In Vitro Studies of the Combined Effect of Ampicillin and Sulfonamides on *Nocardia asteroides* and Results of Therapy in Four Patients

MIKE G. ORFANAKIS, HOWARD G. WILCOX, AND CHARLES B. SMITH

Department of Medicine, University of Utah College of Medicine, and Salt Lake Veterans Administration Hospital, Salt Lake City, Utah 84112

Received for publication 24 November 1971

The minimal inhibiting concentrations (MIC) of ampicillin, trisulfapyrimidines, and various combinations of these drugs were determined for four clinical isolates of *Nocardia asteroides* on Mueller-Hinton agar. The combination of ampicillin and sulfonamides was found to be synergistic for three of the four isolates when an inoculum of 10^8 to 10^4 organisms per ml was used. (Synergism was defined as occurring when the MIC of each of the drugs in combination was one-quarter or less of the MIC of each drug alone.) All four isolates were inhibited by concentrations of the drugs in combination that were clinically achievable. Combined therapy with ampicillin and sulfonamides was used in four immunologically suppressed renal transplant patients who had pulmonary infection with *N. asteroides*. Clinical improvement was noted within 2 weeks in all patients, and the pulmonary infiltrates cleared by 6 months in three patients. One patient died from an unrelated illness 10 days after starting therapy. The disease was arrested in two patients who were maintained on full immunosuppressive therapy.

In recent years, *Nocardia* infections have been increasingly common in that group of patients whose resistance to infection has been altered by immunosuppressive drugs, corticosteroids, anti-metabolites, or neoplastic disease (10).

Sulfonamides have remained the most popular chemotherapeutic agent for *Nocardia* infections; however, response to sulfonamide therapy has not always been satisfactory, particularly in patients who have lowered resistance to infection (10, 11, 14; C. A. Presant and A. A. Serpick, Abstr., Amer. Rev. Resp. Dis. 103:882, 1971). To increase the effectiveness of therapy, some authors have advocated simultaneous use of two or more antimicrobial agents in treating *Nocardia* infections. Utz and Treger (14) recommended the combination of penicillin and sulfonamides, and Pellegrino and Henderson (12) described a patient who failed to respond to sulfonamide therapy alone but responded to the combination of sulfonamides and penicillin.

Wilson and Williams (15) reported that ampicillin was more effective than penicillin against *Nocardia* in vitro, suggesting to us that the combination of ampicillin and sulfonamides might be effective for treatment of *Nocardia* infections.

In the past year, we treated pulmonary *N. asteroides* infection in four renal transplant patients with the combination of ampicillin and sulfonamides. This report describes in vitro studies of the susceptibility of *N. asteroides* to ampicillin and sulfonamides, singly and in combination. In addition, the results of therapy with this combination are discussed.

MATERIALS AND METHODS

The four *N. asteroides* strains were isolated from percutaneous lung aspirate material in the microbiology laboratory of the University of Utah Hospital. The strains were identified as *Nocardia* on the basis of gross morphological characteristics and were subsequently identified as *N. asteroides* by the Mycology Section of the Center for Disease Control, Atlanta, Ga.

The inoculum for in vitro studies of drug susceptibility was grown in Mueller-Hinton broth in shaker flasks at 37 C for 3 to 5 days. The resulting cloudy culture contained many clumps or mats of organisms, and this was homogenized in a tissue grinder to aid in the dispersion of organisms. Persistent large particles in the broth were removed by gentle centrifugation, and the cloudy supernatant fluid was then diluted to give a final inoculum of 10^8 to 10^4 colony-forming units per ml.

In vitro susceptibility was determined by streaking 0.3 ml of the inoculum into Mueller-Hinton agar plates which contained various concentrations of ampicillin, trisulfapyrimidines, or both. The sulfapyrimidines (sulfamerazine, sulfamethazine, and sulfadiazine, kindly supplied by Eli Lilly & Co., Indianapolis, Ind.) were mixed together in equal proportions to simulate the oral preparation available for clinical use.

The bactericidal effect of ampicillin and trisulfapyrimidines was evaluated by inoculating 10^6 colony-forming units of *Nocardia* into shaker flasks containing ampicillin and trisulfapyrimidines alone and in combination. Samples from each flask were titrated for viable organisms at regular intervals over a 48-hr period.

Sulfonamide concentrations in serum were determined by the method of Bratton and Marshall (5).

RESULTS

In vitro studies. The minimal inhibiting concentrations (MIC) of trisulfapyrimidines varied from 5 to 75 $\mu\text{g/ml}$, and those of ampicillin varied between 6.25 and 250 $\mu\text{g/ml}$ (Table 1). In repeated tests with each organism, the MIC values did not differ more than twofold.

