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In Vitro Antagonism by Erythromycin of the Bactericidal Action of Antimicrobial Agents Against Common Respiratory Pathogens

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Ten strains each of *Staphylococcus aureus, Haemophilus influenzae, Enterobacter* spp., *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa,* and *Streptococcus pneumoniae* were tested in vitro against erythromycin combined with ampicillin, cefamandole, or gentamicin. Antagonism by erythromycin occurred with 47% of the combinations involving strains of *S. aureus* and to a lesser degree with *H. influenzae.* Synergy occurred most commonly with *H. influenzae* (27%). The high frequency of antagonism and synergy with these organisms was associated with a broad range of bacteriostatic action by erythromycin against these same bacteria. The implications for the treatment of pneumonia are discussed.

The list of erythromycin-susceptible microorganisms includes *Legionella pneumophila* (3, 4, 7, 13, 17). *Mycoplasma pneumoniae* (10), and most strains of *Streptococcus pneumoniae* (9). The first two organisms, as well as some strains of the latter, are resistant to penicillin and other commonly employed antimicrobial agents. Because of this spectrum of activity, the low toxicity of erythromycin, and the well-recognized difficulty of establishing immediately the etiology of many cases of pneumonia, particularly those caused by *Legionella* spp. or *Mycoplasma* spp., physicians have increasingly considered erythromycin for inclusion in empiric antimicrobial regimens for serious pneumonias of uncertain etiology. One potentially important aspect of this course of therapy is the effect of erythromycin on the activity of other antimicrobial agents. This aspect has not been adequately examined.

Almost 30 years ago, Jawetz and Gunnison divided antimicrobial agents into two categories based on their bactericidal and bacteriostatic activities (11). Penicillin, streptomycin, neomycin, and polymyxin B were classed as bactericidal (group I). Chloramphenicol, tetracycline, erythromycin, and the sulfonamides were classed as bacteriostatic (group II). Synergy was thought to occur most commonly with combinations of group I drugs, occasionally with combinations of group I and II drugs, but not with combinations of group II drugs. More important in the above setting, group II drugs were found to be capable of inhibiting the bactericidal action of group I antibiotics (11, 12).

It therefore seemed valuable to examine the effect of erythromycin on the bactericidal action of common broad-spectrum antimicrobial agents. We were particularly interested to see whether erythromycin would inhibit their bactericidal activity against frequently isolated respiratory pathogens. If one of these organisms was, in fact, the primary pathogen in an episode of pneumonia, we wanted to see whether there was in vitro evidence that erythromycin could potentially impede recovery. Microtiter techniques were employed so that the results could be correlated with antibiotic concentrations.

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MATERIALS AND METHODS

Organisms. We selected 10 strains each of *S. pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Enterobacter* spp. (7 strains of *Enterobacter aerogenes,* 2 strains of *Enterobacter cloacae,* and 1 strain of *Enterobacter agglomerans*), and *Proteus mirabilis* as being representative of the most common bacterial pathogens isolated in clinical pneumonias (2, 5, 18, 21). Organisms were obtained from the clinical microbiology laboratory of the Thomas Jefferson University Hospital. Care was taken to select isolates from different patients. The organisms were initially recovered from cerebrospinal fluid, wound cultures, blood cultures, and predominant growth in sputum specimens. Identification was done by the usual methods and was confirmed by a separate technologist (6). Isolates were kept on refrigerated chocolate agar slants and were

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subcultured on the day before testing to check for purity and viability. Three to five colonies from the fresh subculture were used for susceptibility testing.

Media and reagents. Tests for synergy and antagonism of antimicrobial activity against *S. pneumoniae* were done with brain heart infusion broth plus sterile bovine serum at a concentration of 7.5%. Tests for synergy and antagonism of activity against *H. influenzae* were done in Mueller-Hinton broth supplemented with 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.) and 5% Fildes enrichment (Difco Laboratories, Detroit, Mich.) (22). All other organisms were tested in Mueller-Hinton broth. Cation supplements were added to give a final concentration of approximately 3.2 and 7.5 mg/dl for Mg^{2+} and Ca^{2+}, respectively (23).

Erythromycin base, gentamicin, and cefamandole antimicrobial standard powders were kindly supplied by Abbott Laboratories, North Chicago, Ill., Schering Corp., Bloomfield, N.J., and Eli Lilly & Co., Indianapolis, Ind.

Plate preparation. Stock solutions of each antimicrobial agent were prepared, and twofold serial dilutions were then made in broth to provide gradients of antimicrobial concentration.

