

IN VITRO ACTIVITY AGAINST *ESCHERICHIA COLI* OF CGP 9000,
A NEW ORAL CEPHALOSPORIN

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(Received for publication May 15, 1978)

The activity against *Escherichia coli* of a new oral cephalosporin (manufacturer's code: CGP 9000) has been evaluated *in vitro*. The intrinsic lytic activity of the new compound was greater than that of cephalexin, but less than that of cephalothin. As judged by regrowth studies using ampicillin resistant *E. coli* strains, the β -lactamase stability of the new cephalosporin was somewhat less than that of cephalexin.

When tested in an *in vitro* model in conditions simulating those of the treatment of bacterial cystitis, cephalosporin CGP 9000 suppressed growth of an ampicillin sensitive *E. coli* strain for a therapeutically acceptable period of time, but exhibited reduced activity against an ampicillin resistant *E. coli* strain.

Few cephalosporins are well absorbed when given by mouth. Those which are, like cephalexin and cephradine, generally have low intrinsic lytic activity against Gram-negative bacilli and are consequently only slowly bactericidal to these bacteria.

CGP 9000, 7 β -[D-2-amino-2-(1,4-cyclohexadienyl)-acetamido]-3-methoxy-ceph-3-em-4-carboxylic acid, is a new oral cephalosporin developed by Ciba-Geigy. In the present study, the activity of the new compound against *Escherichia coli* has been assessed according to turbidimetric and morphological criteria previously used in the evaluation of other β -lactam antibiotics¹⁾. In addition the activities of CGP 9000, cephalexin and cephalothin have been compared in an *in vitro* model designed to simulate the conditions in which bacteria are exposed to antibiotics in the treatment of bacterial cystitis²⁾.

Materials and Methods

Antibiotics

CGP 9000 was provided by CIBA Laboratories Ltd; cephalexin by Glaxo Research Ltd; cephalothin by Eli Lilly and Co., Ltd.

Growth medium

'Complete' broth, the composition of which has been described elsewhere³⁾ was used throughout. The medium has a pH of 6.8 and an osmolality of about 325 mOsm (milli-Osmoles) per kg.

Bacterial strains

Seven strains of *E. coli*, designated ECSA 1, ECSA 2, Ara, Far, Bur, Gen and Hos, were examined. All were originally isolated from infected urine and all except *E. coli* strain Ara, which was a fresh clinical isolate, have been used in previous studies of the comparative activity of β -lactam antibiotics.

Static turbidimetric system

Turbidimetric studies were carried out using the multichannel continuous opacity monitoring device described by MACKINTOSH *et al*⁴⁾. Antibiotic was added to bacterial cultures when growth had reached a standard opacity level of either 10 per cent or 30 per cent of maximum, equivalent to an inoculum of *ca* 10⁷ and 5 \times 10⁷ bacteria per ml, respectively.

Bladder model

The design and use of the bladder model have been fully described elsewhere⁵⁾. In brief, 20 ml

of an overnight broth culture of bacteria are diluted with fresh broth at a rate of 1 ml per minute, simulating the diurnal rate of urine flow into the bladder. At intervals (1 hour in the present experiments) controlled by an automatically resetting clock, a pump empties the 'bladder', simulating micturition, leaving behind a residual volume of 20 ml.

Two types of experiment were performed. In the first a single pulse of antibiotic, achieving an instantaneous initial concentration of 500 $\mu\text{g/ml}$ was added to the system after the fourth hourly 'micturition'. In the second type of experiment a gradient forming device (MixoGrad; Gilson Medical Electronics) was used to add the antibiotic with the broth inflow in changing concentration. In these experiments, the concentration of antibiotic started to rise immediately after the fourth hourly 'micturition' and reached a peak value of 500 $\mu\text{g/ml}$ 2 hours later; the drug concentration then fell exponentially over a further ten hour period after which dilution was continued with antibiotic-free broth. A second, identical cycle of exposure was commenced when persisting bacteria had reestablished the bacterial population to its original level.

