0066-4804/86/08270-04$02.00/0
Copyright © 1986, American Society for Microbiology

Therapy of Experimental Cerebral Nocardiosis with Imipenem, Amikacin, Trimethoprim-Sulfamethoxazole, and Minocycline

MYLES E. GOMBERT,* TARYN M. AULICINO, LORRAINE DUBOUCHET, GARY E. SILVERMAN, AND WILLIAM M. SHEINBAUM

Division of Infectious Diseases, Department of Medicine, State University of New York Downstate Medical Center, Brooklyn, New York 11203

Received 12 November 1985/Accepted 21 May 1986

A mouse model of cerebral nocardiosis was used to determine relative antibiotic efficacy by reducing bacterial colony counts per gram of brain tissue. The antimicrobial agents employed were demonstrated in vitro to be inhibitory to most strains of Nocardia asteroides at very low concentrations. The agents used in this study were imipenem-cilastatin, amikacin, trimethoprim-sulfamethoxazole, and minocycline. Antibiotics were administered every 4 h for 72 h before animal sacrifice. Bacterial colony counts were assayed at various time points before the completion of therapy. Imipenem-cilastatin and amikacin were the most effective agents tested. Trimethoprim-sulfamethoxazole was less effective than imipenem and amikacin but more effective than minocycline. Minocycline did not eradicate intracerebral organisms and was similar to saline (control) in its effects.

Nocardia asteroides is being increasingly recognized as a pathogen of normal and immunologically incompetent hosts (7, 19). The most common sites of infection are the lungs, central nervous system, and skin (3). A central nervous system infection usually implies single or multiple brain abscesses, yet meningitis has been reported alone (14). Intracerebral nocardiosis, in contrast to other forms of nocardiosis in the immunologically incompetent patient, can be a rapidly progressive and fulminating infection and has been associated with mortality rates of up to 90% (15). Therapy for all forms of nocardiosis usually consists of a sulfonamide alone or in fixed combination with trimethoprim and these have been advocated by some authors as the drugs of choice (20, 22). There are reports of cures with other antibiotics for those patients who have either failed on sulfonamides or experienced serious side effects which resulted in therapy being discontinued. These antibiotic regimens have included minocycline (23), amikacin, ampicillin (6), and erythromycin in combination with other agents (2). These alternative forms of therapy usually have been based on in vitro susceptibility testing of the specific strain of N. asteroides isolated from the patient and on previous anecdotal reports.

Recent studies have shown that many new beta-lactam antibiotics are active at very low concentrations against a wide range of N. asteroides clinical isolates (11). Further studies have shown that synergism can be established with several antibiotic combinations (12). Unlike susceptibility testing with uniformly growing gram-positive and gram-negative bacteria for which in vitro activity can reasonably predict clinical efficacy, it has been shown that in vitro susceptibility testing of nocardia cannot necessarily predict clinical efficacy (1, 17).

The present study was undertaken to assess the relative in vivo efficacy of several antibiotics in the therapy of a mouse model of cerebral nocardiosis. The antibiotics were chosen on the basis of their in vitro activity against the challenge strain used. The combination of trimethoprim-sulfamethoxazole (TMP-SMX) in a fixed-weight ratio of 1:5 was selected as this represents one of the more common forms of treatment and would provide a basis for comparison with other agents.

MATERIALS AND METHODS

Animals. Female Swiss Webster mice weighing approximately 20 g were used in this study. The animals were received from Charles River Breeding Laboratories, Inc., Wilmington, Mass., at 6 weeks of age and were used several days thereafter. The mice were housed in standard cages and fed food and water ad libitum.

Bacteria. A strain of N. asteroides isolated from a patient with cerebral nocardiosis at the Downstate Medical Center was used throughout this study. The isolate was maintained on Sabouraud agar slants and transfers to new agar slants were performed periodically. Inoculum preparation was performed by the method of Beaman and Maslan (5) and was modified for the present study. Briefly, a loopful of N. asteroides cells was subcultured into 100 ml of brain heart infusion (BHI) broth and was grown overnight in a rotating incubator at 150 rpm at 37°C. A homogeneous suspension of organisms was obtained, and 1 ml of this suspension was inoculated into 100 ml of new BHI broth and grown overnight in a rotating incubator under the same conditions. This resulted in a homogeneous suspension of single organisms in coccobacillary form as ascertained by repeated Gram staining of the suspension throughout the period of incubation. The duration of this second incubation period assured that these organisms were in log-phase growth as determined by a previous set of experiments. Organisms in this growth phase have been shown to be more virulent than organisms that are not rapidly dividing (5). This suspension was then centrifuged, and the pellet was resuspended in saline for animal inoculation. The number of bacteria in the inoculum was 10⁶ CFU/ml. This particular isolate was chosen from our collection on the basis of its uniform and rapid growth and because it demonstrated an affinity to infect cerebral tissue following intravenous injection. This was determined in several pilot studies with this strain and is not uncommonly seen with other isolates of N. asteroides. MICs were deter-
mired by the agar dilution technique as previously reported (11).