The antinocardial effect of combinations of ampicillin and trisulfapyrimidines is shown in Fig. 1. The isobolograms for all four strains are concave in shape, indicating at least some advantage of the combination for all strains. For three strains, the MIC of each drug in the combinations was one-fourth or less of the MIC of each antimicrobial agent alone; for these strains, the combinations could be called "synergistic."

TABLE 1. Susceptibility of *Nocardia* to trisulfapyrimidines and ampicillin^a

Strain	Test no.	MIC ($\mu\text{g/ml}$)	
		Sulfa	Ampicillin
D.D.	1	18.7	250
	2	—	125
	3	18.7	250
A.B.	1	5	—
	2	—	6.25
	3	9.3	—
	4	9.3	12.5
D.C.	1	18.7	125
	2	32.5	125
	3	18.7	250
R.V.	1	—	125
	2	75	250

^a Inoculum: 10^3 to 10^4 colony-forming units/ml.

FIG. 1. Combined antinocardial activity of trisulfapyrimidines and ampicillin. Synergistic combination defined as fourfold or greater decrease in the MIC of each antimicrobial when used in combination.

FIG. 2. Bactericidal activity of ampicillin and trisulfapyrimidines alone and in combination against *Nocardia*. The combination was more rapidly and completely bactericidal.

The combination of ampicillin and trisulfapyrimidines was also more bactericidal than either agent alone (Fig. 2). Colony-forming units of strain R.V. in the control culture increased 100-fold by 32 hr, whereas trisulfapyrimidines and ampicillin each caused 100-fold decreases in numbers of viable organisms by 32 hr. Between 32 and 48 hr, growth resumed in the flasks containing trisulfapyrimidines or ampicillin alone, indicating a net bacteriostatic effect but not a bactericidal effect. In contrast, in the flask containing both trisulfapyrimidines and ampicillin the rate of killing was greater, and at 24 hr and subsequently no growth was obtainable.

CASE REPORTS

Patient no. 1 (Fig. 3). D. D., a 16-year-old girl, was seen 4 months after renal transplantation complaining of cough of 7 days' duration. Chest film revealed nodular infiltrates in the left lower lobe, and, when sputum culture grew out predominantly *Staphylococcus aureus*, she was treated with nafcillin. Over the next 10 days, she failed to respond to this therapy; her temperature increased to 104 F (40 C), and she became more dyspneic and slightly cyanotic. Chest X rays revealed some increase in the size of the left lower lobe infiltrates. At this time, a percutaneous needle aspirate of the left lower lobe was obtained; and stains of the aspirate revealed gram-positive, acid-fast, branching, rodlike organisms. The presumptive

diagnosis of nocardiosis was later confirmed by culture.

She was initially treated orally with sulfadiazine, 4 to 8 g/day with bicarbonate, and intravenously with ampicillin, 2 g/day. Over the next 3 weeks, she gradually improved and became afebrile. At this time, her urine sediment revealed sulfa crystals; thus, sulfadiazine therapy was discontinued and replaced by trisulfapyrimidines, 2 to 4 g/day, and the route of administration of ampicillin was changed to oral.

At 5 weeks after therapy was started, she was much improved; the chest film showed reduction in the extent of the infiltrates, and ampicillin therapy was stopped. One week later she was discharged from the hospital on trisulfapyrimidines, 2 g/day, and prednisone.

Her course over the next 7 months, during which the sulfonamide concentrations in her blood were maintained between 10 and 15 mg/100 ml, was characterized by complete clearing of her pulmonary infiltrates and no further pulmonary infections. Five months after discharge, she rejected her transplant kidney and received a second transplant. Despite immunosuppression with antilymphocyte globulin, azathioprine, and corticosteroids, this second transplant was also rejected, and she died suddenly during peritoneal dialysis. During this period, she was maintained on trisulfapyrimidines, and there was no evidence for reactivation of her *Nocardia* infection during the

FIG. 3. Clinical course of patient 1.

second course of immunosuppression and transplantation. No autopsy was obtained.

Case no. 2 (Fig. 4). A. B. is a 24-year-old woman who was admitted to the hospital 6 months after renal transplantation complaining of left-sided pleuritic chest pain. Chest film revealed a diffuse infiltrate in the left lower lobe. After sputum examination revealed gram-positive, acid-fast, branching rods that resembled *Nocardia*, a percutaneous lung aspirate was performed. Similar organisms were seen in this specimen, and *N. asteroides* was cultured.

