THE *IN VITRO* ACTIVITY OF CEFTEZOL (DEMETHYLCEFAZOLIN) AGAINST DENSE POPULATIONS OF *ESCHERICHIA COLI*

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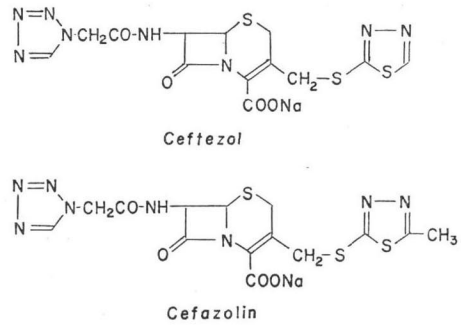
The activity of ceftezol was examined by continuous turbidimetric monitoring of dense populations of *Escherichia coli* exposed to the drug. Although ceftezol was found to be very active against strains of *E. coli*, its activity was consistently less than that of the closely related antibiotic, cefazolin. This difference was also found when strains of *E. coli* were examined in a dynamic system which simulates some of the conditions in which bacteria and drug interact in the treatment of bacterial cystitis.

Evidence is presented that the difference in activity between the two cephalosporins resides in a differential ability to induce certain morphological changes in *E. coli* and in a differential rate of destruction by escherichial β-lactamases.

The antibacterial activity of different β-lactam antibiotics often varies more than conventional *in vitro* tests indicate, most notably in their morphological effects on sensitive bacteria and their susceptibility to certain enterobacterial β-lactamases[1,2]. Cephalosporins in particular often exhibit similar antibacterial activity in conventional titrations, but differ substantially when dense bacterial populations are examined by continuous turbidimetric monitoring[1,3,4]. In order to investigate these differences, we have developed methods of studying the response of dense bacterial populations to β-lactam antibiotics by turbidimetry in static culture[1,5] and in a mechanical model which simulates the hydrokinetic features of the urinary bladder[6,7,8,9].

Fig. 1. Structures of ceftezol and cefazolin.



In the present study we have used these methods to assess the *in vitro* activity of a new cephalosporin, ceftezol (Fig. 1), a compound closely related structurally to cefazolin which has also recently been examined in this way[10].

Materials and Methods

Antibiotic: Sodium ceftezol was provided by Fujisawa Pharmaceutical Co., Ltd. Suitable concentrations were freshly prepared as required in sterile distilled water.

Growth medium: The 'complete' broth having an osmolality of about 325 milliosmoles per kilogramme, described elsewhere[1], was used.

Bacterial strains: Seven strains of *Escherichia coli*, all of which were originally isolated from infected urine, were examined. Two of the strains (ECSA 1 and ECSA 2) were sensitive to 4 μg ampicillin per ml, the remaining strains (Bur, Gen, Obr, Kin and Hos) were resistant

to 500 μg ampicillin per ml as judged by conventional titration in broth.

Static turbidimetric system: Cultures were grown from small inocula in broth in the multichannel bacterial growth monitoring device described by MACKINTOSH *et al.*[11] in which the opacity of twelve independent bacterial cultures can be continuously recorded. Antibiotic was added at a standard point in the logarithmic growth phase equivalent to 30 per cent maximum opacity (viable count *ca* 5×10^7 organisms per ml).

Bladder model: The design and operation of the *in vitro* bladder model have been described in detail elsewhere[6,7,8,9]. In this system 20 ml of an overnight broth culture of bacteria are diluted with fresh broth at 1 ml per minute, simulating the rate of ureteric urine flow into the bladder. At preset intervals (1 hour in the present series of experiments) a 'micturition' episode empties the system of accumulated broth leaving a residual 20 ml volume. Arrangements are made to mix the culture and the turbidity is continuously monitored photometrically. In the present study antibiotic was added as a single pulse, to achieve an initial concentration of 500 μg per ml, immediately after the fourth hourly 'micturition'.

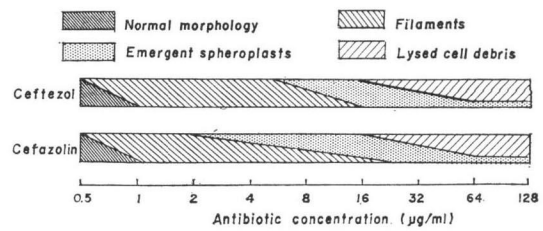
Microscopy: Microscopical observations were made after 1 hour's exposure to antibiotic by interference contrast microscopy.

