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Effect of *N*-Acetylcysteine on Antibiotic Activity and Bacterial Growth In Vitro

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The antibiotic bacterial inactivity of *N*-acetylcysteine (NAC) and its interaction with penicillin and aminocyclitol antibiotics was evaluated. NAC inhibited growth of both gram-negative and gram-positive bacteria. Strains of *Pseudomonas aeruginosa* were more susceptible than other microorganisms tested. *P. aeruginosa* strains were inhibited synergistically by NAC and carbenicillin or ticarcillin. However, NAC antagonized the activity of gentamicin and tobramycin. These findings have implications for the combined clinical use of NAC and aerosolized antibiotics and are also important for the processing of sputum specimens in the microbiology laboratory.

N-acetylcysteine (NAC; Mucomyst) is an effective mucolytic agent in vitro (2) and is frequently used to facilitate the processing of sputum specimens for bacteriological examination (5). It is effective in reducing sputum viscosity in patients with cystic fibrosis and chronic obstructive pulmonary disease when administered by aerosol (3, 7).

NAC, although an effective mucolytic agent, may be antagonistic to the activity of several antibiotics (1, 4, 6). Since NAC is widely used in patients with chronic infections of the lower respiratory tract, especially in patients with cystic fibrosis and other conditions where *Pseudomonas aeruginosa* is a prominent pathogen, we decided to evaluate the antibacterial activity of NAC. We also investigated the interaction of NAC with penicillin derivatives and aminocyclitol antibiotics active against the gram-negative microorganisms found in respiratory infections.

MATERIALS AND METHODS

NAC was used as the commercially available 20% solution (Mucomyst, Mead-Johnson Laboratories, Evansville, Ind.), containing 0.05% disodium ethylenediaminetetraacetic acid as a preservative. Crystalline NAC (Mead-Johnson Laboratories) was used to make a 20% preservative-free solution in distilled water. Dithiothreitol (Sputolysin, Calbiochem, La Jolla, Calif.) was used as a comparison mucolytic, diluted 1:100 with distilled water. Solutions were made fresh daily, filtered through a sterile 0.22-μm membrane filter (Millipore Corp.), and used within 2 h of preparation. The pH of all solutions was adjusted to 7.0.

Microorganisms studied were all isolated from clinical specimens of patients admitted to Columbia-Presbyterian Medical Center. Strains of *P. aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae,* and *Staphylococcus aureus* were identified according to standard laboratory techniques and maintained on brain heart infusion agar slants (Difco) at room temperature prior to use.

Growth curves were determined by optical density methods and serial colony counts. For optical density studies, 10^5 colony-forming units (CFU) from an overnight shaking broth culture suspension in nutrient broth (BBL) were inoculated into 30 ml of nutrient broth containing NAC in concentrations of 0, 0.2, 2, and 20 μg/ml. The resulting suspensions were incubated at 35°C in a rotary water bath shaker, and optical densities were read hourly by a Klett colorimeter. Growth curves were done in a similar fashion, with NAC present at concentrations of 0, 2, 8, 14, and 20 μg/ml of nutrient broth. The number of CFU was determined by inoculating 0.1 ml of the appropriate dilution of timed aliquots onto Mueller-Hinton agar (BBL) plates. Plates were then incubated at 35°C for 24 h. Results obtained in nutrient broth were compared with results using Mueller-Hinton broth and nutrient broth supplemented with $MgCl_2$ (2 mM) and $CaCl_2$ (5 mM).

Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined by the tube dilution technique. The MBC was determined by the absence of growth or less than five colonies when 0.01 ml was transferred to agar plates. NAC was used in concentrations of 0, 2, 8, 14, and 20 μg/ml in nutrient broth. Dithiothreitol was used in similar concentrations. Each dilution was prepared and inoculated with 10^5 CFU of test organism. MIC and MBC values were determined after 24 h of incubation at 35°C.

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Synergy studies were performed in nutrient broth using NAC in combination with gentamicin, tobramycin, carbenicillin, and ticarcillin by the tube dilution checkerboard technique. Antibiotics were used in twofold dilutions, and NAC was used in concentrations of 0, 2, 8, 14, and 20 μg/ml. MIC and MBC values were determined after 24 h of incubation at 35°C.

RESULTS

Growth curve experiments demonstrated that NAC inhibited the growth of both gram-negative and gram-positive microorganisms. Strains of *P. aeruginosa* were most susceptible, and growth rate could be inhibited by as little as 2 μg of NAC per ml (Fig. 1). Increasing the concentration of NAC further inhibited growth, and a concentration of 20 μg/ml achieved 95% killing at 12 and 24 h.

NAC was less inhibitory to the growth of staphylococci and the members of the *Enterobacteriaceae* tested. Although delay in growth occurred with 20 μg of NAC per ml, growth was visible at 24 h and few organisms were inhibited by 20 μg of NAC per ml (Table 1). Susceptibility to NAC was not related to the susceptibility of the microorganisms to penicillin derivatives or aminocyclitol antibiotics.

