefuroxime has been contrasted and correlated with the viable count at intervals after the addition of the antibiotic and with the biophotometer tracings.

[1] Present address: Microbiology Department, Royal Perth Hospital, Box X 2213, GPO Perth, Western Australia 6001.

MATERIALS AND METHODS

Bacterial strains and their beta-lactamase activities. Isolates of *Escherichia coli*, *Klebsiella* spp., and *P. vulgaris* were obtained from clinical specimens at Northwick Park Hospital Harrow, London, England. The strains studied in the biophotometer were selected to cover the range of speed of beta-lactamase production as shown by the chromogenic cephalosporin 87/312 (8). One drop of a solution of 87/312 (500 μg/ml) was added to an overnight broth culture of each organism at 37°C; cleavage of the beta-lactam ring was shown by a color change from yellow to red, and the time taken for this change was noted.

MICs. Serial dilutions of cefoxitin, cefuroxime, and cephalothin were made in Sensitest Agar (Oxoid, London); within 48 h of being poured, plates were inoculated from a multiple inoculator that delivered approximately 0.03 ml of a suspension containing 1×10^5 or 2×10^7 bacteria/ml. After incubation for 18 h the lowest concentration showing no growth was regarded as the MIC.

Growth medium. The medium of Greenwood and O'Grady (6), which has an osmolality of about 325 mosmol/kg, was used.

Turbidimetric system. Cultures were grown from 0.04 ml of an overnight broth culture inoculated into 8 ml of medium to produce a concentration of approximately 3×10^6 bacteria/ml. This inoculum was

chosen because it produced a short lag phase but still allowed 100% transmission. Six stirred broth cultures, at 37°C, were continuously monitored in a Jouan (Paris) biophotometer. To one culture antibiotic was not added, and to each of the others a different antibiotic concentration was added at the same point in the logarithmic growth phase; before the antibiotic was added, a sample was taken for a viable count of each culture. The concentrations of antibiotic were usually two, four, and eight times the MIC for the denser population of the organism (see MICs above). In some experiments a viable count was done at hourly intervals after the addition of the antibiotic, wet preparations of the organisms were examined by bright-field microscopy, and their morphological appearance was noted.

Assay of antibiotic in the culture medium. Antibiotic assays were done at intervals after the addition of the antibiotic. Experiments identical to those described above were performed, but for the antibiotic assay all of the broth mixture was removed and centrifuged, and the supernatant was passed through a membrane filter (Millipore; pore size, 0.45 μm) to remove remaining bacteria. The filtrate, undiluted and at suitable dilutions, was placed in wells in agar plates. For the assay of cephalothin, a suspension of *Staphylococcus aureus* (NCTC 6571) was flooded over a plate of Sensitest Agar (Oxoid) and the plate was dried; for the assay of cefoxitin and cefuroxime, a suspension of *Bacillus subtilis* spores was incorporated into a plate of nutrient agar (10). Each plate also included three wells filled with known concentrations of the antibiotic being assayed. After overnight incubation the diameters of the zones of inhibition were measured. A graph was constructed from the results of the standards, and the antibiotic concentration of each test solution was calculated.

Resistance of antibiotics to commercial beta-lactamase. A commercial beta-lactamase preparation (Whatman Biochemicals) made from *B. cereus* 569H contained beta-lactamases I and II. Each vial contained 50 U of beta-lactamase II (cephalosporinase). The contents of the vial were dissolved in 5 ml of distilled water, and 0.1 ml of this solution was used for each test. Solutions containing 20 μg of each antibiotic per ml were prepared in nutrient broth; 0.1 ml of beta-lactamase solution was added to 0.5 ml of antibiotic; the pH of all mixtures was 7. Each was assayed immediately and after 10, 30, 60, and 120 min.

All were incubated at 37°C between samplings. The antibiotic solutions, without beta-lactamase, were also assayed. The assays were performed by the method described above.

RESULTS

Beta-lactamase activity. The chromogenic cephalosporin 87/312 (Fig. 1), used to estimate beta-lactamase activity, undergoes a distinct change from yellow to red when it is hydrolyzed by beta-lactamases. The time taken for this to occur depends on the amount of lactamase produced by the strain being tested. Some of the strains we examined and the times taken for the color change to occur were: *E. coli* strains 17, 150, and 103, 5, 5, and 10 min, respectively; *Klebsiella* strains 19 and 21, 30 and 120 min. *P. vulgaris* strains failed to produce a color change, even after overnight incubation.

MICs. The MICs with two different inocula are shown in Table 1; the larger inoculum was the concentration of organisms found at the time of antibiotic addition in the turbidimetric studies. The larger inoculum resulted in a two- to fourfold increase of MIC in most strains.

