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Therapy of Pulmonary Nocardiosis in Immunocompromised Mice

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We compared the bactericidal efficacies of various antimicrobial agents and combinations thereof in experimentally induced *Nocardia asteroides* pneumonia in immunocompromised mice. Cortisone acetate treatment, which produced impaired cell-mediated immune function, was followed by nasal inoculation of 5×10^4 CFU of *N. asteroides* into each mouse. Therapy was begun 24 h after inoculation and continued for the next 96 h. Dosages of antimicrobial agents resulted in concentrations approximating levels in human serum. Animals from each of nine treatment groups were sacrificed every 24 h. The pulmonary tissue obtained was homogenized and quantitatively cultured. Results were calculated to indicate the number of CFU per gram of lung tissue. Amikacin and imipenem were the two most effective single agents studied. Sulfadiazine and ciprofloxacin were ineffective, and ceftriaxone reduced bacterial counts modestly. Combination therapy did not enhance the bactericidal activities of the agents tested. We conclude that amikacin and imipenem, as well as select broad-spectrum cephalosporins, represent therapy superior to the sulfonamides in this experimental model and may represent alternative treatment for patients who cannot tolerate sulfa agents (e.g., human immunodeficiency virus-infected patients) or who fail primary treatment.

Nocardia asteroides is a ubiquitous soil organism that causes infection in immunocompromised patients, particularly those patients with impaired cell-mediated mechanisms due to corticosteroids, inherited or acquired disorders of immunity, or antirejection treatment of organ transplantation (10). This organism has also been increasingly reported as a pathogen in patients with human immunodeficiency virus infection (1, 7, 9). Standard therapy remains the prolonged administration of trimethoprim-sulfamethoxazole or sulfadiazine (11). Trimethoprim-sulfamethoxazole is available in intravenous form and is preferable for patients who are seriously ill and cannot tolerate oral treatment. Sulfa therapy is generally poorly tolerated in patients with human immunodeficiency virus disease, even when the course of therapy is brief (14). For this reason, as well as failure of primary therapy, alternate therapy is necessary for some patients.

The in vitro susceptibility of *N. asteroides* to a variety of antimicrobial agents has been determined elsewhere (13). In a previous report, there was good correlation between the activities of some of those agents determined in vitro and the abilities to reduce colony counts in target organs in an experimental model (5). The antimicrobial agents tested to date in vitro which were found to be active at low concentrations are amikacin, imipenem, minocycline, ciprofloxacin, and several of the broad-spectrum cephalosporins, including cefotaxime and ceftriaxone. These agents have been shown to be useful therapeutic alternatives in patients who have not responded to or have developed adverse effects from sulfa drugs. Indeed, there have been patients treated with the aforementioned antimicrobial agents as primary therapy (unpublished observations).

A murine model of nocardiosis was developed to determine the distribution of organisms in various target organs (2). This model was modified as described by Beaman in order to determine the in vivo efficacy of antimicrobial therapy in reducing *N. asteroides* in cerebral infections (5). These models used immunologically competent mice, and there was a tendency for the infections to spontaneously resolve after many days. In mice treated with corticosteroids, persistent infection develops and is associated with a high mortality rate (3). This model is more applicable to humans.

In the present study, we treated mice which had cortisone-induced cell-mediated immune deficiencies with antibiotics in order to compare their abilities to eradicate *N. asteroides* in an experimental pulmonary infection.

MATERIALS AND METHODS

Organism. A clinical isolate of *N. asteroides* from a patient with pneumonia treated at Kings County Hospital, Brooklyn, N.Y., was used in these studies. This strain differed in origin from the strain we used in previous experiments but did not differ in growth characteristics. This organism was maintained on Sabouraud agar slants and frequently subcultured to new slants. Growth characteristics and virulence in mice were also periodically ascertained.