Microscopy

Antibiotic-induced morphological changes in bacteria were observed in untreated 'wet' preparations by interference contrast microscopy.

Results

Minimum Inhibitory Concentrations

The minimum inhibitory concentrations (MIC) of CGP 9000, cephalixin and cephalothin for the seven *E. coli* strains as judged by conventional titration in broth, using a bacterial inoculum of 10^5 organisms per ml, are listed in Table 1. Enterobacteria have been divided into six distinct categories of resistance to β -lactam antibiotics⁶. On the basis of this categorization, 3 of the *E. coli* strains used here (ECSA 1, ECSA 2 and Ara) belong to category 2 (fully sensitive to ampicillin; slightly less sensitive to cephalosporins), the remainder belong to category 5 (β -lactamase producing strains that are fully resistant to ampicillin and whose sensitivity to cephalosporins is greatly affected by the size of the bacterial inoculum).

Morphological Response Profile

The morphological changes induced in an ampicillin sensitive *E. coli* strain (ECSA 2) by various concentrations of CGP 9000 are shown in Fig. 1. Similar effects of cephalixin and cephalothin reported elsewhere¹ are included for comparison.

Turbidimetric Response Profile

Ampicillin-sensitive Strains

The general form of the response of fully sensitive *E. coli* strains to CGP 9000 was identi-

Table 1. Minimum inhibitory concentrations (MICs) of CGP 9000, cephalixin and cephalothin for the seven *E. coli* strains.

Strain	MIC ($\mu\text{g/ml}$)		
	CGP 9000	Cephalixin	Cephalothin
ECSA 1	16	32	16
ECSA 2	16	16	8
Ara	16	16	8
Far	32	8	8
Bur	16	4	4
Gen	16	8	8
Hos	32	16	32

Fig. 1. Morphological response profiles of an ampicillin sensitive strain of *E. coli* (ECSA 2) after 1 hour's exposure to cephalixin, cephalothin or CGP 9000.

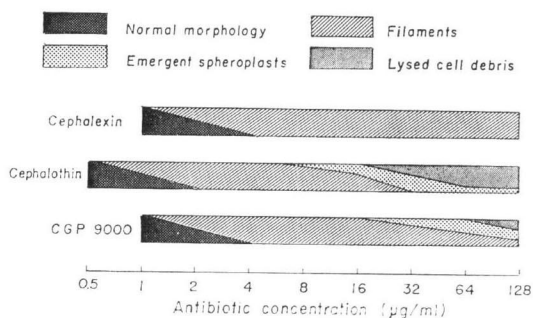


Fig. 2. Turbidimetric response profiles of an ampicillin sensitive strain of *E. coli* (ECSA 1) showing the time elapsing after the addition of cephalixin (CEX), cephalothin (CTN) or CGP 9000 before lysis of the culture occurred. Antibiotic added at an opacity level of 30 per cent of maximum.

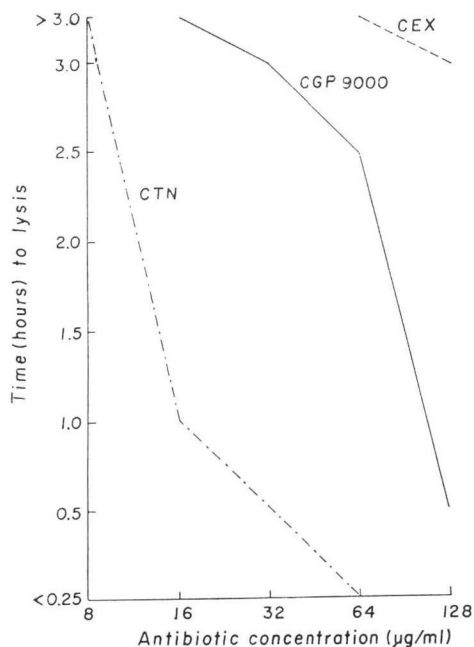
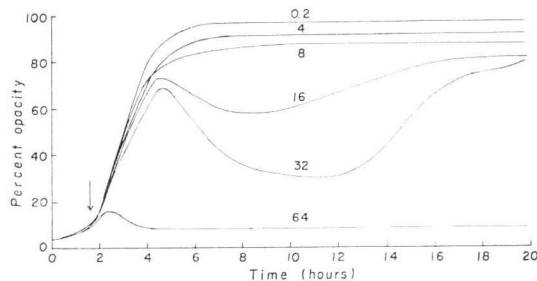


