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# Antibacterial Activity of Trospectomycin (U-63366F) and Initial Evaluations of Disk Diffusion Susceptibility Tests

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**The in vitro activities of trospectomycin sulfate were compared with those of spectinomycin against 632 aerobic microorganisms, including 66 *Neisseria gonorrhoeae* isolates. Against species other than gram-negative bacilli, trospectomycin was about 4- to 16-fold more active than spectinomycin. For disk diffusion tests, a 30-μg disk is recommended, with tentative zone size breakpoints of ≤13 mm for resistance (MIC, ≥64 μg/ml) and ≥17 mm for susceptibility (MIC, ≤16 μg/ml).**

Trospectomycin sulfate (U-63366F) is an analog of spectinomycin (6, 7; D. R. White and G. A. Cain, 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 264, 1987). The in vitro activity of trospectomycin has been reported to be superior to that of spectinomycin (5, 8, 9). Both aminocyclitol antibiotics function by binding to 30S ribosomal subunits, thus inhibiting protein synthesis (A. L. Laborde and R. J. Mourey, 27th ICAAC, abstr. no. 266, 1987). The greater potency of trospectomycin might be explained by the increased affinity of the drug for the ribosomal subunits.

In humans, trospectomycin has been found to be well tolerated when administered intravenously in a single 20-min infusion. Following infusion of a 1,000-mg dose, the average peak concentration in serum was 81 μg/ml and the half-life in serum was 2.2 h (E. Novak, L. M. Paxton, G. E. Zurenko, and S. F. Francom, 27th ICAAC, abstr. no. 271, 1987). Trospectomycin readily penetrates most tissues and is eliminated very slowly; i.e., the half-life in tissue is approximately 3 days (M. Burrows, T. A. Osgood, and L. G. Dring, 27th ICAAC, abstr. no. 273, 1987). Binding to proteins in serum is approximately 10 to 14%, depending on the assay method (R. Brown, personal communications).

In this study we compared the in vitro activity of trospectomycin with that of spectinomycin against 632 aerobic bacterial isolates selected from the stock culture collections of our two institutions. We also describe results of preliminary studies that were designed to select the most appropriate disk potency for susceptibility tests and to propose very tentative interpretive criteria to be used during clinical evaluations.

TABLE 1. In vitro activity of trospectomycin and spectinomycin against *Neisseria* species

| Microorganism (no. of isolates) | Antimicrobial agent | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| *N. meningitidis* (30) | Trospectomycin | 4.0–8.0 | 8.0 | 8.0 |
| | Spectinomycin | 8.0–32 | 32 | 32 |
| *N. gonorrhoeae* | | | | |
| Spectinomycin resistant (11) | Trospectomycin | >32 | >32 | >32 |
| | Spectinomycin | 128–>256 | >256 | >256 |
| Spectinomycin susceptible | | | | |
| β-Lactamase positive (23) | Trospectomycin | 1.0–32 | 2.0 | 4.0 |
| | Spectinomycin | 8.0–32 | 16 | 16 |
| β-Lactamase negative, penicillin resistant[a] (11) | Trospectomycin | 2.0–8.0 | 4.0 | 8.0 |
| | Spectinomycin | 8.0–32 | 16 | 32 |
| β-Lactamase negative, penicillin susceptible (21) | Trospectomycin | 1.0–2.0 | 1.0 | 1.0 |
| | Spectinomycin | 8.0–32 | 16 | 16 |

[a] Benzylpenicillin MICs were 2.0 to 16 μg/ml.

Trospectomycin sulfate and spectinomycin were both provided by The Upjohn Co. (Kalamazoo, Mich.). Three disk potencies (30, 75, and 100 μg) were evaluated. Agar diffusion susceptibility tests were performed by the procedure described by the National Committee for Clinical Laboratory Standards (NCCLS) (1). Diffusion tests were not performed with *Neisseria gonorrhoeae* isolates, since a different medium and different zone size criteria would be needed. Also, *Neisseria meningitidis* isolates were tested only by the microdilution procedure. Broth microdilution tests were performed by the procedures described by the