Inoculum preparation. The method for preparing the organisms for inoculation into mice has been reported previously (5). Briefly, the isolate was subcultured from the agar slant into 100 ml of brain heart infusion broth and incubated in a rotary shaker at 150 rpm at 37°C. Twenty-four hours later, a sample of homogeneous growth was subcultured into new brain heart infusion broth and similarly incubated. This method of incubation causes the organisms to grow in coccobacillary form (not in clumps), which was ascertained by repeated Gram staining. After 24 h, the log-phase growing organisms were centrifuged and the pellet was suspended in phosphate-buffered saline for animal inoculation. The number of bacteria in the inoculum was approximately 10^6 CFU/ml.

Mice. Female Swiss Webster mice (4 to 6 weeks old) weighing approximately 20 to 22 g were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass. The mice were caged and fed food and water without restriction.

Immunosuppression. Subcutaneous injections of cortisone

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acetate (125 mg/kg of body weight per day) were administered to mice for 3 consecutive days in order to produce defects in cell-mediated immunity. This dosage was reported to decrease total leukocyte, monocyte, and lymphocyte counts in mice (3). Pilot studies performed prior to the current investigation confirmed these findings. Additionally, those mice which received corticosteroids and were then subsequently infected developed persistent pulmonary nocardiosis and suffered a high mortality rate.

Pulmonary nocardiosis. Pulmonary nocardiosis was produced by placing 50-μl droplets of phosphate-buffered saline containing 5×10^4 CFU of *N. asteroides* on the anterior nares of lightly anesthetized mice. These mice have forceful inhalations, and material placed on the nose is quickly and fully aspirated to the lower respiratory tree. Pilot studies, in which mice were sacrificed approximately 5 min after the aspiration procedure described above, showed virtually total recovery of the initial inoculum and the absence of other infecting pathogens. All experiments were performed in duplicate, and the results represent the mean of two investigations.

Antibiotics and susceptibility studies. The antimicrobial agents used in the study were amikacin, ceftriaxone, imipenem, ciprofloxacin, and sulfadiazine. All agents were supplied by their manufacturers, except for sulfadiazine, which was purchased as laboratory assay powder from Sigma Chemical Co., St. Louis, Mo. These agents were selected because of their in vitro activity against *N. asteroides* and their clinical utility in human infection. The combinations of amikacin plus ceftriaxone, amikacin plus imipenem, and ciprofloxacin plus sulfadiazine were also used, since these combinations exhibited synergy against the challenge strain. Agar dilution methods were used for the susceptibility and synergy studies. Synergy was defined as a fourfold or greater reduction in the MICs of both antibiotics against the tested isolate.

Therapy. The cortisone-treated mice were randomized to one of eight antibiotic-regimen groups or one saline-treated control group. Therapy was begun 24 h postinoculation so that we would be treating animals with established infection. Pilot studies indicated that organisms inoculated into pulmonary tissue in the manner described above were able to reproduce and cause pneumonitis, as evidenced by histologic appearance and culture results.

The dosages of antimicrobial agents administered were based upon pilot pharmacokinetic studies. Individual antibiotics were administered to groups of 10 mice each to determine half-lives and peak concentrations in serum. Levels in serum were assayed by the agar well diffusion technique at various time points from 5 min to 3 h following subcutaneous injection. The dosages of antimicrobial agents resulted in concentrations in serum severalfold higher than the MICs of the agents against the challenge strain. The half-life of each agent in mice was much shorter than that in humans, and this necessitated giving therapy every 4 h for the duration of the investigation. The following dosages were administered by subcutaneous injection (in milligrams per kilogram of body weight): amikacin, 25; ceftriaxone, 50; imipenem, 10; ciprofloxacin, 20; sulfadiazine, 150.