Antibiotics. The antibiotics used in this study were imipenem-cilastatin (Merck Sharpe & Dohme, Rahway, N.J.), amikacin (Bristol-Myers Inc., Syracuse, N.Y.), minocycline (Lederle Parenterals, Inc., Carolina, P.R.), and TMP-SMX (Hoffman-La Roche Inc., Nutley, N.J.). Imipenem, amikacin, and minocycline were chosen on the basis of their superior in vitro activity against the challenge strain of *N. asteroides*. TMP-SMX was chosen because it is one of the more common forms of treatment for most types of nocardial infection (20).

Pharmacokinetic studies. In several pilot studies, cisternal punctures were performed on groups of 10 mice each to determine the concentrations of antibiotics in cerebrospinal fluid (CSF) following subcutaneous injection. Concentrations in CSF were assayed at various time points from 5 min to 3 h after subcutaneous injection of each agent. The method of cisternal puncture in mice was adapted from Kim and Anthony (13). The doses of antibiotics used resulted in levels in CSF above the MIC of each antimicrobial agent. The following doses were administered by subcutaneous injection: imipenem-cilastatin (1:1, fixed-weight ratio), 0.5 mg; amikacin, 0.75 mg; minocycline, 0.3 mg; TMP-SMX, 0.1 and 0.5 mg. Concentrations of antibiotics in CSF were determined by the agar well diffusion technique (9). Based upon our determination of the half-lives of the tested antibiotics in serum in mice, we administered therapy every 4 h.

Intracerebral nocardiosis. Intracerebral nocardiosis was produced by injection of approximately $1.8 \times 10^5$ CFU of *N. asteroides* (total volume, 0.1 ml) into the tail vein of each mouse. All organisms were in log-phase growth at the time of inoculation. The mice were then returned to their cages and remained there between 12 and 18 h before therapy was instituted. This delay period of incubation before beginning therapy was determined in a previous set of experiments in which mice were clinically ill yet survival was expected with the inoculum injected. In this study, the mice were treated with the above doses of antibiotics every 4 h for the following 72 h. A 72-h period of therapy was chosen because the number of viable organisms isolated from mouse cerebral tissue tends to diminish after 72 to 96 h following tail vein injection without specific therapy (Fig. 1). These experiments were performed in duplicate.

Bacterial quantitation. At the end of the last dosing inter-}

FIG. 1. Growth curve of *N. asteroides* in cerebral tissue following intravenous inoculation of untreated mice. Each point represents the mean from a group of 10 mice.

val the animals were killed, and blood was aseptically removed from the heart of each mouse and placed into sterile tubes containing BHI broth containing sodium polyanethol sulfonate. Serial dilutions of this mixture were made and were cultured quantitatively on Mueller-Hinton agar plates. Colonies were counted after 48 h of incubation at 37°C. The brain was removed, weighed, and placed in sterile tubes. Sterile saline was added to each tube to give a total volume of 3 ml. The brain tissue was homogenized by a high-speed tissumizer (SDT Tissumizer, Tekmar Co., Cincinnati, Ohio) at 20,000 rpm for 20 s. The homogenate was inoculated into Mueller-Hinton agar in appropriate dilutions so that the number of colonies per gram of brain tissue could be calculated. These plates were incubated for 48 h at 37°C, and the colonies were counted for each different antibiotic group. Statistical analysis was performed by the Student *t*-test.

RESULTS

In vitro and pharmacokinetic studies. The susceptibility of the *N. asteroides* challenge strain to the drugs in question expressed in terms of the MIC (micrograms per milliliter) was as follows: imipenem, 0.5; amikacin, 0.5; TMP-SMX, 0.5/10; minocycline, 2.0. The mean peak concentrations of imipenem, amikacin, trimethoprim, and minocycline in CSF were 9.75, 7.45, 1.33, and 6.39 μg/ml, respectively.