Fig. 3. Continuous opacity records of an ampicillin sensitive strain of *E. coli* (ECSA 2), showing the effect of various concentrations (μg per ml) of CGP 9000 (added at arrow) on growing cultures.



cal to that described for other β -lactam antibiotics³): low concentrations caused delayed bacterial lysis and high concentrations caused rapid lysis. Fig. 2 compares the times elapsing before a fall in opacity was detectable following exposure of *E. coli* ECSA 1 to various concentrations of CGP 9000, cephalixin and cephalothin. Judged in this way, the intrinsic lytic activity of CGP 9000 was intermediate between that of cephalixin and that of cephalothin.

When cultures of *E. coli* are exposed to predominantly filament-forming β -lactam antibiotics, such as cephalixin, at the standard 30 per cent opacity level, no effect is recorded turbidimetrically because the bacteria enter the stationary phase before sufficient damage has been caused to lead to the death and degeneration of the bacteria. Lysis can be detected, however, if the antibiotic addition is made earlier in the logarithmic growth phase at an opacity level of 10%¹). In order to compare CGP 9000 with the closely related cephalixin, therefore, additional experiments were performed in which antibiotic was added at the earlier growth point. The response obtained is depicted in Fig. 3. In these circumstances, a decline in opacity was observed following exposure to CGP 9000 concentrations of 16 $\mu\text{g}/\text{ml}$ and above, and at concentrations of 16 and 32 $\mu\text{g}/\text{ml}$ regrowth occurred during the overnight incubation period. The behaviour of cephalixin in these circumstances has been described elsewhere^{1,3}): lysis of *E. coli* is very much delayed, and regrowth occurs, even at a cephalixin concentration of 128 $\mu\text{g}/\text{ml}$.

Ampicillin-resistant Strains

When added to cultures of ampicillin-resistant *E. coli* strains Gen and Hos at the 30% opacity level, 128 μg CGP 9000 per ml caused no deviation from the normal growth curve. When tested against *E. coli* strains Far and Bur, a concentration of 128 μg CGP 9000 per ml caused a transient lysis, with rapid regrowth; lower levels achieved no lytic effect.

Somewhat greater activity was revealed in experiments in which the antibiotic was added to cultures at the 10% opacity level. Figs. 4 and 5 compare the turbidimetric response profiles of CGP 9000,

Fig. 4. Turbidimetric response profiles of an ampicillin resistant strain of *E. coli* (Bur) showing the time elapsing after the addition of various concentrations of cephalixin (CEX), cephalothin (CTN) or CGP 9000 before (A) lysis; (B) regrowth, of the culture occurred. Antibiotic added at an opacity level of 10% of maximum.

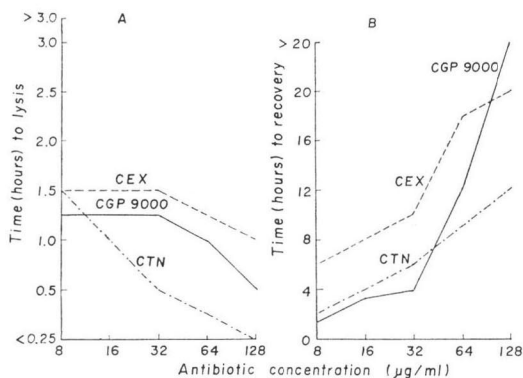
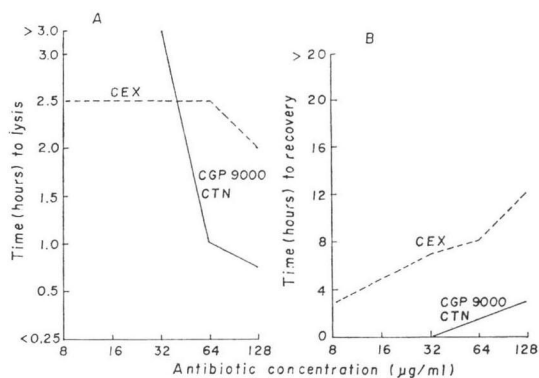


