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Antagonism of Carbenicillin and Cefamandole by Cefoxitin in Treatment of Experimental Infections in Mice

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The ability of cefoxitin to antagonize the in vivo efficacy of cefamandole and carbenicillin as predicted by in vitro assays was analyzed in experimental infections in mice. Cefoxitin was administered in a nonprotective dose either at the time of challenge or simultaneously with the protective drug, 1 and 3.5 h postchallenge. In mice infected with *Enterobacter cloacae*, median 50% protective doses of cefamandole and carbenicillin were markedly increased by cefoxitin, especially when the latter was given at the time of challenge. The antagonistic effect was also associated with increased numbers of challenge bacteria present in animal heart blood within a 6.5-h period after infection. In infections with *Pseudomonas aeruginosa*, cefoxitin antagonized carbenicillin; however, the effect was less dramatic than that seen with *E. cloacae*. Antagonism in this model was pronounced with simultaneous administration of antagonizing and protective drugs. The antagonistic effects observed in all in vivo tests were not due to the selection of stable resistance to the protective drugs, but appeared to be due to a reversible induction of beta-lactamases by cefoxitin.

Studies from this and other laboratories have demonstrated the ability of cefoxitin, a relatively new semisynthetic cephamycin, to antagonize the in vitro activity of a wide range of beta-lactam antibiotics (2, 3, 6–9, 14, 15, 17). Recently, Kuck et al. have shown that such antagonisms also occur in vivo (9). However, important questions still remain regarding the relationship of these in vitro antagonisms to the outcome of therapy when cefoxitin is used with another beta-lactam antibiotic. Therefore, the current study was designed to assess the therapeutic efficacy of drug combinations clearly shown to be antagonistic by in vitro disk approximation tests (6, 14, 15). Since the preceding in vitro study had shown that antagonism may occur by at least two different mechanisms (15), the strains and drugs selected for this study were those that appeared to involve different mechanisms. Cefamandole was chosen for study as it represented a beta-lactam antagonized by cefoxitin via the induction of drug-inactivating enzymes. Carbenicillin was chosen for study as it represented a beta-lactam antagonized by cefoxitin via the induction of drug-binding (but not inactivating) enzymes that create a barrier to target proteins. *Enterobacter cloacae* and *Pseudomonas aeruginosa* were selected because both species characteristically possess inducible beta-lactamases (12, 16). Both mechanisms of antagonism appear operative in *Enterobacter* spp., whereas the barrier mechanism appears to be of primary importance in *Pseudomonas* spp. (15). Since the in vitro study indicated that antagonism by both mechanisms involved the reversible induction of beta-lactamases, the ability of cefoxitin to antagonize the therapeutic efficacy of carbenicillin and cefamandole was assessed when (i) the drugs were administered simultaneously and (ii) cefoxitin was administered first so as to allow induction before exposure of the cells to the second drug.

MATERIALS AND METHODS

Bacterial strains. Two challenge strains of clinical origin were used: (i) *E. cloacae* susceptible in vitro to cefamandole and carbenicillin (minimal inhibitory concentration [MIC], 3.1 μg/ml), but resistant to cefoxitin (MIC, >50 μg/ml); and (ii) *P. aeruginosa* susceptible in vitro to carbenicillin (MIC, 50 μg/ml), but resistant to both cefamandole and cefoxitin. In vitro antagonism assays were performed by a disk approximation procedure described previously (14). Beta-lactamase assays were performed as described in the preceding paper (15).

Infection-treatment protocols. Experimental infections were induced as described previously (4). Male CF-1 mice (20 to 25 g of body weight) were infected intraperitoneally with cells suspended in 4% hog gastric mucin, sufficient to kill 100% of untreated animals within 24 or 48 h. At 1 and 3.5 h postinfection, the protective drug (either cefamandole or carbenicillin) was administered intramuscularly at five different dosages to groups of 10 animals each. The ability of a totally nonprotective dose (PD_0) of cefoxitin (25 mg/kg) to antagonize the efficacy of the protective drug was then determined, using two treatment regimens: (i) simultaneous administration of cefoxitin and the