Antibiotic efficacy of experimental cerebral nocardiosis. Imipenem-cilastatin and amikacin were superior to minocycline and TMP-SMX in reducing bacterial colony counts in this test model. The mean number of CFUs per gram of brain tissue assayed before antibiotic treatment and at different time intervals during treatment is shown in Fig. 2. Quantitative blood cultures at the time of death grew extremely low numbers of *N. asteroides*, indicating that there was no significant blood-borne bacterial contamination of brain tissue. Most mice had 0 to 10 organisms per ml of blood, and no mouse had >100 organisms per ml of cultured blood. At the completion of the 72-h treatment schedule, the mean log$_{10}$ CFU per gram of brain tissue for each group of 10 mice was as follows: imipenem-cilastatin, 2.44 ± 0.49; amikacin, 2.25
272

GOMBERT ET AL.


± 0.50; TMP-SMX, 3.36 ± 0.61; minocycline, 4.9 ± 0.84; saline-treated controls, 5.32 ± 0.79. Imipenem-clislatin and amikacin were significantly more effective in reducing bacterial colony counts at all times tested than was a saline-treated control group (P < 0.05). Moreover, imipenem-clislatin and amikacin were significantly more effective in reducing colony counts than was TMP-SMX (P < 0.05). TMP-SMX was more effective than the control (P < 0.05). Minocycline was significantly less effective than any other treatment group in this model and was not statistically different than the saline-treated control group. There was no statistical difference in the effectiveness of therapy between amikacin and imipenem-clislatin (P > 0.05).

DISCUSSION

The mortality rates for all forms for cerebral nocardiosis including brain abscesses and meningitis remain high with current therapy, particularly in immunocompromised patients (10, 16). There have been several reports in which standard sulfonamide therapy was either ineffective or the patient could not tolerate the side effects of the therapy (6, 7, 23). The need for evaluating forms of therapy other than that of the sulfonamides alone or in combination with trimethoprim is warranted. Reliable and valid clinical trials of antibiotic efficacy are difficult to accomplish because of the paucity of patients with identical clinical syndromes and lack of sufficient number of patients seen at any one institution. Therefore, treatment is usually based on accumulated clinical experience and on anecdotal reports. In addition, in contrast to other bacteria for which in vitro testing can, in part, predict in vivo efficacy, the same cannot be said for nocardial isolates. For these reasons a valid and reproducible animal model of several forms of nocardiosis is useful in evaluating antibiotic efficacy both alone and in combination with other agents. Several recent in vitro susceptibility studies indicate that there are several new agents which are active against many nocardial isolates at very low concentrations (8, 11). Many of these agents are more active than the currently advocated sulfonamides.

Other animal models of experimental N. asteroides infection have been reported (17, 18, 21). None of these studies has attempted to determine antibiotic efficacy in such a quantitative fashion. Rather, the only measures of outcome were mortality rates of animals in acute and chronic models of infection. The present study was undertaken to quantitatively determine the efficacy of several antibiotics which are active in vitro against many strains of N. asteroides by reducing bacterial colony counts in an infected target organ. Many of the variables encountered in previous studies have been eliminated. Each animal was given a homogeneous suspension of the same organism at the same inoculum. The 72-h period of therapy employed in this study allowed us to assess the efficacy of four antimicrobial agents alone without the host defense mechanisms killing intracerebral organisms. Our mice were able to clear organisms from cerebral tissue after 72 to 96 h without specific therapy (Fig. 1). This phenomenon has been observed in other species of mice (4). The short period of incubation (12 to 18 h) of disease in these mice was sufficient to achieve large numbers of organisms in the brain (Fig. 1) and to cause a marked inflammatory response as determined by histopathologic examination. The rise in serum amikacin was statistically significant and was not statistically different than the saline-treated control group. There was no statistical difference in the effectiveness of therapy between amikacin and imipenem-clislatin (P > 0.05).

This ratio may not be optimal against all organisms and may not be optimal for the strain of N. asteroides used in this study. However, changing the fixed ratio and assessing its in vitro effectiveness is time-consuming, and the results may not predict clinical efficacy. Therefore, the usual ratio of the combination was used in these studies.

The results of therapy in the present study show that the widely used combination of TMP-SMX was less effective than imipenem-clislatin or amikacin in decreasing bacterial colony counts in mouse brain tissue. These results were statistically significant. TMP-SMX, however, was significantly more effective in killing N. asteroides than was the saline solution given the control mice. Minocycline was ineffective in this model. A previously reported model of acute N. asteroides intra-abdominal infection showed TMP-SMX to be virtually ineffective (21). The improved results with amikacin were consistent with those reported in a previous study in which amikacin was compared with TMP-SMX (21). Further investigation of bactericidal activity of other antimicrobial agents or combinations of agents in an experimental model of N. asteroides is warranted.

LITERATURE CITED