Fig. 5. Turbidimetric response profiles of an ampicillin resistant strain of *E. coli* (Hos) showing the time elapsing after the addition of various concentrations of cephalixin (CEX), cephalothin (CTN) or CGP 9000 before (A) lysis; (B) regrowth, of the culture occurred. Antibiotic added at an opacity level of 10% of maximum.



cephalexin and cephalothin in such experiments in which *E. coli* strains Bur and Hos were the test organisms. In these experiments the time to regrowth was also plotted for each antibiotic concentration. This value may be used as an index of the stability of the antibiotic to β -lactamase elaborated by the bacteria, provided a comparable degree of lysis is initially achieved¹. In the case of *E. coli* strain Bur (Fig. 4) the lytic activity of CGP 9000 was similar to that of cephalixin, but considerably less than that of cephalothin. Taking into account the different degrees of lysis achieved, CGP 9000 and cephalixin both appeared superior to cephalothin in terms of β -lactamase stability. When tested against *E. coli* strain Hos (Fig. 5) various concentrations of CGP 9000 and cephalothin achieved virtually identical degrees of lysis and growth suppression; the lytic activity of both agents was superior to that of cephalixin and the β -lactamase stability somewhat inferior.

Bladder Model

Two representative *E. coli* strains, ECSA 1 and Hos, were examined in this system. Addition of sufficient CGP 9000, cephalixin or cephalothin to achieve an initial concentration of 500 $\mu\text{g/ml}$, after the fourth hourly 'micturition' caused a rapid fall in opacity, with recovery occurring as persisting bacteria resumed growth when the drug level had fallen to below an inhibitory level. The times taken, following antibiotic addition, for the opacity to reattain its original level is shown for each strain and cephalosporin in Table 2. The 3 antibiotics achieved very similar degrees of growth suppression of both bacterial strains.

In experiments designed to simulate more closely the conditions in which antibiotic is presented to the culture in the treatment of bacterial cystitis (see methods), a fall in opacity occurred as the antibiotic concentration rose to an inhi-

Table 2. 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 CGP 9000, cephalixin or cephalothin.

Strain	Time (hours) to recovery after a single pulse (500 $\mu\text{g/ml}$) of		
	CGP 9000	Cephalixin	Cephalothin
ECSA 1	7	6	6.5
Hos	4	3.5	4

bitory level and suppression of bacterial growth continued during the period when the antibiotic level again declined. After the 12-hour cycle of exposure to antibiotic was completed, dilution was continued with antibiotic-free broth and the opacity eventually started to rise again as persisting bacteria resumed growth. The antibiotic susceptibility of the bacteria surviving antibiotic exposure was tested by commencing a second, identical, cycle of drug exposure when growth had reattained its original level. A comparison of the degree of growth suppression achieved by each of the two 'doses' of CGP 9000, cephalixin and cephalothin is shown in Fig. 6. Using the ampicillin sensitive *E. coli* strain ECSA 1 as the test organism, each antibiotic suppressed growth for a similar period of time (9~10.5 hours after the completion of 'treatment'); when challenged with a second 'dose' survivors exhibited a slightly reduced susceptibility to the drugs, which was most marked in the case of cephalixin.

The degree of growth suppression of *E. coli* strain Hos achieved by sequential 'doses' of cephalixin was similar to that obtained with the ampicillin sensitive strain ECSA 1, but the activity of CGP 9000 and cephalothin appeared considerably lower (Fig. 6).

