Susceptibility In Vitro and In Vivo of *Pseudomonas pseudomallei* to Rifampin and Tetracyclines

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Received for publication 19 March 1971

Tests were performed on 64 strains of *Pseudomonas pseudomallei* to compare rifampin, various tetracyclines, and other antibiotics for inhibitory activity in vitro. Rifampin minimum inhibitory concentration (MIC) values generally fell between 25 to 50 μg/ml. For doxycycline, methacycline, tetracycline, and minocycline, MIC means ranged from 1.3 to 2.7 μg/ml. Delayed treatment tests in subacute mouse infections revealed a better rifampin activity than was expected from its weak activity in vitro, whereas of the others, minocycline appeared superior. None of these five antibiotics demonstrated fully curative effectiveness in terms of mouse survival or eradication of residual infection in organs.

Although the acute form of human melioidosis with its attendant high mortality has long been a distinct therapeutic problem (1, 8), additional concern has been expressed recently for improved therapy of the persistent, inapparent, and potentially recrudescent forms of infection by *Pseudomonas pseudomallei* (4, 7, 14, 16). In particular, there continues to be a need for therapeutic agents and regimens which offer hope for an eradicative action in these chronic forms of melioidosis.

The present studies were undertaken because a preliminary report of mouse tests and tests in vitro on a new antibiotic, rifampin, suggested outstanding effectiveness (9). Also, since tetracycline has been indicated for clinical use as a preferred drug (15), it was included along with several related derivatives for tests of comparative activity. Moreover, the attempt to correlate susceptibility data derived in vitro with controlled experimental infection test results was a desirable objective in these studies.

This report is part of a larger program which involved many strains, many antibacterials, and the development of reproducible acute and chronic infections in mice. These studies, which will be reported elsewhere, revealed that the use of acute infection models (with or without mucin) for drug testing not only tended to provide exaggerated drug effects but also failed to permit a proper examination of eradicative effects, the development of resistance, and the evaluation of drug treatment regimens and combinations. In these present tests, therefore, persistent types of nonadjuvanted experimental infections were used, and, to add to clinical relevance, treatment was withheld to permit firm establishment of the bacterial infection. In addition, treatment was by drug-diet, to provide some constancy of dosage without excessive manipulation of the infected mice.

**MATERIALS AND METHODS**

Tests in vitro were performed with 64 strains of *P. pseudomallei*, including a number of recent clinical isolates as well as cultures obtained from human and animal sources dating as far back as 1946 (8). All strains were of confirmed identity through appropriate cultural, morphological, and biochemical tests.

**Susceptibility tests in vitro.** Using tryptic soy broth, minimal inhibitory concentrations (MIC) determinations were carried out in tube-dilution tests based upon inocula of approximately 10⁶ colony-forming units (CFU) per tube, 5 ml of broth per tube, 37°C incubation temperature, and end point readings made at 24 hr. Rifampin was lot D1281 (Pittman-Moore); doxycycline HCl, lot 85525-58002 (Pfizer); methacycline HCl, lot 93868-56010 (Pfizer); minocycline HCl, lot 7412B-56 (Lederle); and tetracycline HCl, lot 174-009 (Lederle).

As a check on the potency of lot D1281 rifampin used in these tests, MIC determinations were carried out against a number of bacterial species other than *P. pseudomallei*. The results of such tests indicated that this rifampin preparation possessed expected antibacterial potency, and details are accordingly not given here.

**Mouse chemotherapy tests.** Female CD-1 (Charles River) mice, 18 to 22 g, were used throughout. Mice were housed in groups of 10 in glass or plastic cages and were sustained on a water and ground pellet diet. Treatment was by drug diet, with powdered antibiotic added to ground mouse diet to provide various drug...
concentrations. Mice were started on freshly prepared drug diets three days after challenge and continued for 14 days on these diets. After 14 days on drugs, surviving mice were placed on standard drug-free diets for 14 to 20 additional days. After this time, survivors were sacrificed, and spleens, livers, kidneys, and any grossly apparent lesions were cultured for possibly residual \textit{P. pseudomallei}.