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TABLE 2. In vitro activity of trospectomycin and spectinomycin against 536 bacterial isolates that were also used to evaluate trospectomycin disk susceptibility tests

| Microorganism (no. of isolates tested) | Antimicrobial agent | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| *Staphylococcus* spp. | | | | |
| *S. aureus* (55) | Trospectomycin | 4.0–32 | 16 | 16 |
| | Spectinomycin | 32–>128 | 128 | 128 |
| Other species (49)[a] | Trospectomycin | 4.0–64 | 8.0 | 32 |
| | Spectinomycin | 16–>128 | 64 | >128 |
| Methicillin resistant (20)[b] | Trospectomycin | 4.0–64 | 32 | 64 |
| | Spectinomycin | 32–>128 | >128 | >128 |
| *Enterococcus* spp. | | | | |
| *E. faecalis* (28) | Trospectomycin | 8.0–32 | 16 | 32 |
| | Spectinomycin | 128 | 128 | 128 |
| Other species (22)[c] | Trospectomycin | 4.0–32 | 16 | 32 |
| | Spectinomycin | 16–128 | 64 | 128 |
| *Streptococcus* spp. | | | | |
| *S. agalactiae* (30) | Trospectomycin | 32–64 | 32 | 64 |
| | Spectinomycin | >128 | >128 | >128 |
| *S. pyogenes* (30) | Trospectomycin | 8.0–32 | 16 | 16 |
| | Spectinomycin | 64–>128 | 128 | >128 |
| Serogroups C and G (20)[d] | Trospectomycin | 2.0–4.0 | 4.0 | 4.0 |
| | Spectinomycin | 16–32 | 32 | 32 |
| *S. pneumoniae* | | | | |
| Penicillin susceptible (20) | Trospectomycin | ≤0.25–2.0 | 2.0 | 2.0 |
| | Spectinomycin | 2.0–32 | 16 | 16 |
| Penicillin resistant (10) | Trospectomycin | 2.0–4.0 | 2.0 | 4.0 |
| | Spectinomycin | 8.0–64 | 16 | 32 |
| *Listeria monocytogenes* (30) | Trospectomycin | 4.0–32 | 8.0 | 8.0 |
| | Spectinomycin | 32–128 | 32 | 64 |
| *Corynebacterium jeikeium* (8) | Trospectomycin | 1.0–32 | 8.0 | —[e] |
| | Spectinomycin | 8.0–32 | 16 | — |
| *Bacillus* spp. (10) | Trospectomycin | 8.0–16 | 16 | 16 |
| | Spectinomycin | 32–128 | 64 | 128 |
| *Branhamella catarrhalis* (20) | Trospectomycin | 1.0–4.0 | 2.0 | 4.0 |
| | Spectinomycin | 4.0–8.0 | 4.0 | 4.0 |
| *Haemophilus influenzae* (57) | Trospectomycin | ≤0.25–16 | 1.0 | 2.0 |
| | Spectinomycin | 2.0–128 | 16 | 32 |
| *Acinetobacter calcoaceticus* (5) | Trospectomycin | 16–64 | 32 | — |
| | Spectinomycin | 64–>128 | 128 | — |
| *Pseudomonas* spp. (34) | Trospectomycin | 2.0–>128[f] | >128 | >128 |
| | Spectinomycin | 16–>128[f] | >128 | >128 |
| *Enterobacteriaceae* family (108) | Trospectomycin | 4.0–>128 | 64 | 128 |
| | Spectinomycin | 16–>128 | 32 | >128 |

[a] Included 28 *S. epidermidis*, 7 *S. warneri*, 4 *S. simulans*, 4 *S. saprophyticus*, 3 *S. hominis*, and 3 *S. capitis* isolates.
[b] Included 10 *S. aureus* and 10 coagulase-negative isolates that were resistant to oxacillin as well as methicillin.
[c] Included 10 *E. faecium*, 7 *E. durans*, and 5 *E. hirae* isolates.
[d] Included 10 serogroup C and 10 serogroup G streptococcus isolates.
[e] —, $MIC_{90}$s were not calculated when there were <10 isolates.
[f] Of three *P. stutzeri* isolates, two were susceptible to trospectomycin (MIC, 2.0 μg/ml) and their spectinomycin MICs were 8.0 and 128 μg/ml.

NCCLS (2). The inoculum was approximately $5 \times 10^5$ CFU/ml, and MICs were recorded after 18 to 20 h at 35°C in ambient air. Cation-supplemented Mueller-Hinton broth was used throughout the study. For testing nutritionally fastidious species, 2 to 3% lysed horse blood was added. *Haemophilus* test medium was used for broth microdilution tests with *Haemophilus influenzae*. An agar dilution procedure was used for testing *Neisseria gonorrhoeae* isolates (2). GC agar base (Difco Laboratories, Detroit, Mich.) contained a defined supplement (Prepared Media Laboratories, Tualatin, Oreg.). The latter supplement was identical to the product which is sold as IsoVitaleX by BBL Microbiology Systems (Cockeysville, Md.), except that cysteine was omitted. The cysteine in such products might inactivate some β-lactam compounds (unpublished data) and is not essential for growth of the gonococci.