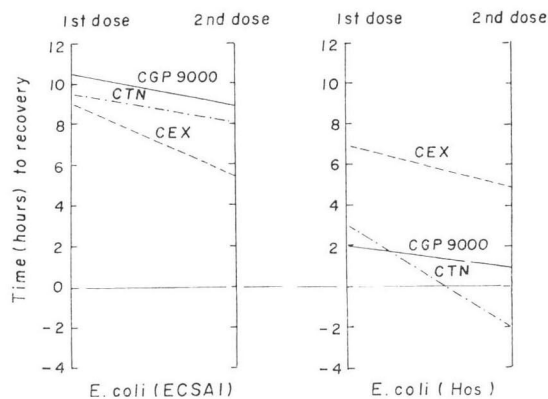
Discussion

The intrinsic lytic activity of the new cephalosporin, CGP 9000, as shown by continuous turbidimetric monitoring of dense cultures of *E. coli* exposed to the drug, appears to be intermediate between that of cephalothin and that of cephalixin; this level of activity is also reflected in the concentrations required to cause various morphological alterations in *E. coli* (Fig. 1).

The dense bacterial inocula of the turbidimetric experiments also revealed the level of the inoculum effect observed with the new cephalosporin. Fig. 3 shows that the MIC (the concentration suppressing growth overnight) of CGP 9000 for *E. coli* strain ECSA 1, using a bacterial inoculum of 10^7 bacteria per ml, was $64 \mu\text{g/ml}$, four-fold higher than the value obtained in conventional tube titrations using an inoculum of 10^5 bacteria per ml. This inoculum density effect is less than that observed with cephalothin and cephalixin^{1,3}) and may indicate that CGP 9000 is somewhat more stable to the slow-acting β -lactamase, characteristic of ampicillin-sensitive *E. coli* strains, which is thought to be largely responsible for the considerable inoculum effect observed with many cephalosporins^{8,6}). As with most other cephalosporins, a larger inoculum effect was observed when CGP 9000 was tested against ampicillin-resistant *E. coli* strains. The extent of this inoculum effect may be gauged by comparing the low inoculum MIC (Table 1) with the concentration needed to suppress bacterial growth for 20 or more hours in the turbidimetric system (where the inoculum is 100-fold higher) shown in Figs. 4 and 5.

An obvious clinical application for an oral cephalosporin is in the treatment of urinary infection and the activity of the new compound was therefore tested in an *in vitro* model of the infected urinary bladder. In simple "pulse" experiments designed to compare the intrinsic activity of antibiotics, CGP 9000, cephalixin and cephalothin all achieved similar degrees of suppression of bacterial growth. This activity was, however, considerably lower than that of ampicillin against the sensitive *E. coli* strain ECSA 1⁵) and that of the parenteral cephalosporin cefuroxime against the ampicillin-resistant Hos⁷).

Fig. 6. Comparison of the times taken for cultures of *E. coli* ECSA 1 and Hos to recover following exposure to sequential 'doses' of cephalixin (CEX), cephalothin (CTN) or CGP 9000 in the bladder model.



In simulated therapeutic experiments in which the renal excretion of a 'dose' of antibiotic is simulated⁹⁾, a single cycle of exposure to CGP 9000 suppressed growth of *E. coli* strain ECSA 1 for periods that far exceed the normal interdose interval. Less striking activity was observed with the ampicillin resistant strain Hos as test organism, against which cephalixin was more successful, probably reflecting greater stability to the β -lactamase of this strain.

High concentrations of β -lactam antibiotics are achievable in urine, but in the treatment of urinary infection, the antibiotic has to contend with dense bacterial populations which may have the capacity to hydrolyse the antibiotic. In such circumstances conventional tests of antibiotic sensitivity may not give a true picture of therapeutic effectiveness. The new cephalosporin CGP 9000 has comparable intrinsic activity to some other presently available cephalosporins, but, like many other antibiotics of this group, its activity against dense bacterial populations of β -lactamase producing *E. coli* appears to be considerably less than conventional tests would predict.

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