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Dual-Action Cephalosporin Utilizing a Novel Therapeutic Principle

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A new cephalosporin is described that overcomes, in a novel way, the general susceptibility of this group of agents to enterobacterial β-lactamases. The new compound carries a substituent that is released on cleavage of the β-lactam ring and then exhibits antibacterial activity in its own right. The possible therapeutic benefits of such an antibiotic are discussed.

Many cephalosporins have marked activity against a wide spectrum of gram-negative bacilli when tested by conventional minimum inhibitory concentration (MIC) methods using low inocula. When tested against dense populations of enterobacteria, however, their activity is much less impressive (1, 2, 11). This inoculum effect is particularly marked in those strains of enterobacteria that are resistant to ampicillin, and, when tested against dense populations of such strains, cephalosporins often cause only a transient antibacterial effect due to their relative susceptibility to enterobacterial β-lactamases (2, 4).

One approach to the enhancement of the antibacterial effect of this group of agents is to combine them with a β-lactamase inhibitor, and this can be very effective in vitro, providing a potent, broad-spectrum β-lactamase inhibitor is used (5).

A new cephalosporin synthesized by Glaxo Research Ltd. (9) offers a totally different solution to the problem. This compound, when attacked by β-lactamases, loses a substituent, omadine, normally carried at the 3-position of the cephalosporin molecule, which itself possesses antibacterial activity in the free, but not in the bound, state. The present paper discusses this novel principle and presents some results of in vitro tests that explore the potential of this type of agent.

MATERIALS AND METHODS

(6R,7R)-7-[(2R)-2-hydroxy-2-phenylacetamido]-3-(pyrid-2-yl-*N*-oxide) thiomethylceph-3-em-4-carboxylic acid (MCO) (Fig. 1) was synthesized and provided by Glaxo Research Ltd. Suitable concentrations were freshly prepared in sterile distilled water as required.

Sodium omadine was provided by Kingsley and Keith (Chemicals) Ltd. as a 40% solution.

Turbidimetric studies were made using the multichannel opacity monitoring device described by Mackintosh et al. (8). Strains of *Escherichia coli*, of which the response to β-lactam antibiotics has been extensively studied in previous investigations (2, 3, 6, 7), were grown in complete broth (2) in the opacity monitoring device. Antibacterial agent was added at a standard point (30% maximum opacity) in the mid-to-late logarithmic growth phase. Drug-induced morphological changes in the exposed bacteria were observed by interference contrast microscopy.

Cephalosporin MCO was also used to treat "bacterial cystitis" in a mechanical model that simulates the hydrokinetic features of a urinary bladder (3, 6, 10). In the model, 20 ml of a fully grown broth culture is diluted at 1 ml/min with fresh broth, simulating the diurnal flow of urine into the bladder. At preset intervals (1 h in the present experiments), a "micturition" episode empties the "bladder," leaving a residual 20-ml volume. In the present series of experiments, a single pulse of antibiotic, to achieve an initial concentration of 500 μg/ml, was added to the system after the fourth hourly micturition.

RESULTS

The MICs of cephalosporin MCO, a number of other β-lactam antibiotics, and sodium omadine, tested against several strains of *E. coli*, are shown in Table 1.

Figure 2 shows a typical continuous opacity trace of an ampicillin-susceptible strain of *E. coli* (ECSA 1) exposed to various concentrations of cephalosporin MCO. The morphological changes exhibited by the bacteria after 1 h of exposure are depicted in Fig. 3. Rapid lysis of the culture occurred at an antibiotic concentration of 128 μg/ml, a biphasic response (associated with part of the population lysing, part transforming to the spheroplast form, and part undergoing filamentation) was induced at 64 μg/ml, and a delayed lysis (associated with spheroplast formation and filamentation) occurred at 32 μg/ml. Little or no lysis occurred at lower concentrations although filamentation of the bacteria was demonstrated microscopically

at antibiotic concentrations of less than 1 μg/ml. In striking contrast to the finding with other cephalosporins (2, 4, 7), no regrowth of cultures that had succumbed to antibiotic lysis occurred during the 20-h period of observation.

