ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1990, p. 1597–1599
0066-4804/90/081597-03$02.00/0

Vol. 34, No. 8

# In Vitro Susceptibilities of Oral Pigmented *Bacteroides* Species to Trospectomycin and Other Selected Antimicrobial Agents

CAROLINE C. JOHNSON,* MICHAEL WENDELER, AND MATTHEW E. LEVISON

*Division of Infectious Diseases, Department of Medicine, The Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129, and Veterans Administration Medical Center, Philadelphia, Pennsylvania 19104*

Received 13 December 1989/Accepted 16 May 1990

**The in vitro activity of trospectomycin against 97 clinical isolates of oral pigmented *Bacteroides* species was compared with the activities of five other antimicrobial agents. At 4 μg/ml, more than 90% of isolates were inhibited by trospectomycin. Overall, strains that produced β-lactamase ($n$ = 41) were more resistant to trospectomycin, penicillin G, cefoxitin, piperacillin, and tetracycline but not to clindamycin. In this study, trospectomycin had excellent in vitro activity against oral pigmented *Bacteroides* species.**

Trospectomycin (6[1] propylspectinomycin sulfate pentahydrate) is a new aminocylitol antibiotic that is derived from spectinomycin. In comparison to the parent molecule, trospectomycin has an expanded antimicrobial spectrum and enhanced potency (11). It has excellent in vitro activity against most gram-positive cocci, as well as against *Haemophilus influenzae*, *Haemophilus ducreyi*, *Neisseria gonorrhoeae* (both β-lactamase-positive and -negative strains), *Mycoplasma* species, and *Chlamydia trachomatis* (8, 9; L. K. Klein, D. H. Batts, A. D. Hall, and R. J. Yancey, Jr., Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 269, 1987; G. L. Ridgway, G. Mumtaz, and G. Gabriel, 27th ICAAC, abstr. no. 267, 1987). Its activity against species of the family *Enterobacteriaceae* is moderate, comparable to that of spectinomycin. In addition, many anaerobic bacteria are susceptible to trospectomycin in vitro. The MIC for 90% of *Bacteroides* isolates has been reported to be 4.0 μg/ml (7). Because of this broad spectrum of activity, trospectomycin might be a useful drug in the treatment of mixed aerobic-anaerobic infections. In particular, infections that derive from the flora of the oropharynx, such as aspiration pneumonia, empyema, and neck space infections, might be amenable to treatment with trospectomycin. Most of the aerobic organisms involved in these infections are susceptible to trospectomycin in vitro; however, the susceptibility of upper respiratory tract anaerobes is not well documented. Pigmented *Bacteroides* species are among the most commonly isolated and most antibiotic resistant of the anaerobes associated with these types of infections (3, 4). The present study was undertaken to determine the in vitro activity of trospectomycin against strains of oral pigmented *Bacteroides* species and to compare its activity with those of five other antimicrobial agents.

Bacteria used for susceptibility testing were isolated from subgingival plaque samples of outpatients with periodontal disease. After routine processing of the samples, strains were selected on the basis of dark pigmentation or red fluorescence after 48 to 72 h of anaerobic incubation. All isolates were strictly anaerobic, bile-sensitive, gram-negative bacilli that did not produce catalase (6). Further identification to species was accomplished using the An-Ident system (Analytab Products, Plainview, N.Y.). MICs were determined by an agar dilution method (10). Standard powders of the antimicrobial agents were supplied by the manufacturers as follows: trospectomycin and clindamycin, The Upjohn Co., Kalamazoo, Mich.; penicillin G and tetracycline, Sigma Chemical Co., St. Louis, Mo.; cefoxitin, Merck Sharp & Dohme, Rahway, N.J.; and piperacillin, Lederle Laboratories, Pearl River, N.Y. Antibiotics were prepared fresh on the day of the experiment according to the manufacturers' recommendations. Twofold dilutions of each were added to brucella agar supplemented with laked sheep blood (5%) and vitamin K (0.005%). After overnight incubation in supplemented thioglycolate broth, each isolate was diluted to approximately $10^8$ CFU/ml in brain heart infusion broth. A Steers replicator was used to deliver an inoculum of $10^5$ CFU to the surface of the antibiotic-containing agar. After 48 h of anaerobic incubation, MICs were read as the lowest concentrations of drug yielding either no growth, a single colony, or a barely visible haze. All isolates were tested for production of β-lactamase by using a nitrocefin disk (Cefinase) (5).

