LYSIS ENHANCEMENT: A NOVEL FORM OF INTERACTION BETWEEN β -LACTAM ANTIBIOTICS

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BETA-LACTAM antibiotics are well known to exert a number of concentration-dependent effects on Gram-negative bacilli that are reflected in different morphological changes in the bacteria. In a recent comprehensive comparison of the changes of opacity and bacterial morphology produced in cultures of Gram-negative bacilli by exposure to various β -lactam antibiotics (Greenwood and O'Grady, 1973a) we showed that the concentration ranges over which the different morphological effects occurred varied markedly between agents. Cephalixin, which is known to be a potent inducer of filament formation in Gram-negative bacilli—a response characteristic of low concentrations of other β -lactam antibiotics—was anomalous among those tested in that filamentation was the sole response of *Escherichia coli* to this agent over a very wide range of concentrations.

In the present study cephalixin was again shown to be unusual in that it showed synergy of a novel kind with other penicillins and cephalosporins.

MATERIALS AND METHODS

Cephalosporins were supplied by Glaxo Laboratories Ltd and ampicillin by Beecham Research Laboratories Ltd. Suitable concentrations of antibiotic were freshly prepared in sterile distilled water as required.

Strains of *E. coli* were isolated from infected urine in the diagnostic bacteriology department of this hospital. Two strains, designated ECSA1 and *Far*, which are respectively ampicillin sensitive and ampicillin resistant, were intensively studied.

Growth medium was the "complete" broth having an osmolality of *c.* 325 mOsmoles per kg previously described (Greenwood and O'Grady, 1973a). Turbidimetric studies were made with the 12-channel opacity-monitoring device described by Mackintosh *et al.* (1973). Antibiotic was added at a point in the mid-to-late logarithmic phase of growth corresponding to a viable count of either *c.* 5×10^7 organisms per ml (30% maximum opacity) or 10^7 organisms per ml (10% maximum opacity).

Combinations of antibiotic were added together at the 30% opacity point. Where they were added separately, the first addition was made at 10% and the second at 30% opacity.

RESULTS

The results were expressed as the intervals between antibiotic addition and a sharp drop in opacity indicating lysis of the culture.

As previously reported (Greenwood and O'Grady, 1973a) addition of cephaloridine or cephalothin to the ampicillin-sensitive strain ECSA1 or the ampicillin-resistant strain *Far* caused lysis after an interval that depended on the concentration of the agent. Sufficient cephalixin to produce a concentration of 8 μ g per ml caused delayed lysis of the culture after 1½ to 2 h if added at 10% opacity, but was without effect on the growth curve if added at 30% opacity. The characteristic filamentation response of *E. coli* to this antibiotic was nevertheless demonstrated microscopically in both cases. When sufficient cephalixin to produce a concentration of 8 μ g per ml was added at the same time, the concentration of cephaloridine or cephalothin required to produce a given lytic effect was approximately halved (table I).

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When the same concentration of cephalixin (8 μ g per ml) was allowed to act on the culture for about 40 min. before the addition of the second agent the concentration of cephaloridine or cephalothin needed to produce a given lytic effect was reduced to approximately one quarter (table I). With the ampicillin-sensitive strain ECSA1 similar results were obtained with cephalixin and ampicillin, but when the antibiotic additions were made in the reverse order—ampicillin before cephalixin—enhancement of the early lytic effect was not found (table II).

The results obtained with the two strains reported here were confirmed, with minor differences, for three other strains of *E. coli*, two of which were resistant to ampicillin.

DISCUSSION

Three types of interaction are now known to occur between β -lactam agents. The best known of these is the β -lactamase inhibition effect (Abraham and Newton, 1956; Hamilton-Miller, Smith and Knox, 1964; Sutherland and Batchelor, 1964) where some β -lactam antibiotics, such as methicillin, which are inactive against Gram-negative bacilli, are nevertheless stable to enterobacterial penicillinase for which they have a high affinity. Such agents may be used to sequester the penicillinase of a resistant organism and allow a penicillinase-sensitive β -lactam agent to act.