She was initially treated orally with trisulfapyrimidines, 4 g/day, and intravenously with ampicillin, 4 g/day, while prednisone and azathioprine were continued. After 2 weeks of treatment, during which sulfonamide concentrations in her blood were maintained between 12 and 15 mg/100 ml, there was a noticeable clearing of the pulmonary infiltrate; she was sent home on trisulfapyrimidines therapy, 2 g/day, and ampicillin was discontinued.

One week later, repeat chest film showed worsening of the infiltrate. She was readmitted, and combined therapy with trisulfapyrimidines and intravenous ampicillin was resumed. The sulfonamide concentration in her blood at the time of readmission was 6 mg/100 ml. Over the next 2 weeks, the infiltrate again improved, and she was this time discharged on oral trisulfapyrimidines, 2 g/day, and ampicillin, 2 g/day, while immunosuppression was maintained with azathioprine and prednisone.

Eight months after diagnosis of her *Nocardia* infection, her chest film was stable with only min-

imal scarring. Combined therapy was maintained for 9 months, and sulfonamide therapy was maintained for 18 months.

Case no. 3. D. C., a 41-year-old man, was admitted to the hospital 3 months after renal transplantation with a chief complaint of pleuritic chest pain. Films of the chest revealed nodular infiltrates in both lower lobes. Percutaneous needle aspiration of the lung revealed *Nocardia*-like organisms on smear, and *N. asteroides* was subsequently identified on culture. Therapy was begun with trisulfapyrimidines, 6 g/day, and intravenous ampicillin, 4 g/day. One week later, the chest films indicated slight improvement; however, at this time the patient developed acute pancreatitis which was rapidly fatal.

Autopsy revealed multiple nocardial abscesses of the right lung with empyema, acute pancreatitis, and marked fat necrosis. *N. asteroides* was cultured from the lungs and pleural fluid. Gram-stained sections of the pancreas failed to reveal *Nocardia*.

Case no. 4 (Fig. 5). R. V., a 43-year-old man, 6 weeks after renal transplantation was noted to have a nodular infiltrate of the upper lobe of the right lung on routine X-ray examination. He was asymptomatic and, except for cushingoid features, examination was unremarkable.

Percutaneous needle aspiration of the lung revealed *Nocardia*-like organisms, and *N. asteroides* was subsequently cultured from the lung aspirate, sputum, and gastric washings. Treatment for no-

FIG. 4. Clinical course of patient 2.

FIG. 5. Clinical course of patient 4.

cardiosis was begun with trisulfapyrimidines, 4 g/day, and ampicillin, 2 g/day; prednisone and azathioprine were continued for immunosuppression. Serum sulfonamide concentrations were maintained between 14 and 24 mg/100 ml. There was noticeable improvement in the chest film after 2 weeks of therapy, and after 2 months only residual scarring was noted. Combined therapy was maintained for 7 months, at which time ampicillin was discontinued. Trisulfapyrimidines therapy was continued for 18 months.

DISCUSSION

Nocardia infections in the untreated patient are usually fatal (8). Most reviewers agree that sulfonamide therapy has reduced mortality from *Nocardia* infections; however, estimated mortality in treated patients is still sizable, ranging from 50% (9) to 10% (7). For this reason, the addition of a second antimicrobial agent to sulfonamide therapy is often recommended (2, 11, 14). There is less agreement regarding the choice of the second agent; choices have included cycloserine (7), penicillin (12, 14), and broad-spectrum antimicrobials (2, 11). Although Runyon (13) has pointed out that the in vitro susceptibility of *Nocardia* to antimicrobial agents may not correlate with in vivo effectiveness in the animal model, it still seems reasonable to base the choice of the second agent on in vitro studies.

Our decision to evaluate ampicillin as the second antimicrobial agent in treating *Nocardia* infections was based on the report of Wilson and Williams (15), which indicated that ampicillin was the most effective of the penicillins, inhibiting 40% of various *Nocardia* strains at a concentration of 25 µg/ml. Black and McNellis (4) recently reported that six of nine strains of *N. asteroides* were inhibited by 25 µg or less of ampicillin/ml. Ampicillin was also chosen in our study because of its relative safety in patients with abnormal renal function.

Although only one of the four strains of *N. asteroides* tested in the present study was inhibited by 25 µg or less of ampicillin/ml, our in vitro assays of the effect of the combination of trisulfapyrimidines with ampicillin indicated an enhanced effect of the combination against all four strains of *Nocardia* tested. The MIC of ampicillin in combination with trisulfapyrimidines was consistently 62.5 µg or less/ml, levels which should be attainable in the serum of patients with decreased renal function (1). We have been unable to find other reports of in vitro studies of the susceptibility of *Nocardia* to the combination of sulfonamides with penicillins. Black and McNellis (3) were unable to show by in vitro tests that the

combination of sulfonamides and trimethoprim had an advantage over either agent alone.