Bacterial quantitation. Five animals from each group of mice were sacrificed, at 5 min and at 1, 24, 48, and 96 h after initiation of therapy. Blood was obtained for culture, and the lungs were aseptically removed, washed in saline, and placed in sterile tubes. This tissue was then homogenized with a high-speed tissue homogenizer (Tekmar, Cincinnati, Ohio). Serial 10-fold dilutions were made of the homogenate, and 1-ml samples were inoculated into Mueller-Hinton agar and incubated for 48 h at 37°C. After this period of incubation, the number of viable organisms in pulmonary tissue following therapy could be determined and calculated as the number of organisms per gram of tissue. Statistical analysis was performed by the Student Neuman Keuls procedure.

RESULTS

In vitro and pharmacokinetic studies. The in vitro susceptibilities of the challenge strain of *N. asteroides* to the antimicrobial agents used in this study (expressed in terms of the MIC in milligrams per liter) were as follows: amikacin, 1.0; ceftriaxone, 4.0; imipenem, 0.5; ciprofloxacin, 0.5; sulfadiazine, 0.5. The combinations of amikacin plus ceftriaxone, amikacin plus imipenem, and ciprofloxacin plus sulfadiazine showed synergy against the challenge strain on the basis of the above definitions. The mean peak concentrations in serum (in milligrams per liter) were as follows: amikacin, 42; ceftriaxone, 150; imipenem, 43; ciprofloxacin, 3.7; sulfadiazine, 68. The calculated half-lives of amikacin, ceftriaxone, imipenem, ciprofloxacin, and sulfadiazine were 10, 32, 12, 40, and 120 min, respectively.

Effect of cortisone on pulmonary infection. The 50% lethal dose for the saline-treated mice was 9.0×10^7 CFU. Cortisone acetate treatment lowered the 50% lethal dose to 5.0×10^4 CFU, which was the initial nasal inoculum of *N. asteroides* given each mouse. The lungs of cortisone-treated mice exhibited extensive purulent exudate and hemorrhage 24 h after the inoculum was administered. Similarly, numerous abscesses were present in the cortisone-treated mice sacrificed at 48 h. In contrast, the control mice had grossly normal lungs at 24 and 48 h.

Antibiotic efficacy in experimental nocardiosis. The mean numbers of CFU of *N. asteroides* per gram of lung tissue at various time intervals before and during therapy for each treatment group are shown in Fig. 1.

The datum points represent the mean ± standard error $\log_{10}$ CFU per gram of lung tissue from groups of 10 mice. Blood cultures obtained at the time of sacrifice contained very low numbers of *N. asteroides*, indicating that there was no significant blood-borne contamination of lung tissue. The mean ± standard error of the $\log_{10}$ CFU per gram of lung tissue at 96 h for each group is shown in Fig. 1.

Amikacin reduced bacterial colony counts of *N. asteroides* in this model more effectively than any other treatment regimen. Imipenem was the next most effective single agent, as it reduced colony counts by approximately 3.5 $\log_{10}$ CFU/g compared with controls. Ceftriaxone caused a modest reduction in the numbers of organisms recovered, and both ciprofloxacin and sulfadiazine caused counts not statistically different from those of the controls. The addition of imipenem or ceftriaxone to amikacin did not further enhance bactericidal activity.

DISCUSSION

Pneumonia caused by *N. asteroides* may be associated with a high mortality rate, especially in the immunocompromised patient (8), although one more recent review of nocardiosis in heart transplant patients showed improved outcome with early recognition and treatment (10). Sulfonamides are frequently poorly tolerated by certain groups of patients and may be associated with primary therapeutic failure. A previous report in an experimental model has shown sulfonamides to be bacteriostatic (5). In addition,