Turbidimetric studies. A typical example of the changes in turbidity of *E. coli* is shown in Fig. 2. After the addition of cephalothin and cefoxitin to *E. coli* 103, lysis of the organism occurred within 15 to 30 min. With 32 μg of cephalothin per ml, twice the MIC against the larger inoculum, the organism started to regrow at 2.5 h after the addition of antibiotic; with a concentration four times the MIC, regrowth was delayed for 6 h. With cephalothin, similar results were obtained for all of the strains of enterobacteria that produced beta-

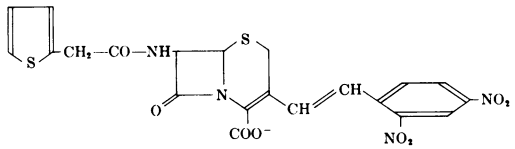

FIG. 1. *Structure of the chromogenic cephalosporin 87/312.*

TABLE 1. *MICs of three cephalosporins against two sizes of inocula*

Species	Strain	MIC (μg/ml) against:					
		1 × 10⁵ organisms/ml			2 × 10⁷ organisms/ml		
		Cephalothin	Cefuroxime	Cefoxitin	Cephalothin	Cefuroxime	Cefoxitin
E. coli	17	4	8	4	8	8	4
E. coli	55	8	4	2	12	8	8
E. coli	103	8	4	4	16	8	4
E. coli	150	8	8	4	12	12	8
Klebsiella	19	2	2	2	8	8	8
Klebsiella	21	2	2	2	2	1	4
P. vulgaris	24	4	4	4	8	2	4

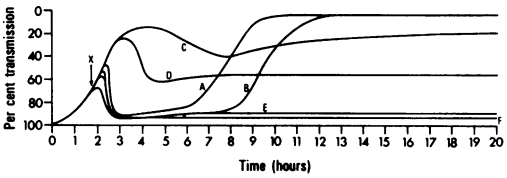

FIG. 2. *Copies of biophotometer tracings of six cultures of E. coli 103, showing the effect of addition, at time x, of: (A) cephalothin, 32 μg/ml, twice the MIC; (B) cephalothin, 64 μg/ml, four times the MIC; (C) cefuroxime, 16 μg/ml, twice the MIC; (D) cefuroxime, 32 μg/ml, four times the MIC; (E) cefoxitin, 8 μg/ml, twice the MIC; and (F) cefoxitin, 16 μg/ml, four times the MIC.*

lactamase: after lysis, regrowth occurred even with concentrations eight times the MIC, up to 128 μg/ml, although with the slowest beta-lactamase producer, *E. coli* 55, regrowth was delayed for up to 12.5 h. Regrowth did not occur after lysis by cefoxitin. After the addition of cefuroxime at 16 and 32 μg/ml, turbidity continued to increase for 2.5 and 1.5 h, respectively. This was followed by partial lysis, and turbidity fell to an intermediate level that was maintained until the end of the experiment.

When each antibiotic, at a concentration twice the MIC for the larger inoculum, was added to *E. coli* 150, cephalothin almost disappeared from the culture medium within 4 h, but cefuroxime and cefoxitin remained in their initial concentrations (Table 2). The disappearance of cephalothin was accompanied by regrowth of the organism, commencing at 3 h after the addition of the antibiotic, when the antibiotic concentration had fallen below the MIC for the smaller inoculum. A similar correlation between the concentration of the antibiotic and regrowth of the organism, as shown by a biophotometer tracing, was found for the other organisms and antibiotics.

The time between the addition of antibiotic and the recommencement of growth after lysis, as shown by turbidity measurements, is illustrated graphically in Fig. 3. After the addition of some concentrations of antibiotic, lysis did not occur; for example, *E. coli* 17 with cephalothin at the lowest concentration. The turbidity measurements suggested that, with a concentration of cefuroxime twice the MIC and *E. coli* 17, multiplication of the organism continued without a decrease in turbidity; however, viable counts during the first few hours after the addition of antibiotic showed that there was a 10-fold decrease in the viable count, with a suppression of regrowth and the formation of very long filamentous forms (see below).