A typical continuous opacity trace obtained with an ampicillin-resistant *E. coli* strain (*Gen*) is shown in Fig. 4. With this strain, partial lysis occurred at an antibiotic concentration of 128 μg/ml, but again no regrowth was seen on prolonged incubation.

Data obtained from these experiments together with similar data obtained in experiments with other β-lactam agents are plotted for strains ECSA 1 and *Gen* in Fig. 5 and 6, which show the time elapsing after the addition of various concentrations of each antibiotic before lysis of the cultures occurred.

The effect of sodium omadine on an exponentially growing culture of *E. coli* ECSA 1 is shown in Fig. 7. A concentration of 4 μg/ml, equivalent to the conventionally determined MIC, caused an abrupt cessation of growth, with little subsequent increase in opacity over the period of observation.

Bladder model. The general form of the growth/dilution response of *E. coli* strains in the bladder model and the influence of β-lactam antibiotics upon the normal response have been described elsewhere (3, 6, 10). In the model, bacterial culture and antibiotic are subjected to dilution and periodic discharge. The hydrokinetic washout effect thus produced depends on the "urine" flow rate, the interval between micturition episodes, the residual volume of culture left after micturition, and the bacterial growth rate. An overnight bacterial culture, initially in the lag phase, enters a "fluctuating equilibrium" state (in which the same cycle of opacity changes occur between each micturition) after about 6 h of dilution and hourly micturition (3). In the equilibrium state the bacterial population is maintained at a near-climax level, in which the viable count is in excess of 10^8 organisms/ml. Antibiotic added as a single pulse after a micturition episode is subsequently diluted 1:4 during each hourly cycle, but in the case of β-lactam agents enzymatic destruction may also serve to reduce the concentration of active antibiotic (6).

Table 2 compares the ability of a single pulse (sufficient to achieve an initial concentration of 500 μg/ml) of MCO or of several other β-lactam agents to suppress growth of two strains of *E. coli* in the bladder model. One of these strains was *E. coli* ECSA 1, also used in the "static"

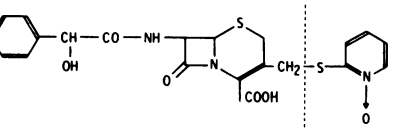

FIG. 1. *Structure of cephalosporin MCO.*

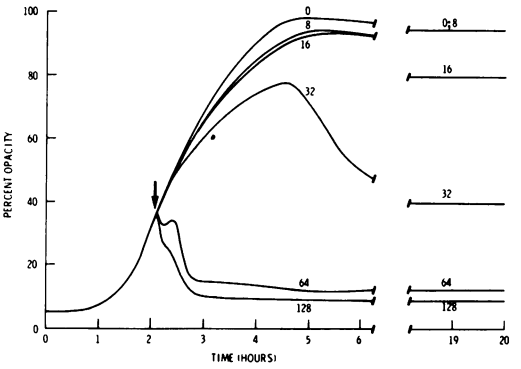

FIG. 2. *Continuous opacity records of an ampicillin-susceptible E. coli strain (ECSA 1). Numbers indicate the concentration (micrograms per milliliter) of cephalosporin MCO added at arrow.*

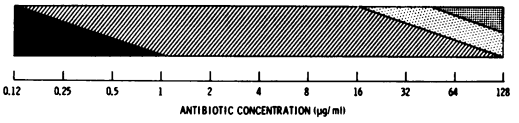

FIG. 3. *Morphological response profile of an ampicillin-susceptible E. coli strain (ECSA 1) after 1 h of exposure to cephalosporin MCO. Black = normal; hatching = filaments; light stippling = emergent spheroplasts; dense stippling = lysed cell debris.*

TABLE 1. *Comparison of the MICs of cephalosporin MCO, other β-lactam agents, and sodium omadine for several strains of E. coli*