Two strains were used for mouse infections: PM-22 [NBL-111 of Redfearn et al. (12) isolated in 1953 from a patient in Malaya], and PM-1, received in 1966 from R. F. Unger, Brooke Army Hospital, where it was isolated from a patient who had acquired melioidosis in Viet Nam. The MIC's for relevant antibiotics for these two mouse-infecting strains, PM-22 and PM-1, respectively, were (in \textmu{g}/ml): rifampin, 12.5 and 25.0; tetracycline, 1.6 and 3.1; minocycline, 1.6 and 1.6; methacycline, 1.6 and 1.6; doxycycline, 0.8 and 0.8. The choice of these two strains came from many previous tests which indicated that highly reproducible, slowly progressive, generally fatal infections in mice could be accomplished for either strain without the concomitant use of minox. Also, strain PM-22 consistently induced a subacute infection, with a median death time in untreated mice of about 13 days after intraperitoneal challenge with approximately 10^6 CFU per mouse. At a similar intraperitoneal challenge level, strain PM-1 provided a more prolonged type of infection, with a median death time of about 24 days. With either strain, preliminary studies showed that these challenges had produced early localized infections after 3 days, principally in the spleen.

**RESULTS**

Results of tests in vitro with up to 64 strains of \textit{P. pseudomallei} are given in Table 1. Because they were almost invariably noninhibitory at 50 to 100 \textmu{g}/ml or more, specific data are not given here for streptomycin, paromomycin, gentamicin, cephalothin, lincomycin, erythromycin, furazolidone, nalidixic acid, colistin, and polymyxin B. Ampicillin, however, showed MIC values of 12.5 to 25.0 \textmu{g}/ml.

Additional tests were carried out with rifampin to determine the influence on MIC values of inoculum size as well as the presence of Tween 80 as a culture medium ingredient. However, because it was found that neither the presence of Tween 80 nor wide variations of inoculum size appreciably affected end points, details are not being reported here.

Results of therapy tests in mice are presented in Tables 2-4, with Table 2 reporting on tests with strain PM-22, Table 3 for strain PM-1, and Table 4 being a summary of all mouse test results. Since the challenge doses were purposely adjusted at a low level to avoid early or acute deaths to provide enough survivors for reference use in postmortem (sacrifice) organ bacteriology, mortality among untreated controls was 77 to 80\%. Accordingly, emphasis was given to the parameters of per cent survival, median survival time, and (of special importance) bacteriological negativity as evidence of eradicate effectiveness. In these tests, rifampin proved to be of respectable activity, although, as for the other drugs, failed consistently to provide either complete protection or fully eradicate effects.

**DISCUSSION**

Insofar as results are concerned from tests in vitro, rifampin proved to be of relatively poor activity. This observation for rifampin was also made by Eickhoff et al. (5) in broth-dilution susceptibility tests. The fact that neither variations in inoculum size nor the presence of Tween 80 failed to reveal inhibition by rifampin at a level less than 6.25 \textmu{g}/ml leaves no accounting for the extraordinary MIC of 0.04 \textmu{g}/ml reported for one strain by Hobby et al. (9). The possibility, therefore, must be considered that these investigators had by chance employed an atypical strain.

**Table 1. Susceptibility in vitro of Pseudomonas pseudomallei strains to various antimicrobial agents**

| Drug               | No. of strains having MIC at (\textmu{g}/ml) | Total no. of strains | Mean MIC (\textmu{g}/ml) |
|--------------------|---------------------------------------------|----------------------|--------------------------|
|                    | \textgreater{}100 | 100 | 50 | 25 | 12.5 | 6.3 | 3.1 | 1.6 | 0.8 |                  |
| Doxycycline        | 0              | 0   | 0   | 0   | 0   | 9   | 9   | 10  | 37  | 56     | 1.3    |
| Methacycline       | 0              | 0   | 0   | 0   | 0   | 5   | 8   | 35  | 8   | 56     | 2.1    |
| Tetracycline       | 0              | 0   | 0   | 0   | 0   | 9   | 19  | 25  | 11  | 64     | 2.6    |
| Minocycline        | 0              | 0   | 0   | 0   | 0   | 5   | 25  | 26  | 0   | 56     | 2.7    |
| Chloramphenicol    | 1              | 8   | 0   | 0   | 6   | 39  | 9   | 1   | 0   | 64     | 6.3^a  |
| Novobiocin         | 0              | 0   | 0   | 0   | 9   | 46  | 8   | 1   | 0   | 64     | 6.6    |
| Kanamycin          | 0              | 2   | 4   | 34  | 22  | 2   | 0   | 0   | 0   | 64     | 24.0   |
| Rifampin           | 0              | 2   | 21  | 28  | 3   | 2   | 0   | 0   | 0   | 56     | 35.7   |
| Sulfisoxazole\(^b\) | 0              | 4   | 8   | 5   | 2   | 4   | 3   | 0   | 0   | 26     | 37.9   |

