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N-Acetylcysteine Inhibits Germination of Conidia and Growth of *Aspergillus* spp. and *Fusarium* spp.

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***N*-Acetylcysteine inhibited hyphal growth and germination of conidia of *Aspergillus* spp. and *Fusarium* spp. *N*-Acetylcysteine inhibited conidial germination as well as or better than L-cysteine. Cysteine-related compounds may provide a potential therapeutic strategy against agriculturally and medically important fungal pathogens.**

Exposure to organic dusts has been known to cause pulmonary disease for several centuries (24). Agricultural environments where cotton, hay, and grain are handled produce respirable-sized, airborne dusts contaminated with bacteria, fungi, and their associated toxins (8, 12, 16, 27). Among the most common medically important fungi recovered from the inanimate environment are *Aspergillus fumigatus* and *Aspergillus flavus* (29). *Fusarium* spp. also may be recovered from soil and have further agricultural importance because they cause infestation of crops and mycotoxicosis of farm animals and produce (17). *Aspergillus* spp. and *Fusarium* spp. are also important causes of respiratory infection in immunocompromised patients, including organ and bone marrow transplant recipients, those with human immunodeficiency virus infection, and patients with prolonged neutropenia or neutrophil dysfunction (1, 6, 13, 15, 17, 20, 21, 28). Germination of *Aspergillus* or *Fusarium* conidia is a critical step in the pathogenesis of pulmonary infections caused by these organisms (2, 25). Strategies for inhibition of conidial germination may lead to novel therapeutic interventions.

N-Acetylcysteine (NAC) is administered as an aerosolized mucolytic drug in patients with cystic fibrosis and chronic obstructive pulmonary disease (14, 22). These patients also are at risk for the development of saprophytic aspergillosis of bronchiectatic airways. NAC possesses antimicrobial activity against respiratory bacterial pathogens (18, 19, 23). Little is known, however, about the potential antifungal effect of NAC against medically important opportunistic respiratory fungal pathogens. We therefore studied the effect of NAC, as well as that of L-cysteine (LC), on the germination of conidia and the growth of hyphae of *Aspergillus* spp. and *Fusarium* spp.

Organisms. One clinical respiratory isolate each of *A. fumigatus*, *A. flavus*, *Aspergillus niger*, *Fusarium oxysporum*, and *Fusarium solani* was studied. Cultures were stored on potato dextrose agar (PDA) slants at 4°C. Isolates were subcultured onto fresh PDA slants and were incubated for 5 days. Isolates of *Aspergillus* spp. were incubated at 37°C, while isolates of *Fusarium* spp. were grown at 30°C. After incubation, 10 ml of 1.0% potato dextrose broth supplemented with 0.01% Triton X-100 was added to each slant (5). Conidia were harvested as a suspension, which was vortexed to disperse aggregates. Conidial suspensions were enumerated with a hemacytometer. Few hyphae were observed by this method, and only conidia were evaluated for germination.

Germination assay. The test compounds NAC (*N*-acetyl-L-cysteine) and LC were obtained from Sigma Chemical Company (catalog numbers C-7755 and A-9165, respectively). The concentrations used were 0.0, 0.1, 1.0, and 10.0% (final concentrations). Test compounds were dissolved in the diluent solution. An aliquot of the conidial suspension was added to the test compound solution to effect a 1:10 final dilution of the conidia. The conidial suspensions containing LC or NAC were vortexed for 10 min prior to plate inoculation. Aliquots (15 μl) of the test suspensions were placed in the center of six circles drawn on the bottom of petri dishes containing 1.5% Noble agar (5). Duplicate plates for each concentration of each test compound assayed were inoculated for each of the time periods tested (4, 6, 8, and 12 h) postinoculation. The plates containing *Aspergillus* spp. and *Fusarium* spp. were incubated at 37 and 30°C, respectively. At the appropriate time, the plates were removed from the incubator. Germination was stopped and germ tubes were fixed and stained by the addition of 30 μl of lactophenolaniline blue, which was added to each inoculated circle (4). Within 18 h, the lactophenolaniline blue had fixed the germinating conidia and the excess medium had soaked into the agar. The conidia were then examined microscopically (×400) for germination. Germination was defined as the development of a hyphal tube of any size arising from a conidium. A total of 100 conidia were observed in each of six randomly selected circles on the duplicate plates containing each of the compounds at each concentration and each control tested per time period. Each experiment was performed in duplicate. A total of 19.200 conidia were observed per fungal species tested per compound.