Table 1 shows the results of in vitro tests with 66 *Neisseria gonorrhoeae* and 30 *Neisseria meningitidis* isolates. Specti-

TABLE 3. Evaluation of trospectomycin disk susceptibility tests by regression analysis comparing zone diameters with broth microdilution MICs

| Disk content (μg) | Regression formula[a] | Correlation coefficient | Calculated zone diam (mm) correlating with a: | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Resistance MIC (μg/ml) of[b]: | | | Susceptibility MIC (μg/ml) of[b]: | | |
| | | | ≥32 | ≥64 | ≥128 | ≤8.0 | ≤16 | ≤32 |
| 30 | $y = 18.1 - 2.8x$ | 0.91 | ≤16 | ≤13 | ≤10 | ≥20 | ≥17 | ≥14 |
| 75 | $y = 18.8 - 2.7x$ | 0.89 | ≤19 | ≤16 | ≤13 | ≥23 | ≥20 | ≥17 |
| 100 | $y = 18.9 - 2.6x$ | 0.88 | ≤20 | ≤17 | ≤14 | ≥24 | ≥21 | ≥18 |

[a] $y$ = MIC as $\log_2 + 9$ (in micrograms per milliliter), and $x$ = zone diameter (in millimeters).
[b] Three possible MIC breakpoints for trospectomycin were applied to tests with each of the disk potencies.

nomycin-resistant gonococci were also resistant to trospectomycin. Zurenko et al. (9) have also reported the complete cross-resistance of these organisms to both drugs. Against spectinomycin-susceptible gonococci and meningococci, on the other hand, trospectomycin was approximately four to eight times more active than spectinomycin. Against β-lactamase-negative, penicillin-resistant gonococci, trospectomycin was about four times less active than it was against penicillin-susceptible strains (MICs for 50% of strains tested [$MIC_{50}$s], 4.0 and 1.0 μg/ml, respectively). Spectinomycin did not differ in its potency against various types of spectinomycin-susceptible gonococci. Similar results have been reported by others (5, 9).

Table 2 summarizes the results of additional tests performed with 536 bacterial isolates, representing a variety of pathogenic species that were also tested by the disk diffusion method. In most cases, trospectomycin was approximately 4 to 16 times more active than spectinomycin. Based on results of preliminary pharmacokinetic studies in humans (E. Novak, L. M. Paxton, G. E. Zurenko, and S. F. Franom, 27th ICAAC, abstr. no. 271, 1987), strains inhibited by ≥64 μg/ml are tentatively considered resistant to trospectomycin, and those inhibited by ≤16 μg/ml are considered susceptible. By those interpretive criteria, trospectomycin appears to be a potentially useful antistaphylococcal drug. β-Lactamase production by the staphylococci did not influence the activity of either aminocyclitol. Methicillin-resistant staphylococci were exceptions, since they were not uniformly susceptible to either drug; i.e., only 9 of 20 strains were inhibited by 16 μg of trospectomycin per ml and by 64 μg of spectinomycin per ml. Most enterococci were only moderately susceptible to trospectomycin ($MIC_{50}$, 16 μg/ml). Our *Streptococcus agalactiae* and *Streptococcus pyogenes* isolates were also moderately susceptible ($MIC_{50}$s, 32 and 16 μg/ml, respectively) compared with other streptococci ($MIC_{50}$s, 2.0 or 4.0 μg/ml). Zurenko et al. (9) have reported eightfold-lower MICs for *Streptococcus agalactiae* and *Streptococcus pyogenes* isolates. Rolston et al. (K. Rolston, D. H. Ho, and G. P. Bodey, 28th ICAAC, abstr. 536, 1988) reported MICs for 90% of strains tested ($MIC_{90}$s) of 2.0 to 8.0 μg/ml for different streptococci. In our hands, the pneumococci, including penicillin-resistant strains, were susceptible to trospectomycin ($MIC_{50}$, 2.0 μg/ml) and spectinomycin ($MIC_{50}$, 16 μg/ml). The gram-positive bacilli were also susceptible to trospectomycin. *Branhamella catarrhalis* isolates were also susceptible to both drugs ($MIC_{50}$s, 2.0 and 4.0 μg/ml, respectively). Ampicillin-resistant and -susceptible isolates of *Haemophilus influenzae* were very susceptible to trospectomycin, but spectinomycin MICs were generally 16-fold greater. Similar trospectomycin data have been reported by other workers (5, 9; B. A. Daley, J. J. Lipuma, and T. L. Stull, 88th Annu. Meet. Am. Soc. Microbiol. 1988, A-143, p. 24). Trospectomycin has also been shown to be effective against *Mycoplasma pneumoniae*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* (8). *Chlamydia trachomatis* and many anaerobic microorganisms have also been reported to be susceptible to trospectomycin in vitro (9; G. Gordon and J. E. Bross, 28th ICAAC, abstr. no. 538, 1988).