Evaluation of the clinical response of patients with nocardiosis to various therapeutic regimens is difficult, as the experience of most investigators involves only a small number of patients. This evaluation is particularly difficult in patients who are receiving corticosteroid, antimetabolic, or immunosuppressive therapy. Presant and Serpick (Abstr., Amer. Rev. Resp. Dis. 103:882, 1971) reported a mortality of 80 to 100% in patients with localized pulmonary nocardiosis who received immunosuppressants, antimetabolites, or corticosteroids. However, our success in three of four patients and that of Cohen et al. (6) in a patient treated with sulfonamides and penicillin support further evaluation of sulfonamide-ampicillin therapy for nocardiosis.

Although sulfadiazine is usually recommended for treating *Nocardia* infections, our experience in one patient who developed renal toxicity with this drug led us to choose the more soluble combination of trisulfapyrimidines for therapy in subsequent patients. The effectiveness and safety of sulfonamide therapy in patients with renal impairment can be improved with the adjustment of dose based on periodic determination of sulfonamide concentrations in serum.

Our experience with four renal transplant patients indicated that *Nocardia* infection of the lung may present as an asymptomatic lung nodule or as rapidly progressive pneumonia. Percutaneous needle aspiration of the lung proved to be a rapid method of making a specific etiological diagnosis and permitted early institution of appropriate therapy.

ACKNOWLEDGMENTS

We acknowledge the technical assistance of Barbara A. Saxon. This work was supported by Public Health Service grants 5 TO1 AI00039, 5 FO2 AI43645, and NIH, RR-64.

LITERATURE CITED

1. Altmann, G., B. Bogokowsky, G. Boner, and H. E. Eliahou. 1970. Blood levels of ampicillin, kanamycin and gentamicin in the uremic patient. *Israel J. Med. Sci.* 6:683-690.
2. American Thoracic Society. 1969. Treatment of fungal diseases. *Amer. Rev. Resp. Dis.* 100:908-910.
3. Black, W. A., and D. A. McNellis. 1970. Sensitivity of *Nocardia* to trimethoprim and sulphonamides *in vitro*. *J. Clin. Pathol.* 23:423-426.
4. Black, W. A., and D. A. McNellis. 1971. Susceptibility of *Nocardia* species to modern antimicrobial agents. *Antimicrob. Ag. Chemother.* 1970, p. 346-349.
5. Bratton, A. C., and E. K. Marshall, Jr. 1939. A new coupling component for sulfanilamide determination. *J. Biol. Chem.* 128:537-550.
6. Cohen, M. L., E. B. Weiss, and A. P. Monaco. 1971. Successful treatment of *Pneumocystis carinii* and *Nocardia asteroides* in a renal transplant patient. *Amer. J. Med.* 50:269-276.
7. Hoepfich, P. D., D. Brandt, and R. H. Parker. 1968. Nocardial

- brain abscess cured with cycloserine and sulfonamides. *Amer. J. Med. Sci.* 255:208-215.
8. Lerner, G., G. C. Tarasidis, and F. S. Jenney. 1967. Nocardiosis: the importance of early diagnosis and treatment. *Amer. Rev. Resp. Dis.* 96:494-500.
 9. Murray, J. F., S. M. Finegold, S. Froman, and D. W. Will. 1961. The changing spectrum of nocardiosis. A review and presentation of nine cases. *Amer. Rev. Resp. Dis.* 83:315-330.
 10. Murray, J. F., H. F. Haegelin, W. L. Hewitt, H. Latta, D. McVickar, A. F. Rasmussen, Jr., and L. G. Rigler. 1966. The UCLA interdepartmental conference: opportunistic pulmonary infections. *Ann. Intern. Med.* 65:566-594.
 11. Peabody, J. W., Jr., and J. H. Seabury. 1960. Actinomycosis and nocardiosis. A review of basic differences in therapy. *Amer. J. Med.* 28:99-115.
 12. Pellegrino, E. D., and R. R. Henderson. 1961. Response of pulmonary nocardiosis to treatment with massive doses of penicillin intravenously. *Amer. Rev. Resp. Dis.* 84:242-255.
 13. Runyon, E. H. 1951. *Nocardia asteroides*: studies of its pathogenicity and drug sensitivities. *J. Lab. Clin. Med.* 37:713-720.
 14. Utz, J. P., and A. Treger. 1959. The current status of chemotherapy of systemic fungal disease. *Ann. Intern. Med.* 51:1220-1229.
 15. Wilson, D. E., and T. W. Williams, Jr. 1966. In vitro susceptibility of *Nocardia* to antimicrobial agents. *Antimicrob. Ag. Chemother.* 1965, p. 408-411.