Determination of MICs. Inhibition of growth was studied by a previously described and standardized macrodilution assay consisting of MOPS (morpholinepropanesulfonic acid)-buffered (pH 7.0) RPMI 1640 with glutamine (BioWhittaer, Inc., Walkersville, Md.); the medium was spectrophotometrically adjusted to contain a final inoculum concentration of 1×10^3 to 5×10^3 conidia, and the temperature of incubation was 35°C (9, 10). MICs were read at 24 and 48 h and were the lowest concentrations inhibiting 80% of the growth control.

Statistical analysis. Statistical analysis was performed by T grouping and analysis of variance (ANOVA). T grouping compares the results obtained with each concentration of com-

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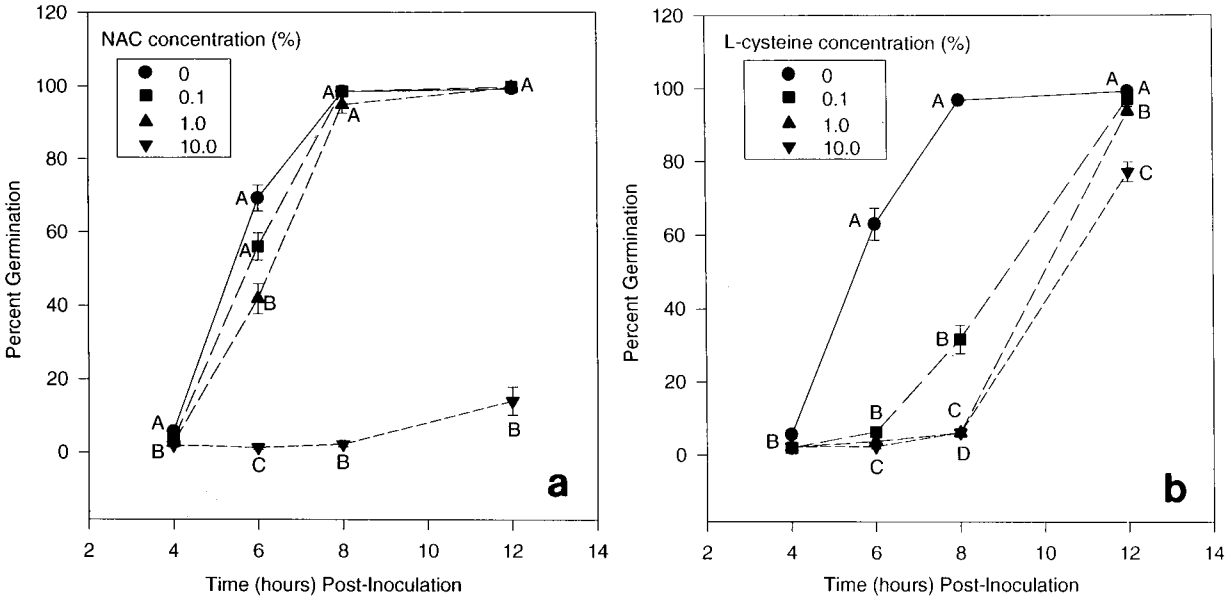

FIG. 1. Effects of NAC (a) and LC (b) on germination of conidia of *A. fumigatus*. Letters refer to T grouping from ANOVA, as follows: A, significantly different from control values; B; $P < 0.05$; C and D, $P < 0.001$.

pound with the results obtained with the remaining concentrations in a pairwise fashion to determine significant differences between each concentration (26). A P value of <0.05 was considered significant.

Results and Discussion. The effects of NAC and LC on inhibition of conidial germination of *A. fumigatus* and *F. solani* are illustrated in Fig. 1 and 2, respectively. Significant inhibition of germination was observed at all concentrations tested. NAC (10%) was the most potent suppressor of germination, causing sustained inhibition over time. Similarly significant patterns of inhibition were consistently observed for *A. flavus*, *A. niger*, and *F. oxysporum* (data not shown). However, NAC and LC were equivalent in their abilities to suppress the germination of *A. niger*. NAC and LC inhibited the growth of all species of *Aspergillus* and *Fusarium* studied at low concentrations of both compounds (Table 1). That the MICs were lower than the concentrations causing inhibition of germination is likely due to the longer exposure of the organisms to NAC and LC in the macrodilution assay.