TABLE 1. Results of laboratory tests on challenge strains

Test	Results[a] on:	
	E. cloacae	*P. aeruginosa*
Disk approximation test for antagonism of:		
Cefamandole	+	
Carbenicillin	+	+[b]
Beta-lactamase assay for inactivation of:		
Cephalothin	89/98	27/97
Cefamandole	0/84	0/20
Carbenicillin	0/0	0/0
Penicillin G	0/35	0/53

[a] Results expressed as nanomoles of drug inactivated per 10^9 cells uninduced/induced with cefoxitin.
[b] Disk approximation test negative when cefamandole disk was used instead of cefoxitin disk.

protective drug intramuscularly into separate leg muscles at 1 and 3.5 h postchallenge, or (ii) pretreatment of infected animals with cefoxitin (intramuscularly), at the time of challenge, followed by the protective drug (administered intramuscularly) at 1 and 3.5 h postchallenge. For all treatment regimens, the PD_{50} was determined by log-probit plot, and the 95% confidence levels were calculated (10).

Postinfection recovery and analysis of challenge bacteria. For all animals dying within 48 h postinfection, isolates of the challenge strain were cultured from heart blood as described previously (4) and tested by dilution assay in Mueller-Hinton broth (MHB; inoculum, 10^5 colony-forming units per ml) for susceptibility to the protective drug used in treatment. For cefamandole and carbenicillin, *E. cloacae* isolates were considered resistant if the MIC of the drug was ≥25 μg/ml.

In one experiment, animals were challenged with *E. cloacae* and treated with 25 mg of cefoxitin per kg at time of challenge. At 1 and 3.5 h postchallenge, cefamandole was administered at a dose of 100 mg/kg. Controls consisted of untreated animals, animals treated with cefoxitin alone, and animals treated with cefamandole alone. At 0.5-h intervals from 0.5 to 6.5 h postchallenge, two animals from each treatment and control group were sacrificed, and heart blood was removed. The heart blood was serially 10-fold diluted in saline and inoculated into Mueller-Hinton agar (MHA) and MHA containing 500 μg of cefamandole per ml to quantify challenge bacteria and to screen for emergence of cefamandole resistance, respectively. All results shown are averages of duplicate counts performed on heart blood from two animals.

TABLE 2. Influence of cefoxitin (25 mg/kg) on the efficacy of cefamandole and carbenicillin in treatment of *E. cloacae* infections[a]

Treatment regimen	PD_{50} (mg/kg)[b]
Cefamandole	72 (51–102)
Cefamandole plus cefoxitin	
Simultaneous	90 (74–100)
Pretreatment	>200
Carbenicillin	9.5 (6.0–15.0)
Carbenicillin plus cefoxitin	
Simultaneous	36.0 (26.0–50.0)
Pretreatment	62.0 (53.0–73.0)

[a] Challenge was 5.6×10^5 colony-forming units.
[b] At 48 h postchallenge, numbers in parentheses are 95% confidence limits.

TABLE 3. Influence of cefoxitin (25 mg/kg) or cefamandole (100 mg/kg) on the efficacy of carbenicillin in treatment of *P. aeruginosa* infections[a]

Treatment regimen	PD_{50} (mg/kg)[b]
Carbenicillin	92 (58–146)
Carbenicillin plus:	
Cefamandole (simultaneous)	105 (76–144)
Cefoxitin (simultaneous)	345 (213–558)
Cefoxitin (pretreatment)	248 (145–425)

[a] Challenge was 3.5×10^6 colony-forming units.
[b] At 24 h postchallenge, numbers in parentheses are 95% confidence limits.

RESULTS

Effect of cefoxitin on the PD_{50} of beta-lactams. In vitro disk approximation tests with *E. cloacae* indicated that the activity of cefamandole and carbenicillin was antagonized by cefoxitin. Assays for beta-lactamases revealed the presence of cefoxitin-inducible enzymes in this strain capable of inactivating cefamandole but not carbenicillin (Table 1). As shown in Table 2, a PD_0 of cefoxitin was able to antagonize the in vivo efficacy of both cefamandole and carbenicillin in animals challenged with this bacterium. The antagonism was most pronounced with carbenicillin when either the simultaneous administration of cefoxitin and carbenicillin or single-dose cefoxitin pretreatment resulted in significantly decreased protection. With both carbenicillin and cefamandole, the greatest antagonism was observed with the single-dose cefoxitin pretreatment regimen.

