In Vitro Susceptibility of Strains of *Pseudomonas pseudomallei* to Rifampin

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Reported high activity of rifampin for *Pseudomonas pseudomallei* could not be verified by extensive in vitro tests conducted with 31 recently isolated strains. Minimal inhibitory concentrations of rifampin were 25 μg/ml for three strains and greater than 25 μg/ml for 28 strains. Rifampin had relatively poor in vitro activity when compared with tetracycline drugs and chloramphenicol antibiotics now commonly used for treating melioidosis.

Tetracycline drugs and chloramphenicol are usually effective and preferred for treatment of subacute forms of melioidosis (9). The use of sulfonamides may also be helpful (4). Treatment best succeeds when drugs are given at relatively high dosage and for prolonged periods (8, 9). The preferred drugs are not reliable for management of patients with acute septicemia or chronic forms of melioidosis (1, 7, 8, 10). The efficacy of other antibiotics is equivocal (8). Even in patients clinically cured by antibiotic treatment, attendant problems in persistence of infection may occur.

In view of needs for alternative treatment regimens in melioidosis, it was deemed important to verify a preliminary report by Hobby et al. (5) that a new antibiotic, rifampin, had a high degree of activity against a single strain of *Pseudomonas pseudomallei*. Our test findings on the relatively low in vitro activity of rifampin for melioidosis strains are reported. Similar in vitro findings by Fisher et al. (3) are concurrently presented.

**MATERIALS AND METHODS**

**Source of cultures.** Thirty-one strains used in tests were isolated from patients who were infected in Southeast Asia. The identity of each strain was confirmed through appropriate cultural, morphological, biochemical, and serological examinations.

**Susceptibility in vitro tests.** A broth-dilution procedure with a Microtiter technique was used (6). Rifampin (lot no. H-2955, activity 983 μg/ml) was obtained from Mann Research Laboratories, New York, N.Y. On the day of the test, the drug was dissolved in either a 20% solution of ethyl alcohol or a 10% solution of N-N dimethyl formamide in a 0.15 M phosphate-buffered physiological salt solution (pH 7.3) to provide a stock solution of 1,000 μg of rifampin per ml. Either stock solution was then diluted 10-fold with phosphate buffer. Further doubling dilutions were made with Mueller Hinton broth in U-bottomed microtitration plates with a 0.05-ml Microtiter loop. Other antibiotics used for comparative tests with rifampin were obtained commercially or from the Food and Drug Administration. The drugs were dissolved in phosphate buffer and diluted in the same way as rifampin. Overnight cultures in Mueller Hinton broth were used as a source of inoculum in initial tests. Subsequently this medium was supplemented with KNO₃ to a final concentration of 0.1%. The addition of KNO₃ to Mueller Hinton broth enhanced growth with little or no formation of a surface pellicle. The density of the cultures was adjusted spectrophotometrically to approximately 5 × 10⁶ cells (i.e., colony-forming units) per ml and then serially diluted 10-fold with broth to provide various concentrations of organisms used in tests. The actual numbers of colony-forming units contained in the cultures were determined by conventional agar-plating techniques. The desired dilution of culture was added to each dilution of antibiotic in the Microtiter wells in 0.05-ml amounts with a calibrated dropper. Microtiter plates were covered with an adhesive transparent plastic tape and incubated at 35 C. Readings were made after 24 and 48 hr of incubation. An appropriate dilution of an overnight broth culture of *Sarcina lutea* was used to affirm the activity of rifampin with the use of the microtitration technique. Diluent, growth, and sterility controls were included for each test.

**RESULTS**

Initially, 31 different strains of *P. pseudomallei* were tested. The concentration of colony-forming units in the test system ranged from 1.2 × 10⁶ to 1.1 × 10⁸ per ml. The minimum inhibitory
concentration (MIC) of rifampin after 24 hr of incubation was 25 μg/ml for 3 strains and greater than 25 μg/ml for 28 strains.