The size of the inoculum altered the NAC inhibition. With an inoculum of 10^5 CFU, 2 μg of NAC per ml was inhibitory; but with an inoculum of 10^7 CFU, growth of *Pseudomonas* was not inhibited by 2 μg/ml. Inhibition of growth was not dependent upon the presence of ethylenediaminetetraacetic acid in the commercial NAC preparation (Mucomyst); identical results were obtained with pure NAC solution in nutrient broth with or without cation supplementation or in Mueller-Hinton broth. Dithiothreitol was also inhibitory; four of five strains of *P. aeruginosa* were inhibited by 0.8% dithiothreitol.

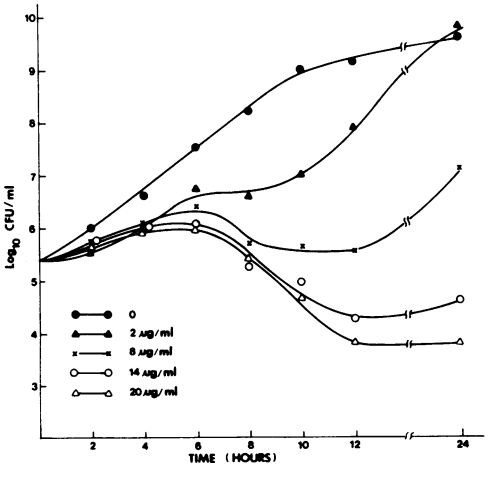

FIG. 1. *Effect of varying concentrations of NAC on the growth of P. aeruginosa in nutrient broth.*

TABLE 1. *In vitro susceptibilities*

Organism (no. tested)	No. inhibited at NAC concn (μg/ml) of:			
	2	8	14	20
Pseudomonas (13)	1	2	9	12
Staphylococcus (8)	0	0	0	1
Klebsiella-Enterobacter (10)	0	0	0	0

The combination of NAC and carbenicillin or ticarcillin was additive or synergistic against strains of *P. aeruginosa* (Fig. 2a, b). However, antagonism of the activity of aminocyclitol antibiotics was found (Fig. 2c, d). In the presence of subinhibitory concentrations of NAC (8 to 14 μg/ml), gentamicin MIC values for *Pseudomonas* rose 2- to 8-fold and tobramycin MIC values rose 8- to 16-fold. Similar results were obtained with *Klebsiella* (Table 2).

DISCUSSION

Although NAC is allegedly without significant antibacterial activity, our results show considerable activity at concentrations that would be achieved either in the upper respiratory tract of patients during aerosolization of NAC or in the laboratory during liquefication of sputum. This activity is predominantly bacteriostatic, is most pronounced against strains of *P. aeruginosa*, and occurs at concentrations less than 10% of commercially available Mucomyst (20% NAC). The activity of NAC is not related to chelation of divalent cations nor to the effect of ethylenediaminetetraacetic acid in the preparation. The effects of NAC are inoculum size and dose dependent. NAC may act by competitively inhibiting amino acid (cysteine) utilization (8) or, by virtue of possessing a sulfhydryl group, may react with bacterial cell proteins. We observed similar inhibitory activity with dithiothreitol.

It has been shown previously that NAC diminishes the activity of aminocyclitol antibiotics, neomycin, streptomycin, and kanamycin (1, 6). Our results show similar data for gentamicin and tobramycin, with a 2- to 16-fold increase in the MIC values of susceptible microorganisms in the presence of low concentrations of NAC.