Similar results were obtained in experiments with mice challenged with *P. aeruginosa* (Table 3). Again, in vitro disk approximation tests were predictive of in vivo outcome, and cefoxitin-inducible enzymes were demonstrable in the strain (Table 1). In contrast to *E. cloacae*, antagonism was statistically significant when both the protective and antagonizing drugs were administered simultaneously. Although *P. aeruginosa* was resistant in vitro to cefamandole, as well as to cefoxitin, substitution of cefamandole for cefoxitin in the simultaneous treatment regimen produced no significant effect on the efficacy of carbenicillin (Table 3). No antagonism

TABLE 4. Recovery of cefamandole-resistant[a] *E. cloacae* from heart blood of animals dying from infection

Treatment regimen	Incidence of cefamandole-resistant isolates[b]
None	1/9 (11)
Cefoxitin at challenge	1/11 (9)
Cefoxitin at 1 and 3.5 h	2/11 (18)
Cefamandole alone	26/30 (87)[c]
Plus cefoxitin (simultaneous)	10/25 (40)
Plus cefoxitin (pretreatment)	20/41 (49)

[a] MIC, ≥25 μg/ml.

[b] Number of animals with resistant isolate/number of animals cultured. Percent is given in parentheses.

[c] Significantly higher than all other values (chi square, Yates correction, $P < 0.05$).

between cefamandole and carbenicillin was seen in disk approximation assays.

Postinfection analysis of therapy. In experiments with both *E. cloacae* and *P. aeruginosa*, challenge bacteria were recovered from the heart blood of nonsurviving animals and tested for their susceptibility to the protective drug used in treatment. As shown in Table 4, cefamandole-resistant isolates were recovered from dead animals in all treatment and control groups. The occurrence of such resistance was minimal (<20%) in untreated control animals and animals receiving only cefoxitin either at challenge or 1 and 3.5 h postinfection. However, animals treated with either regimen of cefoxitin plus cefamandole were less likely to harbor cefamandole-resistant isolates than were animals treated with cefamandole alone.

In cefoxitin-carbenicillin experiments with *E cloacae* (Table 5), few animals (0 to 20%) from treatment and control groups yielded carbenicillin-resistant isolates. Although a somewhat higher percentage of animals treated with carbenicillin alone yielded carbenicillin-resistant organisms than did those receiving both carbenicillin and cefoxitin, this difference was not significant.

Heart blood from 161 animals infected with *P. aeruginosa* was examined for carbenicillin-resistant isolates. No resistant isolates were detected among untreated animals or those treated with cefoxitin or carbenicillin, alone or in combination.

The degree of bacteremia in animals infected with *E. cloacae*, pretreated with cefoxitin, and then treated with cefamandole was determined at 0.5 h-intervals after infection. As shown in Fig. 1, animals receiving only a single dose of cefoxitin at challenge were essentially the same as untreated controls and exhibited steadily increasing bacteremia. Animals receiving cefamandole at 1 and 3.5 h postinfection showed a reduction in the degree of bacteremia after both injections. The antagonistic effect of cefoxitin on cefamandole was reflected by a lack of well-defined reductions in bacterial blood counts after cefamandole administration. Cefamandole-resistant bacteria were cultured from only four animals during the experiment. Three of the animals received only cefamandole, and one was an untreated control (Fig. 1).

DISCUSSION

The ability of cefoxitin to antagonize the efficacy of cefamandole and carbenicillin was demonstrated in animal infection models utilizing two different treatment regimens. These antagonisms confirmed and expanded the observations made in the preceding in vitro study (15). First, disk approximation tests indicating antagonism were predictive of antagonism in vivo. Second, the ability of cefoxitin to antagonize the protective drug either when administered simultaneously with or before the protective drug provided further support for the mechanisms proposed previously. These mechanisms involved the induction of beta-lactamases that either inactivated the protective drug or created a barrier to target proteins. If such mechanisms had been operative in vivo, pretreatment or simultaneous treatment with cefoxitin or both should have been antagonistic. Such antagonism was observed, and both challenge strains possessed cefoxitin-inducible enzymes.