Strain	MIC (μg/ml) of:						
	Ampicillin	Cephaloridine	Cephalothin	Cefazolin	Cephalexin	Cephalosporin MCO	Sodium omadine
ECSA 1	4	8	16	2	32	8	4
ECSA 2	4	4	8	1	16	2	4
Far	>500	8	8	8	8	32	4
Bur	>500	4	4	2	4	16	4
Gen	>500	16	8	4	8	32	2
Hos	>500	16	16	64	16	64	4

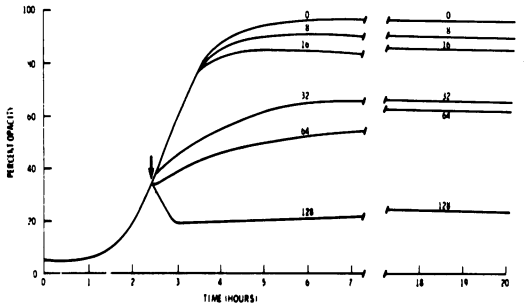

FIG. 4. *Continuous opacity records of an ampicillin-resistant E. coli strain (Gen). Numbers indicate the concentration (micrograms per milliliter) of cephalosporin MCO added at arrow.*

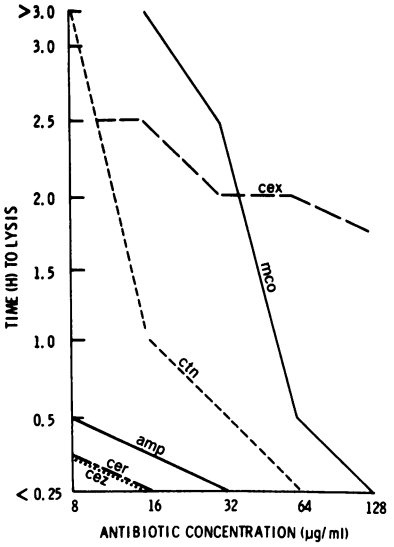

FIG. 5. *Comparison of the lytic activity of cephalosporin MCO and of several other β-lactam antibiotics, showing the time taken for lysis to occur when dense cultures of E. coli strain ECSA 1 were exposed to various concentrations of the antibiotics. amp, Ampicillin; cer, cephaloridine; ctn, cephalothin; cez, cefazolin; cex, cephalexin; mco, cephalosporin MCO.*

experiments; the second strain was an ampicillin-resistant *E. coli* strain (*Hos*) previously shown to be highly resistant to a number of other β-lactam agents in the conditions of the bladder model (6). The results are expressed as the time taken for the opacity to reattain the level at which antibiotic was added. Cephalosporin MCO suppressed the growth of the ampicillin-susceptible strain ECSA 1 for 10 h and that of the ampicillin-resistant strain *Hos* for 5.5 h—longer than any of the other β-lactam agents tested.

DISCUSSION

The in vitro activity of cephalosporins against gram-negative bacilli is generally mitigated by their susceptibility to enterobacterial β-lactamases, including the slow β-lactamase characteristic of ampicillin-susceptible *E. coli* strains (2, 4–7). The new cephalosporin, MCO, overcomes this disability by releasing, on cleavage of the β-lactam ring, a substituent, omadine, which on liberation exhibits antibacterial activity. Thus, although the compound is susceptible to enterobacterial β-lactamases, the initial antibacterial effect of the entire molecule is continued after its destruction by the omadine substituent.