A Cooke-Dynatech MIC-2000 dispenser (Dynatech Laboratories, Inc., Alexandria, Va.) was used to dispense the antimicrobial agents into 96-well (8 by 12) microtiter plates. All possible combinations within the cited ranges were tested. The volume in each of the 96 wells was 0.1 ml. The concentrations (in micrograms per milliliter) of antimicrobial agents for each pairing were as follows: (i) ampicillin at 0, 0.067, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, or 64, with erythromycin at 0, 0.25, 0.5, 1, 2, 4, 8, or 16; (ii) gentamicin at 0, 0.25, 0.5, 1, 2, 4, 8, or 16, with erythromycin at 0, 0.67, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, or 64; (iii) cefamandole at 0, 0.67, 0.125, 0.25, 0.5, 0.5, 1, 2, 4, 8, 16, 32, or 64, with erythromycin at 0, 0.25, 0.5, 1, 2, 4, 8, or 16.

All plates were frozen at −20°C until used, for a maximum of 80 days.

Inoculation and incubation. Several colonies of each test organism were inoculated into Trypticase (BBL) soy broth which was then incubated for 2 to 4 h at 35°C. The turbidity was adjusted to equal a barium sulfate 0.5 McFarland standard, with each tube containing approximately 1×10^8 to 5×10^8 viable organisms per ml. This culture diluted 1:10 in sterile water provided the inoculum. A 1.5-μl amount of inoculum was delivered to each well of the microtiter plate by using a multiprong MIC-2000 inoculator. The final concentration in each well was approximately 1×10^5 to 5×10^5 organisms per ml for all organisms except *S. aureus*, which was 1×10^6 organisms per ml. Plates were incubated at 35°C for 20 h. The CO_2 and humidity were 4.2 and 98%, respectively.

The first wells without visible growth in the rows containing only a single antibiotic marked the minimum inhibitory concentration (MIC) of that antibiotic.

A calibrated loop was used to remove 0.01 ml of inoculum from each well showing no growth. This was streaked onto a blood agar plate, except in the case of *H. influenzae*, where the medium used was chocolate blood agar. The plates were incubated as described for the MIC plates. An endpoint of four colonies or less on the subculture plates was used to determine the minimal bactericidal concentration (MBC), in a manner similar to that used to define the MIC. This is at least a 99% bactericidal level.

Several organisms were reexamined at the conclusion of the study, using identical techniques. The organisms were stored as described above between tests.

Antagonism was defined as a fourfold increase in the MBC of the tested antibiotic in the presence of erythromycin. Synergy was defined as a fourfold decrease in the MBC of the tested antibiotic in the presence of erythromycin at a concentration less than the MBC of erythromycin for that organism.

RESULTS

A summary of our results appears in Table 1. No synergy or antagonism was demonstrated with the tested strains of *P. aeruginosa* or *S. pneumoniae* (data not shown). All results are listed based on changes in MBC. Parenthetically, there were only two instances out of the 240 pairs of antibiotics tested in which the MIC alone of an antibiotic was changed. Both cases involved synergy between erythromycin and gentamicin.

As indicated above, several organisms were retested at the conclusion of our study. In all cases the interactions were confirmed.

Isolated wells that were either positive or negative were discounted. Generally, on subculture the patterns were clear, with definite demarcations between antimicrobial concentrations showing growth and no growth. In a very few cases the patterns were less definite. Analysis of the colony counts on these subculture plates showed that the differences could be attributed to small variations in the actual number of colonies and did not represent a flaw in our method.

The MICs and MBCs for erythromycin alone with each of the 80 organisms (Table 2) were correlated with the amount of synergy or antagonism occurring. As might be expected, 29 of the 41 interactions occurred with the two organisms (*S. aureus* and *H. influenzae*) for which erythromycin at the tested concentrations had the broadest range of bacteriostatic activity. When the data for antagonism alone were analyzed, 21 of 25 cases occurred with these same two organisms. We analyzed in depth our data with *S. aureus* to better define possible mechanisms for the patterns observed and to document the consistency of the results. There were 14 instances of antagonism with this organism. In eight of these, antagonism was observed at a given erythromycin concentration up to the highest tested concentration of ampicillin, cefamandole, or gentamicin. In the remaining six cases, erythromycin antagonism appeared to be overcome by higher concentrations of the tested antibiotics.

TABLE 1. *Interactions of erythromycin with ampicillin, cefamandole, and gentamicin for six common respiratory bacterial pathogens*

Organism	Interaction	No. of strains showing interaction when tested with:		
		Ampicillin	Cefamandole	Gentamicin
S. aureus (10)[a]	Antagonism	4	6	4
	Synergy	0	0	0
H. influenzae (10)	Antagonism	4	3	0
	Synergy	1	3	4
Enterobacter spp.[b] (10)	Antagonism	0	1	0
	Synergy	1	1	2
E. coli (10)	Antagonism	1	1	0
	Synergy	0	0	2
K. pneumoniae (10)	Antagonism	0	0	0
	Synergy	2	0	0
P. mirabilis (10)	Antagonism	1	0	0
	Synergy	0	0	0

[a] Number of strains is given within parentheses.
[b] Synergy occurred three times with two strains of *E. aerogenes* and one strain of *E. cloacae*. Antagonism occurred with one strain of *E. agglomerans*.