Analysis of the emergence of resistance to the protective drug also provided evidence that the in vivo antagonisms were due to a reversible induction process. In no instance were challenge isolates resistant to carbenicillin or cefamandole recovered more frequently from animals treated

TABLE 5. Recovery of carbenicillin-resistant[a] *E. cloacae* from heart blood of animals dying from infection

Treatment regimen	Incidence of carbenicillin-resistant isolates[b]
None	1/23 (4)
Cefoxitin at challenge	0/12
Cefoxitin at 1 and 3.5 h	0/10
Carbenicillin alone	7/35 (20)
Plus cefoxitin (simultaneous)	1/26 (4)
Plus cefoxitin (pretreatment)	7/53 (13)

[a] MIC, ≥25 μg/ml.

[b] Number of animals with resistant isolate/number of animals cultured. Percent is given in parentheses.

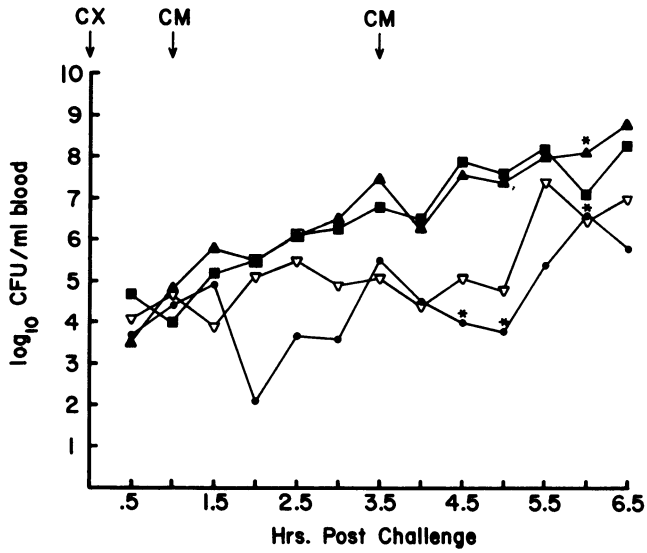

FIG. 1. Degree of bacteremia in animals infected with *E. cloacae* and treated with cefoxitin at time of challenge (■), cefamandole at 1 and 3.5 h (●), cefoxitin at time of challenge and cefamandole at 1 and 3.5 h (▽), no drug (▲). Drug dosages were 100 mg of cefamandole per kg and 25 mg of cefoxitin per kg. Isolation of cefamandole-resistant *E. cloacae* (*). All values are averages of duplicate counts on heart blood from two animals.

with cefoxitin, either alone or in combination with the protective drug. In fact, cefoxitin treatment reduced the incidence of cefamandole resistance in animals infected with *E. cloacae*. Since this analysis was performed on challenge isolates recovered from heart blood of animals dying after therapy, these results indicated that failure of cefamandole therapy in animals treated with cefamandole alone was due primarily to the in vivo emergence of resistance to this drug. Such resistance has been observed previously in other animal infection models (5, 11) and has recently been reported to occur in humans treated with cefamandole (1, 13). This in vivo development of resistance among *Enterobacter* spp. appears to be due to the selection of mutants producing high levels of beta-lactamase and occurs most frequently with strains possessing cefoxitin-inducible enzymes (13). In contrast to this cefamandole-selected resistance, failure of cefamandole therapy in animals also treated with cefoxitin was due to a mechanism that did not produce stable resistance. Such a mechanism probably involved the induction of beta-lactamase. Studies by Gootz et al. have shown that in *E. cloacae*, cefoxitin-induced beta-lactamases confer an unstable resistance to cefamandole that is rapidly lost upon removal of the inducer (T. D. Gootz and C. C. Sanders, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 21st., Chicago, Ill., abstr. no. 675, 1981). Thus cefoxitin's antagonism of cefamandole in vivo appears to be due also to the reversible induction of cefamandole-inactivating beta-lactamases. The similarities observed in tests with carbenicillin, i.e., antagonism after pretreatment with cefoxitin and no increased development of carbenicillin resistance, suggest a reversible induction process in these antagonisms as well.

The clinical significance of these antagonisms is unknown. Since there are currently no clinical data indicating that combinations of beta-lactam antibiotics with cefoxitin are advantageous, they should probably be avoided whenever possible in light of the data presented here and elsewhere that indicate antagonism in vitro and in vivo.

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