We have previously argued (10) that the benefits of high-dose therapy with β-lactam agents may be confined to the first dose of a treatment schedule, in which a large part of the bacterial population is destroyed. Subsequent doses sup-

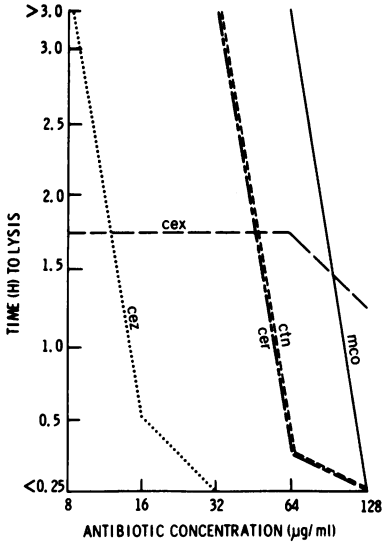

FIG. 6. *Comparison of the lytic activity of cephalosporin MCO and of several other β-lactam antibiotics, showing the time taken for lysis to occur when dense cultures of an ampicillin-resistant E. coli strain (Gen) were exposed to various concentrations of the antibiotics. Abbreviations are the same as those given in the legends to Fig. 5.*

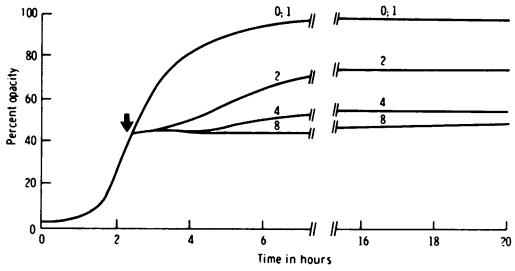

FIG. 7. *Continuous opacity records of an ampicillin-susceptible E. coli strain (ECSA 1). Numbers indicate the concentration (micrograms per milliliter) of sodium omadine added at arrow.*

TABLE 2. *Comparison of the times taken in the bladder model for bacterial cultures to reattain the opacity level at which antibiotic was added after exposure to cephalosporin MCO or to other β-lactam antibiotics*

Strain	Time (h) after antibiotic addition for opacity to reattain original level after a single pulse (500 μg/ml) of:					
	Ampicillin	Cephaloridine	Cephalothin	Cefazolin	Cephalexin	Cephalosporin MCO
ECSA 1	10	8	6.5	11	6	10
Hos	NL[a]	2	4	3	3.5	5.5

[a] NL, No lysis.

port intrinsic clearance mechanisms by maintaining a bacteriostatic effect on the persisting bacteria and need to be present in concentrations only just above the MIC. Cephalosporin MCO provides in a single antibiotic just such prompt bacterial lysis followed by a prolonged bacteriostatic effect, which is maintained even when the cephalosporin moiety is inactivated.

An important feature of a successful cephalosporin of this type must be the ability to destroy by lysis a greater part of the bacterial population before it is itself destroyed by β-lactamases. The minimum concentration level at which cephalosporin MCO caused rapid bacterial lysis was intermediate between that of highly lytic cephalosporins, such as cephaloridine, cephalothin, and cefazolin, and that of α-aminocephalosporins, cephalexin, cephradine, and cephaloglycin, which have little or no lytic activity at therapeutically useful concentrations (2, 7). Furthermore, the lytic activity of cephalosporin MCO, when tested against an ampicillin-resistant *E. coli* strain (Fig. 4 and 6), was very poor, probably indicating that its susceptibility to the more potent β-lactamases is such that it breaks down before extensive bacterial lysis develops.

On the other hand, limited lytic activity at low concentrations may not be an obstacle to use in situations, such as the urine, where high antibiotic concentrations may be achieved. In experiments in a mechanical model simulating the conditions of dilution and periodic discharge that obtain in the urinary bladder, the new cephalosporin was able to suppress growth of an ampicillin-susceptible strain of *E. coli* for as long as the highly active ampicillin and was more active than any other β-lactam agent tested against a very resistant *E. coli* strain (Table 2).

The pharmacokinetics and toxicity of this compound and its cleavage products have not been extensively studied, but toxicity of the omadine substituent is likely to militate against its therapeutic use. Nevertheless, the principle that underlies the antibacterial effect of this novel cephalosporin is one of considerable therapeutic interest.

ACKNOWLEDGMENTS

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