The results of susceptibility tests are summarized in Table 1. Trospectomycin exhibited good activity against strains of oral pigmented *Bacteroides* species. More than 90% of isolates were inhibited by 4.0 μg/ml. Although the breakpoint for determining resistance has not been established, 1.0 g of trospectomycin intravenously yields a mean peak in serum of 81.2 μg/ml and a mean half-life of 2.2 h in humans (A. Bye and L. G. Dring, 27th ICAAC, abstr. no. 272, 1987). Using the breakpoints proposed by the manufacturer (≤16 μg/ml = susceptible, 32 μg/ml = intermediate, and ≥64 μg/ml = resistant), 98% of strains were susceptible and 2.0% were intermediate to trospectomycin in vitro. Clindamycin also showed excellent activity against pigmented *Bacteroides* species. All isolates were inhibited by a concentration of this drug that is readily achievable in the serum. Piperacillin and cefoxitin were slightly less active, while penicillin G and tetracycline were least active against pigmented oral *Bacteroides* species.

No significant differences in antimicrobial susceptibility were observed between the various species of oral pigmented *Bacteroides*. However, strains that produced β-lactamase were significantly more resistant to penicillin, cefoxitin, piperacillin, tetracycline, and trospectomycin (Table 2). Overall, 43% of the isolates were β-lactamase positive. Although there was rough correlation between β-lactamase production and high penicillin MICs, MICs for some strains that produced β-lactamase were low (≤0.062 μg/ml). In contrast, for other strains that did not produce

* Corresponding author.

TABLE 1. Susceptibilities of oral pigmented *Bacteroides* isolates to selected antimicrobial agents

| Organism (no. of isolates) | Antibiotic | MIC (μg/ml) | | % Resistant[a] |
|---|---|---|---|---|
| | | Range | 90%[b] | |
| *Bacteroides* spp., pigmented group (97) | Trospectomycin | 0.125–32.0 | 4.0 | 2 (0) |
| | Penicillin G | ≤0.062–128.0 | 8.0 | 9 |
| | Cefoxitin | ≤0.062–64.0 | 4.0 | 3 |
| | Piperacillin | ≤0.062–128.0 | 8.0 | 2 |
| | Tetracycline | 0.125–64.0 | 16.0 | 18 |
| | Clindamycin | ≤0.062–0.5 | 0.125 | 0 |
| *Bacteroides melaninogenicus* (53) | Trospectomycin | 0.125–32.0 | 4.0 | 2 (0) |
| | Penicillin G | ≤0.062–64.0 | 8.0 | 9 |
| | Cefoxitin | ≤0.062–32.0 | 4.0 | 2 |
| | Piperacillin | ≤0.062–64.0 | 8.0 | 0 |
| | Tetracycline | 0.250–32.0 | 16.0 | 19 |
| | Clindamycin | ≤0.062–0.25 | 0.125 | 0 |
| *Bacteroides intermedius* (22) | Trospectomycin | 0.50–16.0 | 4.0 | 0 (0) |
| | Penicillin G | ≤0.062–128.0 | 8.0 | 9 |
| | Cefoxitin | ≤0.062–64.0 | 4.0 | 5 |
| | Piperacillin | ≤0.062–128.0 | 16.0 | 5 |
| | Tetracycline | 0.125–64.0 | 8.0 | 18 |
| | Clindamycin | ≤0.062–0.25 | 0.125 | 0 |
| *Bacteroides* spp., pigmented (not identifiable to species) (15) | Trospectomycin | 1.0–4.0 | 4.0 | 0 (0) |
| | Penicillin G | 0.125–16.0 | 8.0 | 7 |
| | Cefoxitin | ≤0.062–2.0 | 1.0 | 0 |
| | Piperacillin | 0.25–128.0 | 8.0 | 7 |
| | Tetracycline | 0.125–16.0 | 8.0 | 13 |
| | Clindamycin | ≤0.062–0.125 | 0.125 | 0 |
| *Bacteroides* spp., miscellaneous (7)[c] | Trospectomycin | 1.0–32.0 | 32.0 | 14 (0) |
| | Penicillin G | 0.125–16.0 | 16.0 | 14 |
| | Cefoxitin | 0.25–64.0 | 64.0 | 14 |
| | Piperacillin | ≤0.062–16.0 | 16.0 | 0 |
| | Tetracycline | 0.125–32.0 | 32.0 | 14 |
| | Clindamycin | ≤0.062–0.5 | 0.5 | 0 |

[a] Breakpoints for resistance were as follows: trospectomycin, ≥32 μg/ml (≥64 μg/ml; percent resistant at this breakpoint is shown in parentheses); penicillin G, ≥16 μg/ml; cefoxitin, ≥32 μg/ml; piperacillin, ≥128 μg/ml; tetracycline, ≥8 μg/ml; and clindamycin, ≥8 μg/ml.
[b] 90%, MIC for 90% of isolates.
[c] Miscellaneous *Bacteroides* species were: *B. denticola*, four; *B. corporis*, two; and *B. (Porphyromonas) gingivalis*, one.

β-lactamase, penicillin MICs were relatively high (8.0 μg/ml). These observations suggest that for an individual strain β-lactamase production and the penicillin MIC may be discrepant; therefore, determinations of both are clinically relevant.

Trospectomycin is a new aminocyclitol antibiotic that possesses a broad range of activity. Both parenteral and topical formulations are under investigation. In humans, the apparent serum half-life is 2.2 h following intravenous administration of a 1.0-g dose (Bye and Dring, 27th ICAAC).