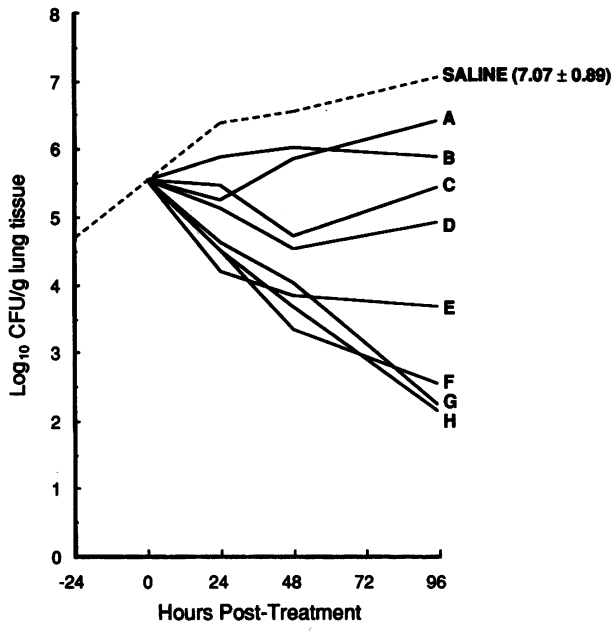

FIG. 1. Effect of antimicrobial therapy on the growth of *N. asteroides* in mouse lung tissue. Each point represents the mean ± standard error $\log_{10}$ CFU per gram for a group of 10 mice treated with the following drug(s): A, ciprofloxacin (6.42 ± 0.49); B, sulfadiazine (5.88 ± 0.39); C, ciprofloxacin plus sulfadiazine (5.46 ± 0.36); D, ceftriaxone (4.96 ± 0.21); E, imipenem (3.68 ± 0.27); F, imipenem plus amikacin (2.56 ± 0.108); G, ceftriaxone plus amikacin (2.25 ± 0.113); H, amikacin (2.2 ± 0.13).

many in vitro data show sulfa agents to be less active than other comparative antimicrobial agents (4). For these reasons, alternative therapies need to be developed and advanced to clinicians caring for patients with nocardiosis.

Since the majority of patients with nocardial infections have some immune defect, the cortisone-treated-mouse model employed in the present investigation may be more applicable to humans. The specific immune defect has been extensively reported, and our pilot projects were confirmatory. Indeed, our 50% lethal dose data were virtually identical to those of Filice and Niewoehner (3).

There have been increasing reports of human immunodeficiency virus-infected patients developing pneumonia due to nocardial species (9). The reports will undoubtedly increase as awareness for this syndrome grows. This population of patients has fixed immune defects and a demonstrated intolerance to sulfonamides. Bactericidal therapy with sulfa alternatives in the treatment of *N. asteroides* infection is highly desirable. Recently, four patients with acquired immunodeficiency syndrome were reported with nocardial pneumonia (9). Three had adverse effects from sulfonamides and were treated with a combination of imipenem, minocycline, and netilmicin, with good results. The report emphasizes the need to develop alternative therapeutic strategies.

In this study, amikacin and imipenem were the two most effective single agents tested. Sulfadiazine was ineffective, as was ciprofloxacin. Possibly, longer treatment courses would alter these results. Ceftriaxone proved to be modestly effective in reducing bacterial colony counts despite its good in vitro activity.

In other reports, combination therapy was shown to be effective in an experimental model of central nervous system nocardiosis (6). This was not the case in the present investigation. The addition of imipenem or ceftriaxone to amikacin was not statistically superior to amikacin alone.

Ciprofloxacin is an oral fluoroquinolone with moderate activity against *N. asteroides*. These features could represent a desirable substitute for long-term oral sulfonamide administration for reasons mentioned above.

To date, there have been published studies involving experimental models showing the superiority of amikacin and imipenem as well as some broad-spectrum cephalosporins to sulfonamides (5, 12, 13). The reasons for this are unclear but in part may be related to the fact that sulfonamides appear to be bacteriostatic in vivo against nocardiae. This indicates that some host immune function might be required for the infection to resolve. In patients with fixed immune defects, the return of immunologic competence cannot be relied upon and the need for bactericidal therapy is present. We believe that imipenem, amikacin, and selected cephalosporins represent alternatives to sulfonamides in the therapy of nocardial pneumonia.

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