In Vitro Synergy of Cefamandole-Tobramycin Combinations

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Twenty-five isolates of *Staphylococcus aureus,* 24 isolates of *Escherichia coli,* and 25 isolates of *Klebsiella pneumoniae* obtained from clinical material were tested *in vitro* for susceptibility to cefamandole, tobramycin and combinations of the two antibiotics utilizing an automated microdilution system. Synergistic or partially synergistic bactericidal effects of the combination were observed against 15 of the *S. aureus* isolates (60%), 23 of the *E. coli* isolates (96%), and 19 of the *K. pneumoniae* isolates (76%) tested. No antagonistic effects of the combination were noted. This study suggests that cefamandole-tobramycin combinations are capable of acting synergistically *in vitro* against certain gram-positive and gram-negative organisms and may have potential usefulness in clinical situations such as gram-negative rod and staphylococcal sepsis.

INTRODUCTION

The enhanced or the diminished efficacy of certain antibiotic combinations compared with single drugs in killing or inhibiting bacteria *in vitro* is now well recognized. Controlled *in vivo* studies utilizing animal models of infection have demonstrated that at least some combinations which are synergistic *in vitro* act synergistically *in vivo* as well [1,2,12]. Furthermore, an appreciation of the kinetics of synergy has enabled predictions of how different classes of antibiotics may interact when combined. Thus, antibiotics which act at different sites in the bacterial cell, such as cephalosporins and aminoglycosides, are often synergistic in combination. As new antibiotics are developed, their interactions with other antibiotics are investigated to anticipate how combinations may be used in eventual therapeutic strategies.

The present studies were undertaken to evaluate two relatively new antibiotics, cefamandole (7-D-mandelamido-3-[(1-methyl-1H-tetrazol-5-yl) thiomethyl] -3-cephem-4-carboxylic acid as the sodium or lithium salt) and tobramycin (0-3-Amino-3-deoxy-α-D-ribo-glucopyranosyl-(1→4)]-0-[2,6-diamino-2,3,6-trideoxy-α-D-ribo-hexopyranosyl-(1→6)]-2-deoxystreptamine) sulfate to determine their potential synergistic activity *in vitro* against *Staphylococcus aureus, Escherichia coli,* and *Klebsiella pneumoniae.*

MATERIALS AND METHODS

Twenty-five strains of *S. aureus,* twenty-four strains of *E. coli,* and twenty-five strains of *K. pneumoniae* were obtained as clinical isolates from different patients at the Yale-New Haven Hospital. All strains were re-identified by standard bacteriologic methods in our research laboratory.

Antibiotics

Cefamandole lithium and tobramycin sulfate standard powders were supplied by Eli Lilly and Company, Indianapolis, Indiana.

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Synergy Studies

For all studies, a checkerboard technique utilizing an automated serial microdilution system (Autotiter III, Canalco Co., Rockville, Md.) was employed as previously reported [1]. All organisms were cultured in Mueller-Hinton Broth (MHB) and the final inoculum in each study contained approximately 10^4 colony forming units/ml.

The minimal inhibitory concentration (MIC) was defined as the lowest concentration of each antibiotic either alone or in combination that prevented growth (detected as turbidity on visual inspection of the wells on each microtitration plate) after 18 hours of incubation at 37° C. The minimal bactericidal concentration (MBC) was determined by transferring 0.05 ml of the contents from each well of the 18 hour cultures to corresponding wells on a fresh microtitration plate containing plain MHB. These subcultures were then read in the same manner as for MICs after 18 hours of incubation at 37° C.

Studies of antibiotic combinations against each isolate were performed in triplicate. The results were recorded as previously described [1,2] insofar as the combination was considered to have a synergistic action when a fourfold or greater reduction of the MIC of both antibiotics occurred as compared to the MIC of each antibiotic alone. A fourfold or greater reduction in the MIC of one antibiotic associated with a twofold reduction of the other was considered to indicate partial synergy. The effect of the combination was considered additive when a twofold reduction of the MIC occurred for both antibiotics and indifferent when the MIC of one drug was unchanged by the combination.

Final concentrations of cefamandole ranged from 0.015 to 128 ug/ml. Final concentrations of tobramycin ranged from 0.012 to 0.75 ug/ml for studies with *S. aureus*; from 0.05 to 3.0 ug/ml for studies with *E. coli*; and 0.19 to 12.0 ug/ml for studies with *K. pneumoniae.*

RESULTS

The susceptibility of the various isolates to cefamandole and tobramycin are shown in Tables 1 and 2 and are expressed as the cumulative percent of isolates killed by each antibiotic at selected concentrations. The MIC of cefamandole and tobramycin when each drug was tested alone and in combination against each of the 25 isolates of *S. aureus* studied are presented in Table 3. The ranges of MBC of each drug alone and in combination against *E. coli* and *K. pneumoniae* are shown in Table 4.