Two of the 31 strains which differed in their sensitivity to rifampin at test level of 25 μg/ml were selected for additional tests to determine whether inoculum size had a bearing on drug inhibitory level. Tests were set up in triplicate with 10⁻³, 10⁻⁴, and 10⁻⁵ dilutions of the standardized overnight cultures in Mueller Hinton broth. The final concentration of colony-forming units of the highest dilutions of the two test cultures were 500 and 650 per ml. After 24 hr of incubation, the MIC of rifampin for one strain successively increased twofold from 6.25 to 25 μg/ml with each 10-fold increase of inoculum size. With the second strain, the MIC level remained stationary at 25 μg/ml with the use of two lower concentrations but increased beyond 25 μg with the highest concentration of colony-forming units. After 48 hr of incubation, the MIC values were usually twofold greater.

Repeat tests with variable concentration of cells were done on the two strains and on four additional strains. The cultures were grown in Mueller Hinton broth containing 0.1% KNO₃. Three each of the cultures produced predominantly smooth and rough colonies, respectively, when plated on nutrient agar containing 3% glycerol. There was no apparent relationship between smooth and rough forms of P. pseudomallei and relative sensitivity to rifampin.

Three strains were selected to compare the activity of rifampin to other antibiotics. One of the strains was known to be chloramphenicol-resistant. A 10⁻⁴ dilution of overnight cultures in Mueller Hinton broth containing 0.1% KNO₃ was used for tests. Results are shown in Table 1. Rifampin had relatively poor activity for P. pseudomallei when compared with tetracycline drugs and chloramphenicol, drugs now commonly used in the treatment of melioidosis.

**DISCUSSION**

By using a broth-dilution technique, Hobby et al. (5) reported that rifampin at a minimal concentration of 0.04 μg/ml inhibited the growth of a single strain of P. pseudomallei. The reported MIC for rifampin was approximately 100- to 200-fold less than those obtained with tetracyclines and chloramphenicol under comparable test conditions. These findings could not be verified in extensive in vitro tests conducted with 31 recently isolated strains of P. pseudomallei. The MIC of rifampin was unusually greater than 25 μg. Even with the use of a small inoculum of 5 × 10⁶ colony-forming units per ml, the lowest observed MIC was 6.25 μg/ml.

Contrary to the observations reported by Hobby et al., we found that rifampin had relatively poor in vitro activity for P. pseudomallei strains when compared with tetracycline drugs and chloramphenicol. The findings in this report are in agreement with those of Fisher et al. (3) and Eickhoff et al. (2).

**LITERATURE CITED**

1. Cooper, E. B. 1968. Treatment of melioidosis. J. Amer. Med. Ass. 204:176.
2. Eickhoff, T. C., J. V. Bennet, P. S. Hayes, and J. Feely. 1970. Pseudomonas pseudomallei: susceptibility to chemotherapeutic agents. J. Infect. Dis. 112:93–102.
3. Fisher, M. W., A. B. Hillegea, and P. L. Nazeeri. 1971. Susceptibility in vitro and in vivo of Pseudomonas pseudomallei to rifampin and tetracyclines. Appl. Microbiol. 21:13–16.
4. Green, R., and D. S. Mankikar. 1949. Afebrile cases of melioidosis. Brit. Med. J. 1:308–311.
5. Hobby, G. L., T. F. Lenert, J. Maier-Engallena, and E. DeNoia-Cicenia. 1969. The control of experimental infection in mice produced by Pseudomonas pseudomallei. Amer. Rev. Resp. Dis. 99:952–954.
6. Marymount, J. H., and R. M. Wentz. 1966. Serial dilution antibiotic sensitivity testing with the microtiter system. Amer. J. Clin. Pathol. 45:548–551.
7. Prevatt, A. L., and J. S. Hunt. 1957. Chronic systemic melioidosis. Review of literature and report of a case, with a note on visual disturbance due to chloramphenicol. Amer. J. Med. 23:810–823.
8. Spotnitz, M. 1968. Treatment of melioidosis. J. Amer. Med. Ass. 204:176.
9. Spotnitz, M., J. Rudnitzky, and J. J. Rambaud. 1967. Melioidosis pneumonia. Analysis of nine cases of a benign form of melioidosis. J. Amer. Med. Ass. 202:950–954.
10. Weber, D. R., L. E. Douglas, W. G. Brundage, and T. C. Stallkamp. Acute varieties of melioidosis occurring in U.S. soldiers in Vietnam. Amer. J. Med. 46:234–244.