TABLE 2. Correlation of antimicrobial susceptibility and β-lactamase activity in oral pigmented *Bacteroides* species

| Antibiotic | $MIC_{90}$[a] (μg/ml) for: | |
|---|---|---|
| | β-Lactamase-positive strains ($n$ = 41) | β-Lactamase-negative strains ($n$ = 56) |
| Trospectomycin | 16.0 | 4.0 |
| Penicillin G | 16.0 | 0.5 |
| Cefoxitin | 8.0 | 1.0 |
| Piperacillin | 32.0 | 2.0 |
| Tetracycline | 16.0 | 2.0 |
| Clindamycin | 0.125 | 0.125 |

[a] $MIC_{90}$, MIC for 90% of isolates.

Approximately 14.5% of the drug is protein bound in human plasma; it is excreted unchanged primarily via the urine. Phase I safety and tolerance studies have shown trospectomycin to be well tolerated in doses up to 1,000 mg. Phase II studies are ongoing.

Trospectomycin may be a useful antibiotic in the management of several types of infections. Because it has activity against *C. trachomatis*, *N. gonorrhoeae*, *Mycoplasma hominis*, and *H. ducreyi*, it should be suitable for treating certain sexually transmitted diseases. In addition, its activity against both *Bacteroides fragilis* group organisms and many members of the *Enterobacteriaceae* gives it promise as a single agent for treating intra-abdominal and pelvic infections. Trospectomycin has been found to be as efficacious as clindamycin and gentamicin for reducing mortality in an animal model of intra-abdominal sepsis (2). It also possesses good activity against experimental subcutaneous abscesses induced with *B. fragilis* (1).

Other infections for which trospectomycin might have promise are those due to oropharyngeal flora. These are typically mixed aerobic-anaerobic infections in which pigmented *Bacteroides* species are prominent pathogens (4). Our results indicate that trospectomycin has excellent in vitro activity against this group of organisms, comparable to the activities of several other antimicrobial agents used to

treat infections involving this organism. Since most aerobic respiratory tract pathogens (*Streptococcus pneumoniae*, *H. influenzae*, *Branhamella* species, and streptococci) are also susceptible to trospectomycin, it should be suitable as single-agent therapy. Further investigations on the activity of trospectomycin are warranted.

Partial funding for this study was provided by grants from The Upjohn Co. and Lederle Laboratories.

## LITERATURE CITED

1. **Brauner, E., and S. Gorbach.** 1989. Comparative efficacy of trospectomycin (U-63366F), clindamycin, and imipenem against *Bacteroides fragilis* in the infected granuloma pouch model. Curr. Ther. Res. **45**:414–420.
2. **Cisneros, R. L., and A. B. Onderdonk.** 1988. Efficacy of trospectomycin for the treatment of experimental intra-abdominal sepsis. Curr. Ther. Res. **43**:456–462.
3. **Edson, R. S., J. E. Rosenblatt, D. T. Lee, and E. A. McVey III.** 1982. Recent experience with antimicrobial susceptibility of anaerobic bacteria: increasing resistance to penicillin. Mayo Clin. Proc. **57**:737–741.
4. **Finegold, S. M., W. L. George, and M. E. Mulligan.** 1985. Anaerobic infections, part I. Diseases-a-Month **31**:53–68.
5. **Gabay, E. L., V. L. Sutter, and S. M. Finegold.** 1981. Rapid beta-lactamase testing in *Bacteroides*. J. Antimicrob. Chemother. **8**:413–416.
6. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
7. **Jacobus, N. V., and F. P. Tally.** 1988. Activity of trospectomycin against *Bacteroides fragilis* and other *Bacteroides* species. Antimicrob. Agents Chemother. **32**:584–586.
8. **Peeters, M., E. Van Dyck, and P. Piot.** 1984. In vitro activities of the spectinomycin analogue U-63366 and four quinolone derivatives against *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **26**:608–609.
9. **Sanson-Le Pors, M.-J., I. M. Casin, M.-C. Thebault, G. Arlet, and Y. Perol.** 1986. In vitro activities of U-63366, a spectinomycin analog; roxithromycin (RU 28965), a new macrolide antibiotic; and five quinolone derivatives against *Haemophilus ducreyi*. Antimicrob. Agents Chemother. **30**:512–513.
10. **Sutter, V. L., D. M. Citron, M. A. C. Edelstein, and S. M. Finegold.** 1985. Wadsworth anaerobic bacteriology manual, 4th ed., p. 79–90. Star Publishing Co., Belmont, Calif.
11. **Zurenko, G. E., B. H. Yagi, J. J. Vavra, and B. B. Wentworth.** 1988. In vitro antibacterial activity of trospectomycin (U-63366F), a novel spectinomycin analog. Antimicrob. Agents Chemother. **32**:216–223.