The mechanism by which cysteine-based compounds inhibit germination is not well understood. A cysteine-rich antimicrobial peptide is present in the mammalian tracheal mucosa (7). In addition, cysteine has been shown to inhibit germination of conidia of *Alternaria* spp. and sporangiospores of *Rhizopus rhizopodiformis* (4, 5, 11). Germinating conidia of *Alternaria* spp. absorb aniline blue and develop a bluish cast, whereas

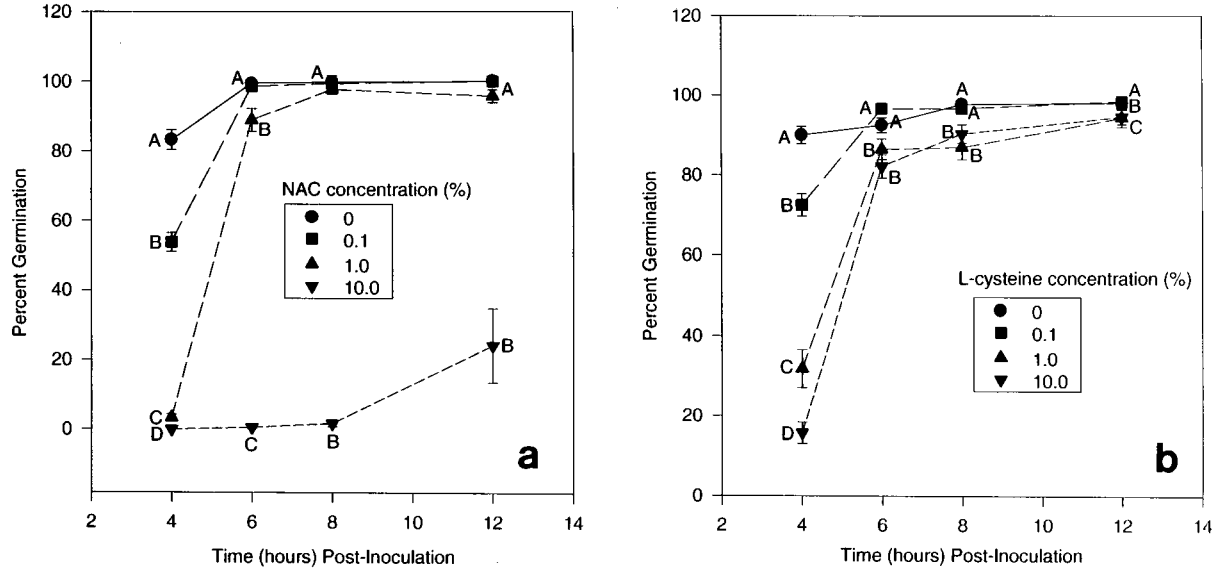

FIG. 2. Effects of NAC (a) and LC (b) on germination of conidia of *F. solani*. Letters refer to T grouping from ANOVA, as follows: A, not significantly different from control values; B, $P < 0.05$; C, $P < 0.001$.

TABLE 1. MICs of NAC and LC for *Aspergillus* spp. and *Fusarium* spp.

Compound (duration of incubation)	MIC (% [wt/vol]; gldl)				
	A. fumigatus	*A. flavus*	*A. niger*	*F. solani*	*F. oxysporum*
NAC (24 h)	0.15	≤0.078	≤0.078	≤0.078	0.31
NAC (48 h)	1.25	0.60	1.25	1.25	2.50
LC (24 h)	≤0.078	≤0.078	≤0.078	≤0.078	0.15
LC (48 h)	0.625	0.30	≤0.078	≤0.078	0.625

conidia treated with cysteine fail to absorb the aniline blue (5). Thus, cysteine may reduce conidial wall permeability to nutrients, thereby affecting germination. Daigle and Cotty (5) concluded that sulfur and amino groups are essential to the ability of cysteine to interfere with conidial germination. The antimicrobial properties of NAC also may be mediated in vivo by enhancement of receptor-mediated phagocytosis by neutrophils (19), as well as by enhancement of intracellular microbicidal activity by alveolar macrophages and polymorphonuclear leukocytes (18).

Aerosolized NAC is indicated for mucolytic therapy of abnormal, viscid, or inspissated secretions in patients with cystic fibrosis and chronic bronchitis. The aerosolized formulation of NAC is delivered in a 10 to 20% solution. Inhibition of growth was achieved by the macrodilution method at concentrations which were less than or equal to 1/10 of the concentrations delivered to the respiratory tract. Moreover, the concentrations at which germination was inhibited in the germination assay were similar to those achieved in the aerosolized formulation of 10 to 20% NAC. While aerosolized NAC has been known to reduce bacterial numbers in human secretions (23), there is a paucity of data on the effect of aerosolized NAC on respiratory fungal pathogens.

The concentration of NAC delivered in aerosolized form equals or exceeds the concentrations required for antifungal activity against *Aspergillus* spp. and *Fusarium* spp. However, the safely achievable concentrations in plasma obtained by oral or parenteral administration of NAC are substantially lower than those found in the present study to be active in vitro (3, 10).

The data from our study indicate the potential application of NAC by aerosolized delivery or topical application for the inhibition of hyphal growth and germination of conidia of *Aspergillus* spp. and *Fusarium* spp. Further investigation of the antifungal properties of NAC and related compounds is warranted.

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