A second type of interaction has recently been described between cephalixin and FL1060 (a 6- β -amidinopenicillanic acid: Lund and Tybring, 1972; Greenwood and O'Grady, 1973*b*) which in combination produce the "typical penicillin effect" of spheroplast formation through discrete cell-wall lesions which neither agent can produce alone (Greenwood and O'Grady, 1973*c*).

The third form of interaction, which is reported here, resembles this in that it also depends on the dominant action of cephalixin on *E. coli*, which is to inhibit division, giving rise to long, snake-like filaments (Muggleton *et al.*, 1969; Russell and Fountain, 1970). With most β -lactam antibiotics, filament formation is seen only at low and generally sub-inhibitory concentrations, but with cephalixin this is the only effect seen over a very wide range of concentrations (Fujii, Konno and Ubukata, 1970; Greenwood and O'Grady, 1973*a*). It is no doubt because of this that exposure to concentrations of cephalixin below 128 μ g per ml produces lysis of *E. coli* only after a long delay, and then only if antibiotic is added sufficiently early in the growth cycle for the filaments to grow long enough to lyse before the culture enters the stationary phase (Greenwood and O'Grady, 1973*a*). Beta-lactam antibiotics such as cephalothin and ampicillin differ from cephalixin in that the concentrations that

TABLE I
*Enhancement of lysis by cephalothin or cephaloridine produced by
prior exposure to cephalixin*

Strain designation	Concentration (μ g per ml) causing lysis within 15 min. of addition of					
	cephalothin			cephaloridine		
	alone	plus	after	alone	plus	after
		cephalexin (8 μ g per ml)			cephalexin (8 μ g per ml)	
ECSA 1	64	32	16	16	8	4
<i>Far</i>	64	32	16	32	16	8

produce filamentation and the emergence of spheroplasts are not widely dissociated (Greenwood and O'Grady, 1973a).

The simplest explanation of the "synergy" of such agents with cephalixin is that they exert a limited lytic activity at lower concentrations than those that cause rapid lysis. If the bacterial population is converted to long, non-septate filaments, this limited activity will exert a more extensive effect because injury at one point of a filament can produce loss of substance equivalent to the lysis of many normal bacteria.

We have previously argued (Greenwood and O'Grady, 1973a) that the concentration-dependent morphological effects of penicillins and cephalosporins stem from the inhibition of two distinct enzymes—one involved in cell division and one involved in generalised cell-wall synthesis (Greenwood and O'Grady, 1973a)—which are differentially inhibited by β -lactam agents over different concentration ranges. According to this hypothesis, rapid lysis is due to osmotic rupture after the simultaneous inhibition of both enzymes. The residual activity postulated to account for the synergy reported here refers to the inhibition of generalised cell-wall synthesis. With cephalixin itself, which inhibits cell division only at concentrations below about 500 μ g per ml and hence does not cause lysis except at very high concentrations, such residual activity should be absent; and in keeping with this, enhancement of lysis was not observed when cephalixin was added to cultures in which filamentation had been previously induced by sub-inhibitory concentrations of ampicillin (table II).

Interesting though this is in relation to the mode of action of β -lactam agents, the need to expose the culture to cephalixin for sufficient time to allow filaments to develop before adding the second agent makes it doubtful whether this kind of interaction offers much therapeutic promise.

SUMMARY

A novel form of synergy has been observed to occur between cephalixin and certain other β -lactam antibiotics. In the presence of cephalixin a reduction was found in the concentration of other β -lactam agents needed to induce lysis of *Escherichia coli*; the effect was particularly marked when cephalixin was allowed to act for a short time before the addition of the second agent.

The basis of this type of synergy—which is not likely to be of therapeutic value—is discussed in terms of a theory previously put forward, which suggests that penicillins and cephalosporins have two distinct sites of action in Gram-negative bacilli.

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TABLE II

Effect of order of adding cephalixin (8 μ g per ml) and ampicillin (4 μ g per ml) on enhancement of lysis of strain no. ECSA1

Antibiotic added at opacity (%) of		Time (h) to lysis
10	30	
Cephalixin	None	2.0
None	Ampicillin	1.5
Cephalixin	Ampicillin	0.25
None	Cephalixin	No lysis
Ampicillin	None	0.75
Ampicillin	Cephalixin	0.75

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