Even at low concentrations of cefoxitin, lysis

TABLE 2. *Antibiotic concentrations remaining at intervals after the addition of cephalothin, cefuroxime, and cefoxitin to E. coli 150*

Antibiotic and concn (μg/ml)	Antibiotic concn (μg/ml) at time (h) after addition of antibiotic:			
	0	1	2.5	4
Cephalothin, 32	32	8	1.5	0.5
Cefuroxime, 25	25	25	25	25
Cefoxitin, 12	12	11	14	12

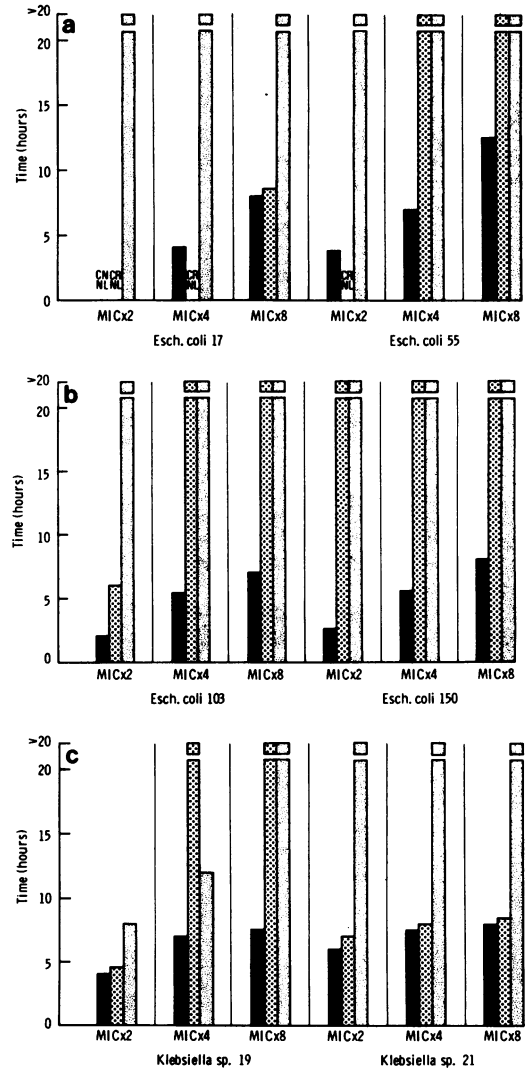

FIG. 3. *Time from addition of antibiotic to recommencement of growth after lysis of: (a) E. coli 17 and E. coli 55; (b) E. coli 103 and E. coli 150; (c) Klebsiella sp. 19 and Klebsiella sp. 21. NL, No lysis. Symbols: ■, cephalothin (CN); ▨, cefuroxime (CR); □, cefoxitin.*

was followed by a complete suppression of regrowth with all strains of *E. coli* and with *Klebsiella* sp. 21; with *Klebsiella* sp. 19, the recovery was delayed for at least 8 h with the lowest concentration of cefoxitin.

Viable counts and morphological appearances. Cephalothin and cefoxitin caused lysis of the strains of *E. coli* and *Klebsiella* spp., and the surviving organisms morphologically were predominantly spheroplasts with occasional rod forms. With these two antibiotics the turbidity measurements correlated with the viable counts; cultures without antibiotic multiplied, reaching up to 1×10^9 organisms/ml, and lysed cultures resulted in counts of 1×10^4 organisms/ml. With *P. vulgaris* 24 the biophotometer tracing did not show evidence of lysis after the addition of cephalothin and cefoxitin, but the turbidity remained at 60% transmission and viable counts revealed that there was suppression of growth; after 4 h without antibiotic the count was 1×10^9 organisms/ml, and with 8 μg of cefoxitin per ml, twice the MIC, the count was 5×10^7 organisms/ml and morphologically all the organisms were spheroplasts.

After the addition of cefuroxime the viable counts did not correlate with the turbidity measurements. For example, for *E. coli* 17 the viable count at antibiotic addition was 3×10^7 organisms/ml, and after 4 h it had fallen to 2×10^6 organisms/ml, but there was increase in turbidity and the final plateau was only slightly lower than that of the culture without antibiotic. At 2 h after the addition of cefuroxime only long filamentous forms were seen in the wet preparation, and after 4 h even longer filamentous forms were seen. With *E. coli* 55 the turbidity measurements showed a decrease to 50% turbidity, and the viable count decreased by 99% to 1×10^5 organisms/ml; with *E. coli* 150 there was a turbidity decrease to 50%, with a viable count after 4 h of 2×10^4 organisms/ml. Regrowth was suppressed, and the turbidity remained the same. Similar results were found with cefuroxime for all of the other strains studied. When a high concentration of cefuroxime, 32 μg/ml, was added to *E. coli* 17, long filamentous forms were seen without any reduction in turbidity.

Antibiotic assays. In incubated broth without any organism, after 18 h of incubation a solution of cefoxitin decreased from a concentration of 16 to 12 μg/ml and a solution of cefuroxime decreased from 8 to 6 μg/ml.

Resistance of antibiotics to commercial beta-lactamase. In mixtures of beta-lactamase (Whatman Biochemicals) with cephalothin and cefuroxime the antibiotic had disappeared completely after approximately 15 s; i.e., there were no zones on the assay plate. After 2 h of incubation the concentration of cefoxitin had fallen from 20 to 12 μg/ml.