Results

Morphological Response Profile

The morphological changes induced in an ampicillin-sensitive strain of *E. coli* (ECSA 1) after one hour's exposure to ceftezol are compared with those induced by cefazolin in Fig. 2. The ability of ceftezol to induce filamentation of *E. coli* was similar to that of cefazolin, but a higher concentration of ceftezol was required to induce spheroplast formation. Similar results were obtained using *E. coli* strain ECSA 2.

Fig. 2. Morphological response profiles of an ampicillin-sensitive strain of *E. coli* (ECSA 1) after 1 hour's exposure to ceftezol or cefazolin.



Turbidimetric Response Profile

The effect of adding various concentrations of ceftezol to an exponentially growing culture of *E. coli* ECSA 1 in the turbidimetric system is shown in Fig. 3. Concentrations of ceftezol exceeding 2 μg per ml induced a decline in the opacity of the culture which occurred sooner as the concentration of antibiotic was increased. Regrowth of the culture occurred after a period which also varied with the anti-

Fig. 3. Continuous opacity records of an ampicillin-sensitive strain of *E. coli* showing the effect of various concentrations (μg/ml) of ceftezol (added at arrow) on exponentially growing cultures.

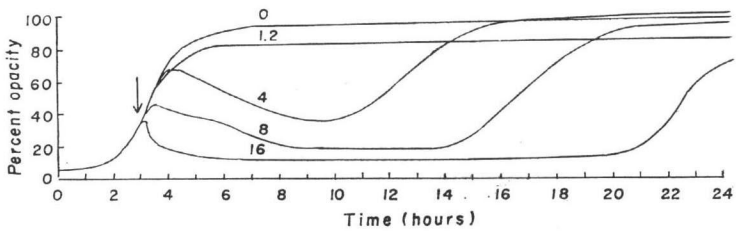


Fig. 4. Turbidimetric response profiles of an ampicillin-sensitive strain of *E. coli* (ECSA 1) showing the time elapsing after the addition of various concentrations of ceftezol or various other β-lactam antibiotics before A) lysis of the culture occurred; B) regrowth of the culture occurred.

Ctz=ceftezol; Cez=cefazolin; Cer=cephaloridine; Amp=ampicillin; Ctn=cephalothin; Cex=cephalexin.

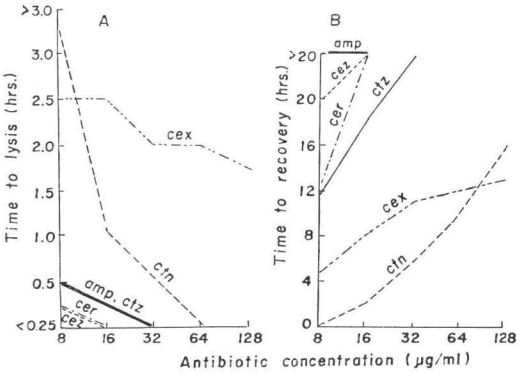


Fig. 5. Turbidimetric response profiles of an ampicillin-resistant strain of *E. coli* (Bur) exposed to five cephalosporins.

Explanation and abbreviations as for Fig. 4.

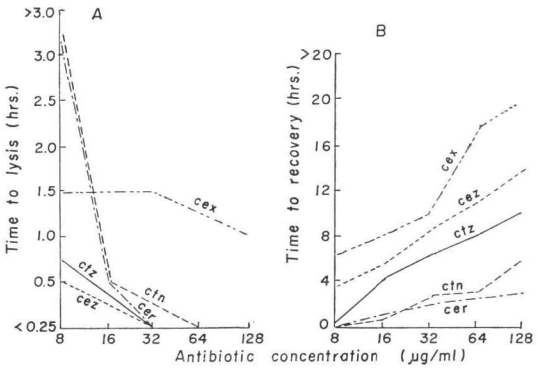
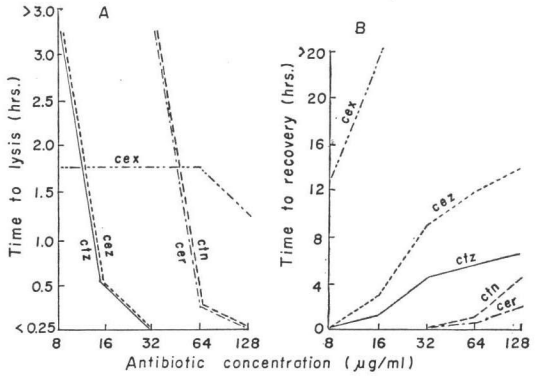


Fig. 6. Turbidimetric response profiles of an ampicillin-resistant strain of *E. coli* (Gen) exposed to five cephalosporins.