Cefamandole

The minimal inhibitory concentration of cefamandole for *Staphylococcus aureus* isolates ranged between 0.5 and 64 ug/ml. *Escherichia coli* isolates were inhibited in a range between 0.5 and 16 ug/ml and *Klebsiella pneumoniae* isolates were inhibited in concentrations ranging from 1.0 to 128 ug/ml. Ranges for MBCs were similar for the three categories of isolates and were 0.5 to 64 ug/ml for *S. aureus,* 0.5 to 16 ug/ml for *E. coli,* and 1.0 to 128 ug/ml for *K. pneumoniae.*

Tobramycin

Minimal inhibitory concentrations of tobramycin ranged from 0.05 to greater than 0.75 ug/ml for isolates of *S. aureus* although 84% of these isolates were killed by 0.375 ug/ml or less. The isolates of *E. coli* were inhibited by concentrations of 0.375 to 1.50 ug/ml of tobramycin, and the *K. pneumoniae* isolates were inhibited in a range between 0.19 to 0.75 ug/ml. The MBCs were in ranges similar to the MICs

TABLE 1
Cumulative Percent of Isolates
Susceptible to Cefamandole

Organism	No. of Isolates	Cumulative percent of isolates susceptible at MBC (ug/ml) of:								
		0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128
S. aureus	25	12	52	68	76	80	84	92	100	—
E. coli	24	17	29	46	79	96	100	—	—	—
K. pneumoniae	25	0	4	20	32	56	84	96	96	100

TABLE 2
Cumulative Percent of Isolates
Susceptible to Tobramycin

Organism	No. of Isolates	Cumulative percent of isolates susceptible at MBC (ug/ml) of:						
		0.05	0.09	0.19	0.375	0.75	1.50	3.00
S. aureus	25	20	52	80	84	84	84	84
E. coli	24	0	0	0	4	75	96	100
K. pneumoniae	25	0	0	4	64	100	—	—

although for individual isolates concentrations of tobramycin required for a bactericidal effect were sometimes two to four times the inhibitory concentrations.

Synergy Studies of Cefamandole-Tobramycin Combination

The combination demonstrated a synergistic or partially synergistic bactericidal effect against many of the isolates tested. Synergism or partial synergism between the two antibiotics was demonstrated by appropriate reduction in minimal bactericidal concentrations for 15 of the 25 *S. aureus* isolates (60%) when compared to MBCs for each drug alone. Synergy or partial synergy in bactericidal effect was demonstrated with 23 of 24 *E. coli* isolates examined (96%) and 19 of 25 *K. pneumoniae* isolates (76%) examined. In many instances a greater than fourfold reduction in the MIC or MBC of both antibiotics was observed when they were combined as compared with their activity when used alone. In no case did we observe antagonism between the two drugs.

DISCUSSION

In recent years a considerable effort by many investigators has been directed at finding combinations of antibiotics that are synergistic against pathogenic organisms. Organisms responsible for bacterial endocarditis and gram-negative rod septicemia have been the focus of much of this investigation. An organism such as the enterococcus which is difficult to eliminate from cardiac vegetations with single drug therapy is more easily dealt with when appropriate antibiotic combinations are utilized. Gram-negative sepsis is also a difficult infection to treat and successful therapy may depend upon rapid elimination of the causative organisms from the blood stream. Gram-negative rod endocarditis is associated with a high mortality even when the causative organisms are sensitive to the antibiotics employed. Combination chemotherapy has

TABLE 3
MICs of cefamandole and tobramycin alone and in combination—action on 25 isolates of *Staphylococcus aureus*

Strain	Cefamandole (ug/ml)	Cefamandole + Tobramycin (ug/ml)		Tobramycin (ug/ml)
Synergy*				
106	4.0	0.5	0.02	0.09
547	0.5	0.125	0.02	0.09
Partial Synergy**				
137	2.0	0.06	0.09	0.19
147	0.5	0.125	0.05	0.09
268	0.5	0.125	0.02	0.05
343	1.0	0.125	0.02	0.05
376	1.0	0.125	0.02	0.05
402	2.0	0.25	0.02	0.05
403	1.0	0.25	0.02	0.05
1403	1.0	0.25	0.05	0.09
Partial Synergy†				
70	1.0	0.5	0.02	0.09
83	2.0	1.0	0.02	0.19
109	2.0	1.0	0.02	0.19
155	8.0	4.0	0.05	0.19
295	0.5	0.25	0.02	0.09
1407	4.0	2.0	0.02	0.09
Additive††				
864	1.0	0.5	0.05	0.09
947	1.0	0.5	0.05	0.09
1347	1.0	0.5	0.05	0.09
Indifference§				
152	1.0	0.5	0.09	0.09
425	64.0	64.0	0.75	0.75
1348	0.5	0.5	0.05	0.05
Indeterminate§§				
108	32.0	32.0	>0.75	>0.75
313	32.0	16.0	>0.75	>0.75
533	32.0	32.0	>0.75	>0.75

*The MIC of each drug was at least four times as effective in combination as the MIC of that drug alone.

**The MIC of cefamandole was at least four times as effective in combination as it was alone while the MIC of tobramycin was at least twice as effective in combination as it was alone.