The method proposed by the NCCLS (3) was used to evaluate the bactericidal activity of both drugs. Broth microdilution panels were inoculated, and viable cell counts were performed to document the inoculum density. After 16 to 18 h of incubation, the MICs were recorded and the trays were shaken and reincubated for a total of 24 h. At that time, two 10-μl samples were subcultured onto blood agar plates. The MBC was defined as the lowest concentration capable of killing 99.9% of the viable cells, using the rejection criteria of Pearson et al. (4). MICs and MBCs of both aminocyclitol compounds were determined for 16 selected isolates (2 *Streptococcus pneumoniae*, 2 *Branhamella catarrhalis*, 2 *Haemophilus influenzae*, 2 *Streptococcus agalactiae*, 2 *Enterococcus faecalis*, 3 *Staphylococcus aureus* [1 methicillin resistant], and 3 *Staphylococcus epidermidis* [1 methicillin resistant]). Against the *Streptococcus pneumoniae*, *Branhamella catarrhalis*, and *Haemophilus influenzae* isolates, both drugs were bactericidal (MBC, one to two times the MIC). No bactericidal activity was observed for the other species (MBC, >128 μg/ml).

Against gram-negative bacilli other than *Haemophilus influenzae*, trospectomycin displayed minimal activity, similar to that of spectinomycin. Among members of the family *Enterobacteriaceae* that were included, trospectomycin MICs were rarely 16 μg/ml, but many isolates were inhibited by 32 or 64 μg/ml. Of 108 enteric bacilli, 5 (4.6%) were susceptible to ≤16 μg/ml, and of 108 strains, only 27 (25%) were very resistant (MIC, ≥128 μg/ml). The clinical significance of this marginal in vitro activity is not clear.

The MIC interpretive criteria that were noted above (MIC, ≤16 μg/ml for susceptibility; MIC, ≥64 μg/ml for resistance) are likely to be applicable to tests with trospectomycin. However, additional clinical experience, pharmacologic information, and dosing decisions will influence the final selection of breakpoints. The tentative MIC breakpoints and two alternative breakpoints were used to evaluate susceptibility tests with three different disk potencies. Regression analyses were applied in order to calculate zone size correlates for each of three different MIC breakpoints (Table 3). If susceptibility is defined as an MIC other than ≤16 μg/ml, appropriate changes in zone size criteria can be made since each $\log_2$ dilution interval in MICs corresponds to a 3-mm change in the zone diameter. All three disks performed satisfactorily, regardless of the MIC breakpoints that were applied to the trospectomycin data. If a susceptibility break-

point of ≤8.0 or ≤16 μg/ml was found to be acceptable, tests with 30-μg disks should be satisfactory. However, in the unlikely event that susceptibility is redefined to include an MIC of ≤32 μg/ml, a 75-μg disk would be preferred.

When the susceptibility MIC breakpoint of ≤16 μg/ml was applied to the disk test data, there were no false-resistance disk tests; but a few false-susceptibility results occurred at the following rates: 3 (0.6%) with 30-μg disks, 8 (1.5%) with 75-μg disks, and 10 (1.9%) with 100-μg disks. Nearly all of the latter very major errors involved tests with methicillin-resistant *Staphylococcus aureus* isolates which were resistant by dilution tests (MIC, 64 μg/ml) but susceptible by disk tests. We conclude that a 30-μg trospectomycin disk can be used for ongoing clinical trials, with tentative interpretive criteria of a zone size of ≤13 mm for resistance (MIC, ≥64 μg/ml) and of ≥17 mm for susceptibility (MIC, ≤16 μg/ml). Standardization of disk tests in which *Neisseria gonorrhoeae* isolates are evaluated against trospectomycin has not yet been accomplished.

In summary, trospectomycin and spectinomycin are nonaminoglycosidic aminocyclitol compounds with similar spectra of activity. However, trospectomycin has a broader spectrum of potentially useful activity because it is significantly more active than spectinomycin in vitro.

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