Explanation and abbreviations as for Fig. 4.



biotic concentration. *E. coli* strain ECSA 2 responded in a very similar fashion when examined in this way.

Data from records such as that shown in Fig. 3, obtained by continuous turbidimetric monitoring, was used to construct turbidimetric response profiles of various ampicillin-sensitive and -resistant *E. coli* strains for ceftezol and, using data reported elsewhere[1,5,10], other β-lactam antibiotics. Typical results are shown in Figs. 4, 5 and 6. These response profiles show the time taken following the addition of various concentrations of antibiotic, for A) lysis; B) regrowth of the culture to occur. The time to lysis reflects the intrinsic activity of the agent, the time to regrowth reflects its susceptibility to β-lactamases of the organism being tested[1,5].

In both resistance to β-lactamase and high intrinsic activity ceftezol closely resembled, but was slightly inferior to, the related cefazolin.

Bladder Model

E. coli ECSA 1 and four ampicillin-resistant *E. coli* strains (Bur, Obr, Kin, Hos) were examined in this system. Addition of sufficient ceftezol to achieve an initial concentration of 500 μg per ml caused an immediate precipitous fall in opacity in all cases with recovery of the culture occurring after a period of time characteristic of the strain. The time taken for the opacity of the culture to reattain the level prevailing at the time of antibiotic addition is shown for each strain in Table 1, in which is included, for comparison, results previously obtained in this system using other β-lactam antibiotics[7,8,10]. According to the criterion of

Table 1. Comparison of the times taken in the bladder model for cultures of *E. coli* to recover to the opacity level prevailing at the time of antibiotic addition after exposure to ceftezol and 5 other β-lactam agents

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

NL=no lytic effect produced; NT=not tested

ability to suppress bacterial growth in this system ceftezol, in common with other cephalosporins tested, was less active than ampicillin against the ampicillin-sensitive strain ECSA 1. Tested against four ampicillin-resistant *E. coli* strains the activity of ceftezol was found generally to be between that of cephaloridine and that of cefazolin.

Discussion

Although ceftezol and cefazolin are closely related structurally, their *in vitro* activities against *E. coli* are not identical. The intrinsic activity of ceftezol against ampicillin-sensitive and -resistant strains of *E. coli* was found to be high, but generally lower than that of cefazolin. This appeared to be related to a diminished ability of the newer cephalosporin to induce spheroplast formation—a function thought to involve the inhibition of two enzymes[12].

When dense bacterial cultures are exposed to cephalosporins regrowth often occurs at antibiotic concentrations which initially cause lysis of much of the population. This regrowth is due to the general susceptibility of cephalosporins to enterobacterial β-lactamases and is seen even with ampicillin-sensitive *E. coli* strains which characteristically possess the ability to hydrolyse cephalosporins comparatively slowly[1]. The time taken for regrowth of the culture to occur under standard conditions of exposure to cephalosporins is chiefly an index of the susceptibility of the antibiotic to β-lactamase[1,5]. When examined in this way ceftezol appeared to be somewhat more susceptible than cefazolin to escherichial β-lactamases, including the 'slow' β-lactamase of ampicillin-sensitive strains.

Ceftezol is structurally demethylcefazolin; these results again highlight the differences which a small modification of structure, apparently remote from the cephalosporin nucleus, can make to the properties of the whole molecule, a situation parallelled among penicillin congeners by differences in activity observed between ampicillin and hydroxyampicillin—amoxycillin[13]. As in the case of ampicillin and amoxycillin, the difference in activity between ceftezol and cefazolin seems to be quite small and is manifested, at least in part, by a differential ability to cause various morphological changes in susceptible Gram-negative bacilli. It is possible that analysis of such differences, induced by minor modifications of the penicillin and cephalosporin molecules, may help in the elucidation of the problem of relating structure to function in this class of antimicrobial compounds.

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