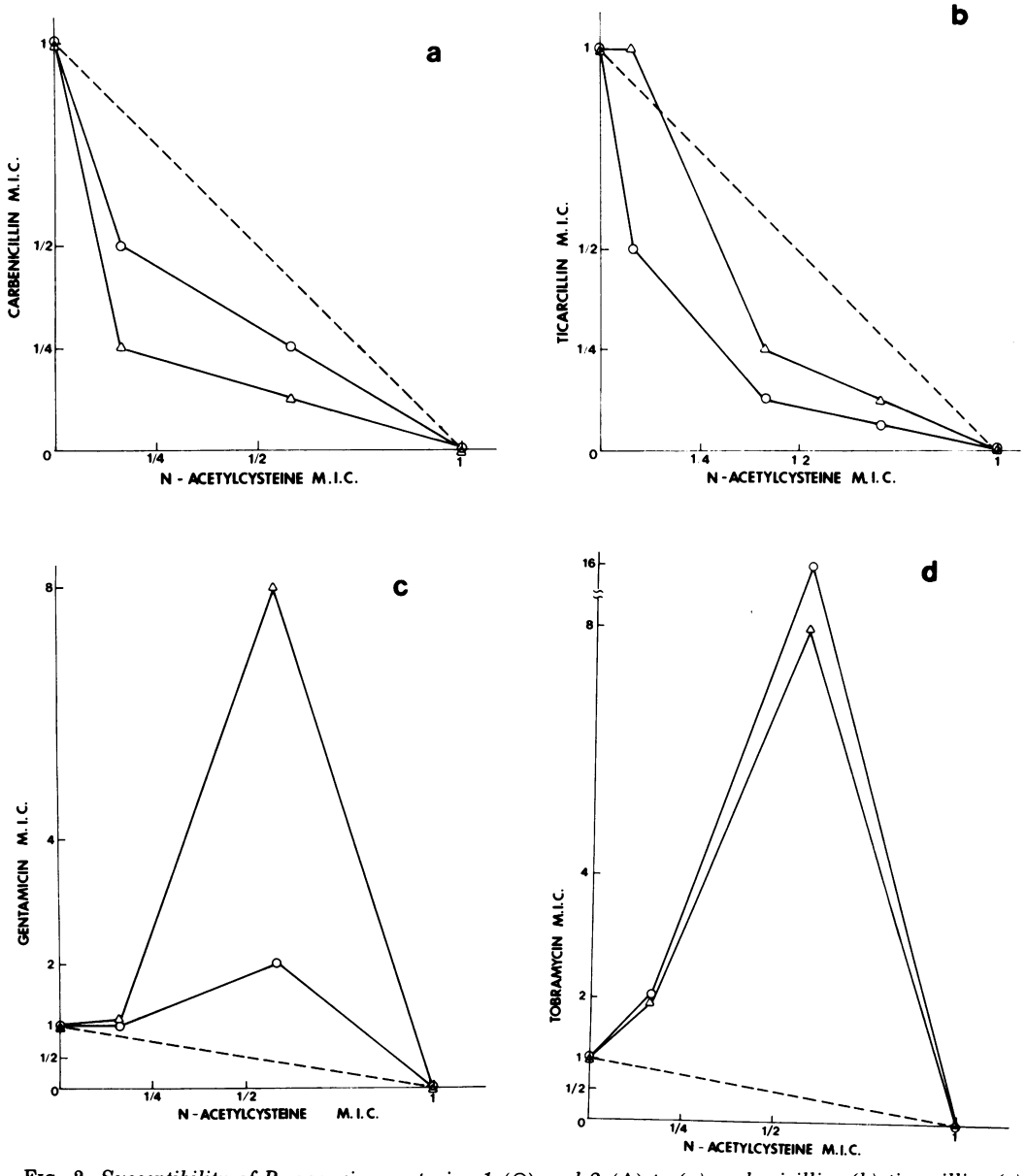

FIG. 2. *Susceptibility of P. aeruginosa strains 1 (○) and 2 (△) to (a) carbenicillin, (b) ticarcillin, (c) gentamicin, and (d) tobramycin. The broken line is the line of additive effect. The concentrations of NAC and antibiotics are expressed as multiples of the MIC.*

TABLE 2. *Effect of NAC on susceptibility of Klebsiella 2851 to gentamicin*

NAC (μg/ml)	Gentamicin (μg/ml)	
	MIC	MBC
0	0.04	0.04
2	0.08	0.08
8	0.31	0.31
14	0.62	1.25
20	0.62	0.62

Several investigators have shown inactivation of ampicillin, methicillin, and isoxazolyl penicillins by NAC (1, 4). The activity of penicillin G may (4) or may not (1) be reduced by NAC. In contrast, we found that the combination of carbenicillin or ticarcillin and NAC was additive or synergistic against the strains of *P. aeruginosa* tested. The explanation for this observation is unknown but does not seem due to choice of microorganism since the same strain

of *Pseudomonas* was not inhibited by gentamicin or tobramycin in the presence of NAC.

These data suggest that the use of NAC or dithiothreitol to liquefy sputum for bacterial culture is likely to inhibit the growth of *Pseudomonas* strains, leading to falsely negative cultures. Indeed, in a study of the clinical effectiveness of ticarcillin and tobramycin in the treatment of pulmonary infection in patients with cystic fibrosis, we found that sputum specimens collected after use of aerosolized NAC often failed to show colonies when examined at 24 h. In contrast, specimens obtained randomly showed rapid growth, and colonies appeared at 48 h on plates collected after NAC aerosol.

The synergy produced by the combination of NAC and penicillins, such as carbenicillin or ticarcillin, may give a false impression that *Pseudomonas* has been cleared from the sputum. In contrast, use of NAC as an aerosolized mycolytic agent may locally inactivate aminocyclitol antibiotics. This is important since many cystic fibrosis groups use aerosols of aminoglycosides and NAC simultaneously. Although they are chemically compatible, we feel that NAC and aminocyclitol antibiotics should not be aerosolized together.

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