\(^a\) Does not include nine resistant strains; mean minimum inhibitory concentration (MIC) of 21.1 if these nine are included.

\(^b\) Tested in chemically defined, antagonist-free liquid medium.
**TABLE 2. Mouse chemotherapy of strain PM-22**

| Treatment       | Per cent drug in diet | Avg drug intake (mg/kg/day) | No. of mice | Per cent surviving | Days STa | No. of surviving mice | Per cent surviving mice with negative cultures |
|-----------------|-----------------------|-----------------------------|-------------|--------------------|---------|-----------------------|---------------------------------------------|
| None (controls) |                       |                             | 70          | 20                 | 13      | 14                    | 64                                          |
| Rifampin        | 0.2                   | 210                         | 20          | 60                 | >30     | 12                    | 92                                          |
|                 | 0.4                   | 500                         | 20          | 90                 | >30     | 18                    | 78                                          |
| Tetracycline    | 0.1                   | 145                         | 20          | 15                 | 15      | 3                     | 0                                           |
|                 | 0.2                   | 300                         | 20          | 20                 | 20      | 4                     | 25                                          |
|                 | 0.4                   | 680                         | 20          | 12                 | 15      | 3                     | 67                                          |
| Methacycline    | 0.1                   | 140                         | 20          | 20                 | 15      | 4                     | 75                                          |
|                 | 0.2                   | 330                         | 20          | 15                 | 15      | 3                     | 100                                         |
|                 | 0.4                   | 560                         | 20          | 50                 | 28      | 10                    | 70                                          |
| Minocycline     | 0.1                   | 170                         | 20          | 60                 | >30     | 12                    | 67                                          |
|                 | 0.2                   | 280                         | 20          | 65                 | >30     | 13                    | 84                                          |
|                 | 0.4                   | 680                         | 20          | 85                 | >30     | 17                    | 71                                          |
| Doxycycline     | 0.1                   | 150                         | 20          | 40                 | 25      | 8                     | 50                                          |
|                 | 0.2                   | 360                         | 20          | 55                 | >30     | 11                    | 82                                          |
|                 | 0.4                   | 690                         | 20          | 50                 | >30     | 10                    | 60                                          |

* Median survival time.

**TABLE 3. Mouse chemotherapy of strain PM-1**

| Treatment       | Per cent drug in diet | Avg drug intake (mg/kg/day) | No. of mice | Per cent surviving | Days STa | No. of surviving mice | Per cent surviving mice with negative cultures |
|-----------------|-----------------------|-----------------------------|-------------|--------------------|---------|-----------------------|---------------------------------------------|
| None (controls) |                       |                             | 105         | 23                 | 24      | 24                    | 54                                          |
| Rifampin        | 0.1                   | 170                         | 10          | 80                 | >32     | 8                     | 75                                          |
|                 | 0.2                   | 300                         | 20          | 40                 | 29      | 8                     | 63                                          |
|                 | 0.4                   | 550                         | 20          | 70                 | >32     | 14                    | 64                                          |
| Tetracycline    | 0.1                   | 140                         | 20          | 20                 | 14      | 4                     | 75                                          |
|                 | 0.2                   | 350                         | 20          | 25                 | 14      | 5                     | 60                                          |
|                 | 0.4                   | 780                         | 20          | 25                 | 13      | 5                     | 80                                          |
| Methacycline    | 0.1                   | 170                         | 20          | 20                 | 10      | 4                     | 50                                          |
|                 | 0.2                   | 280                         | 20          | 40                 | 22      | 8                     | 88                                          |
|                 | 0.4                   | 630                         | 20          | 25                 | 14      | 5                     | 40                                          |
| Minocycline     | 0.1                   | 140                         | 20          | 65                 | >32     | 13                    | 77                                          |
|                 | 0.2                   | 330                         | 20          | 85                 | >32     | 17                    | 77                                          |
|                 | 0.4                   | 600                         | 10          | 100                | >32     | 10                    | 90                                          |
| Doxycycline     | 0.1                   | 140                         | 20          | 20                 | 25      | 4                     | 100                                         |
|                 | 0.2                   | 310                         | 20          | 20                 | 25      | 11                    | 73                                          |
|                 | 0.4                   | 640                         | 20          | 55                 | 30      | 11                    | 73                                          |