TABLE 2. *In vitro susceptibility of erythromycin of 80 organisms representing 10 strains each of eight common bacterial respiratory pathogens*

Organism	MIC (μg/ml) for the following % of strains:			MBC (μg/ml) for the following % of strains:		
	50	80	90	50	80	90
S. aureus	0.25	0.5	0.5	16	>64	>64
H. influenzae	4	16	16	16	64	>64
Enterobacter spp.	>64	>64	>64	>64	>64	>64
E. coli	64	64	>64	>64	>64	>64
K. pneumoniae	64	>64	>64	>64	>64	>64
P. mirabilis	>64	>64	>64	>64	>64	>64
P. aeruginosa	>64	>64	>64	>64	>64	>64
S. penumoniae	<0.067	0.125	0.125	<0.067	0.125	0.25

The mean increase in the MBC was 3.67 wells (range, 2 to 7) for these six organisms.

It might also be expected that if erythromycin antagonizes the bactericidal action of ampicillin, it should also block the activity of cefamandole. We again examined our data with the organism most frequently associated with antagonism, *S. aureus*, to test this hypothesis. In only one instance, where both cefamandole and ampicillin were bactericidal and erythromycin had a broad range of bacteriostatic activity, did antagonism fail to occur. For that particular strain, the MBC for cefamandole was <0.067 μg/ml. We attributed the lack of antagonism by erythromycin to the marked susceptibility of that strain to cefamandole. In all cases, when the MBC and MIC of erythromycin were both low, ≤0.5 μg/ml, no antagonism was noted.

DISCUSSION

In vitro, erythromycin can antagonize or augment the bactericidal action of ampicillin, cefamandole, or gentamicin against some organisms. The mechanism of this dual action is not apparent from our data, but it appears that a minimum of two different mechanisms is necessary to account for the different effects of erythromycin in different settings.

The classic explanation of antimicrobial antagonism is that the bacteriostatic drug inhibits the growth of the target organism so that the action of a bactericidal antimicrobial agent that is dependent on cell growth and replication is blocked. This mechanism is consistent with our results with *S. aureus* and the cell wall-active drugs ampicillin and cefamandole. It does not

explain why in a few instances erythromycin interfered with the action of antibiotics against organisms for which erythromycin alone was not measurably bacteriostatic.

Antimicrobial synergy between bactericidal and bacteriostatic antimicrobial agents may possibly be explained by the inhibition by one drug of the production of enzymes that mediate resistance to the second drug. This mechanism has been demonstrated by Allen and Epp (1) to be present for inducibly resistant strains of *S. aureus*. Griffith et al. (8) demonstrated synergy between erythromycin and cefamandole against *Bacteroides fragilis* and postulated this same mechanism. They also suggested the possibility that cefamandole caused cell wall damage that allowed erythromycin to more easily penetrate bacterial cells.

It is of particular interest that the MBC of ampicillin against one strain of *H. influenzae* was increased 64-fold by 2 μg of erythromycin per ml, whereas this same concentration of erythromycin decreased the MBC of cefamandole from 64 to 2 μg/ml. It would therefore seem reasonable to conclude that erythromycin may have more than one effect on the same organism.

The clinical importance of antimicrobial antagonism has been demonstrated (14-16, 19, 20). In vitro correlation of clinical antagonism has not been commonly performed. It is therefore difficult to extrapolate from our data directly to clinical settings. Factors of host resistance, drug metabolism, drug distribution, and the specific susceptibilities of each microorganism combine to make overly inclusive generalizations unwarranted. Our in vitro evidence of antagonism suggests that clinically important in vivo antagonism may occur. Significant synergy by erythromycin probably does not occur at clinically achievable levels with the antimicrobial agents and microorganisms that we tested.

Clearly, our results support an aggressive approach to obtaining precise microbiological data so that the need for empiric therapy of respiratory tract infections is minimized. Methods for the early diagnosis of infections by *Legionella* spp. and *Mycoplasma* spp. should be developed and widely employed. In locations where penicillin-resistant pneumococci are not uncommon, antimicrobial susceptibility data should be obtained for this organism.

Additional investigation of the in vitro correlates of clinically significant antimicrobial antagonism would also prove to be useful. This might enable a more reasonable prediction from in vitro data as to the possibility of clinically significant antagonism developing between drugs such as erythromycin and other antimicrobial agents against suspected pathogens.

For the moment, the empiric use of erythromycin in a multiple-drug regimen remains a potentially useful strategy for treating pneumonias of uncertain etiology. However, if infection by certain aerobic bacteria, particularly *S. aureus* and *H. influenzae*, is not adequately ruled addition of erythromycin in combination with other antimicrobial agents cannot, a priori, be assumed to be benign.

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