†The MIC of tobramycin was at least four times as effective in combination as it was alone, while the MIC of cefamandole was at least twice as effective in combination as it was alone.

††The MICs of both drugs were twice as effective in combination as each was alone.

§The MIC of one drug was not changed by combining it with the other.

§§No tobramycin end point.

been widely advocated for this disease. In studies done in our laboratory with animal models of gram-negative sepsis, certain antibiotic combinations shown to be synergistic *in vitro* decreased the number of bacteria at the challenge site, reduced the level of bacteremia and significantly improved survival in rats given an intraperitoneal challenge of *Pseudomonas aeruginosa* when compared to controls treated with saline or a single drug [1,2]. We have also observed this in a corresponding model of

TABLE 4
Range of MBCs of cefamandole and tobramycin alone and in combination—action on 24 isolates of *E. coli* and 25 isolates of *K. pneumoniae*

	No. of Isolates	Range of MBCs Cefamandole (ug/ml)	Range of MBCs Cefamandole + Tobramycin (ug/ml)		Range of MBCs Tobramycin (ug/ml)
	E. coli				
Synergy	12	1.00–16.00	0.25–2.00	0.05–0.375	0.375–1.50
Partial Synergy	11	0.50–8.00	0.06–2.00	0.19–0.75	0.75–3.00
Indifference	1	8.00	4.00	0.75	0.75
	K. pneumoniae				
Synergy	9	4.00–16.00	0.06–2.00	0.05–0.19	0.375–0.75
Partial Synergy	10	1.00–32.00	0.06–16.00	0.05–0.19	0.19–0.75
Addition	4	2.00–4.00	1.00–2.00	0.19	0.375
Indifference	2	8.00–128.00	8.00–128.00	0.375–0.75	0.375–0.75

Klebsiella sepsis in rats [12]. Recently, Lumish and Norden have repeated this work in neutropenic rats challenged with *P. aeruginosa* and observed similar results [3]. These considerations have prompted our investigation of other potentially useful antibiotic combinations.

The results of the present study utilizing cefamandole and tobramycin indicate that this combination acts synergistically against the majority of *K. pneumoniae* and *E. coli,* organisms frequently implicated in gram-negative rod septicemia. Furthermore, this synergistic effect was observed at clinically achievable concentrations of these antibiotics. While this study reports only *in vitro* data, our previous experience suggests that this drug combination is likely to show promise *in vivo.*

Although synergy was more frequently demonstrated with the gram-negative organisms studied, synergism was also observed against some *Staphylococcus aureus* isolates. This is of interest since staphylococcal sepsis, especially when it is associated with endocarditis, is a devastating illness and it is desirable to find effective anti-staphylococcal regimens which carry less risk for patients allergic to penicillins. Vancomycin, an effective antibiotic alternative to penicillins for the treatment of staphylococcal endocarditis, is quite toxic, and a recent report records its failure to eliminate *S. aureus* from the blood stream of a patient with endocarditis until it was combined with methicillin and gentamicin [4]. Synergism against staphylococci has been previously demonstrated with combinations of penicillins and aminoglycosides both *in vitro* and *in vivo.* Sande and Courtney, using a rabbit model of staphylococcal endocarditis showed that organisms were cleared more rapidly from cardiac vegetations in animals who received a combination of nafcillin and gentamicin than with nafcillin alone. Gentamicin was not effective alone [5]. Steigbigel and coworkers examining the effect of several antibiotic combinations on mortality in mice infected with staphylococci were able to demonstrate enhanced efficacy of penicillin-aminoglycoside combinations as compared to single drug therapy, but no advantage over single drug therapy was demonstrated with combinations of clindamycin and gentamicin or erythromycin and gentamicin [6]. Watanakunakorn and Glotzbecker examined the *in vitro* activity of several anti-staphylococcal antibiotics alone and in combination with aminoglycosides and observed enhancement of the effect of cephalothin and cefazolin when they were combined with various aminoglycosides including tobramycin [7]. The *in vitro* synergy shown by our work with cefamandole and tobramycin is encouraging since both drugs may be employed in some patients

allergic to penicillins. Comparisons with other cephalosporin-aminoglycoside combinations in animal models seems warranted.

An additional finding which emerged from our studies with *Staphylococcus aureus* was our observation that several isolates were more resistant to cefamandole than has been previously reported. Furthermore, no reduction of the MIC of either drug was observed when the combination was tested with four of these relatively resistant isolates. Bodey and Weaver observed that 100% of the *S. aureus* isolates they tested were killed at concentrations of cefamandole at or below 0.78 ug/ml [8]. Of 56 isolates studied by Neu, none required more than 1.6 ug/ml for inhibition [9]. Several of our 25 isolates of *S. aureus* had MICs greater than 2.0 ug/ml. (These results were confirmed in another laboratory) [10]. Similar resistance to cephalosporins has been observed with methicillin-resistant strains. This resistance does not appear to be related to β-lactamase production but rather to an as yet unexplained intrinsic resistance which extends to other cephalosporins [11].

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