* Median survival time.

In contrast, a different picture developed from the mouse therapy tests. In terms of overall efficacy, rifampin appeared superior to tetracycline, methacycline, and doxycycline; however, minocycline was generally somewhat more effective. More specifically, in the subacute infection by strain PM-22, rifampin and minocycline appeared to be of moderate and comparable activity; tetracycline performed poorly (survival and bacterial eradication percentages equal to or less
TABLE 4. Summary of all mouse chemotherapy tests

| Treatment       | Total no. of mice | Per cent survivors | Per cent of surviving mice cultured negative |
|-----------------|-------------------|--------------------|---------------------------------------------|
| None (controls) | 175               | 22                 | 59                                          |
| Rifampin        | 90                | 67                 | 75                                          |
| Tetracycline    | 120               | 20                 | 54                                          |
| Methacycline    | 120               | 28                 | 63                                          |
| Minocycline     | 120               | 68                 | 73                                          |
| Doxycycline     | 120               | 46                 | 69                                          |

than untreated controls); methacycline and doxycycline were more active than tetracycline, with doxycycline proving somewhat more active than methacycline.

Tests involving the more chronic infection by strain PM-1 revealed generally superior activity by minocycline. In approximate order of activity there followed rifampin, doxycycline, and methacycline. Again, tetracycline treatment groups hardly differed from untreated controls with respect to per cent survival, and, surprisingly, survival times were shorter for the tetracycline groups.

The achievement of 100% negativity, or eradication of organ-sequestered bacteria in all mice, was not characteristic of any of these drugs either as a consistent or a dose-related action. When consideration is given to the fact that most (54 to 64%) of the surviving untreated control mice had succeeded without drug treatment in eliminating challenge residual P. pseudomallei, the performance of all five antibiotics appears even more unimpressive.

A more vexing question may be inferred from the apparent lack of correlation between the data in vitro and in vivo, particularly for tetracycline and rifampin. The failure of tetracycline may be accounted for in part by the knowledge (from tests not reported here) that very little drug is systemically absorbed in mice under the drug-diet procedure used here, as indicated by the lack of detectable blood levels. Minocycline, which was about equal to tetracycline for activity in vitro, provides distinctively high blood levels in mice after ingestion (13), and this may have accounted for its superior activity in these studies.

A more difficult question, however, is posed by rifampin, since it was clearly relatively weak in vitro and yet was definitely active in vivo. However, some absence of a clear in vitro-in vivo correlation for rifampin has also been reported elsewhere for experimental chemotherapy tests against other bacterial species (10). It should not be overlooked, nevertheless, that rifampin fell short of providing a complete eradicative action, and that rifampin dosages were employed here (170 to 550 mg per kg per day) which were far beyond those being used clinically (3). A similar situation pertains to the various tetracyclines since they were also tested at dosages considerably in excess of those employed in human patients (140 to 780 mg per kg per day) and a fully eradicative action still was not obtained.

The results of these studies, therefore, serve to confirm an earlier report of rifampin activity versus P. pseudomallei in vivo, but fail to do so for its activity in vitro (9). The comparative weak activity of rifampin in vitro has also been demonstrated by Alexander and Williams (2) and Eickhoff et al. (5).

ACKNOWLEDGMENT

These studies were supported by U.S. Army Medical Research and Development Command, Office of the Surgeon General, under contract no. DADA 17-67-C-7151.

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