DISCUSSION

Biophotometer tracings are a useful method for continuous monitoring of the interaction between beta-lactam antibiotics and bacteria that produce beta-lactamases. However, the limitations of this method are shown by the viable counts after the addition of an antibiotic, such as cefuroxime, when there may be a 90% reduction of organisms but the tracing shows increasing turbidity and a final turbidity only slightly less than a culture without antibiotic. The morphological response profile of beta-lactam antibiotics has been extensively described by Greenwood and O'Grady (6) and Greenwood et al. (5): these workers used medium with a lower osmolality for *Proteus* than that of the medium for *E. coli* because they found that *Proteus* spheroplasts were protected in the lower osmolality. We preferred to use the same osmolality in all of our experiments, and we found that *P. vulgaris* 24, after the addition of cefoxitin, showed a total absence of rods with a large number of viable spheroplasts, but complete suppression of multiplication.

For strains of *E. coli* and *Klebsiella*, even at an osmolality of 325 mosmol/kg, cefoxitin causes rapid lysis of dense populations of enterobacteria, with complete suppression of regrowth, in contrast to cephalothin which allowed regrowth after a few hours. The resistance of cefoxitin to beta-lactamases is confirmed by its stability in the presence of a commercial beta-lactamase that immediately destroyed cefuroxime and cephalothin.

For clinical use cefoxitin would be expected to be superior to cephalothin, even for strains with a similar MIC, and might allow less frequent administration, for example, twice daily. Cefuroxime has been found to be very similar in action to cephalexin (6), producing long filamentous forms without much lysis in many strains of beta-lactamase-producing *E. coli*. Multiplication of organisms was suppressed and cefuroxime was not destroyed by beta-lactamases, and these features may be sufficient to give a good clinical effect.

ACKNOWLEDGMENTS

We thank R. Blowers for encouragement and advice; Merck, Sharp and Dohme Research Laboratories, West Point, Pa., for the supply of cefoxitin; and Glaxo Laboratories Ltd., Greenford, Middlesex, England, for the supply of cefuroxime and cephalosporin 87/312.

LITERATURE CITED

1. **Brumfitt, W., J. Kosmidis, J. M. T. Hamilton-Miller, and J. N. G. Gilchrist.** 1974. Cefoxitin and cephalothin: antimicrobial activity, human pharmacokinetics, and toxicology. Antimicrob. Agents Chemother. 6:290–299.
2. **Daoust, D. R., H. R. Onishi, H. Wallick, D. Hendlin, and E. O. Stapley.** 1973. Cephamycins, a new family of beta-lactam antibiotics: antibacterial activity and resistance to beta-lactamase degradation. Antimicrob. Agents Chemother. 3:254–261.
3. **Goodwin, C. S., and J. P. Hill.** 1975. Beta-lactamase resistance of cephazolin and other cephalosporins. Scott. Med. J. 20:236–239.
4. **Goodwin, C. S., E. B. Raftery, A. D. Goldberg, H. Skeggs, A. E. Till, and C. M. Martin.** 1974. Effects of rate of infusion and probenecid on serum levels, renal excretion, and tolerance of intravenous doses of cefoxitin in humans: comparison with cephalothin. Antimicrob. Agents Chemother. 6:338–346.
5. **Greenwood, D., C. H. Chan-Teoh, and F. O'Grady.** 1975. Activity of cefazolin against dense populations of enterobacteria. Antimicrob. Agents Chemother. 7:191–195.
6. **Greenwood, D., and F. O'Grady.** 1973. Comparison of the responses of *Escherichia coli* and *Proteus mirabilis* to seven beta-lactam antibiotics. J. Infect. Dis. 128:211–222.
7. **Miller, A. K., E. Celozzi, B. A. Pelak, E. O. Stapley, and D. Hendlin.** 1972. Cephamycins, a new family of β-lactam antibiotics. III. In vitro studies. Antimicrob. Agents Chemother. 2:281–286.
8. **O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shingler.** 1972. Novel method for detection of beta-lactamases by using a chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. 1:283–288.
9. **O'Callaghan, C. H., R. B. Sykes, A. Griffiths, and J. E. Thornton.** 1976. Cefuroxime, a new cephalosporin antibiotic: activity in vitro. Antimicrob. Agents Chemother. 9:511–519.
10. **O'Callaghan, C. H., R. B. Sykes, D. M. Ryan, R. D. Foord, and P. W. Muggleton.** 1976. Cefuroxime—a new cephalosporin antibiotic. J. Antibiot. 29:29–37.