Interpretive Standards for Disk Susceptibility Tests with Sch 21420 and Amikacin

ARTHUR L. BARRY,1* CLYDE THORNSBERRY,2 RONALD N. JONES,3 AND E. HUGH GERLACH4

Clinical Microbiology Laboratory, University of California (Davis) Medical Center, Sacramento, California 95817; Antimicrobial Investigations Section, Center for Disease Control, Atlanta, Georgia 30333; Department of Pathology, Kaiser Foundation Laboratories, Clackamas, Oregon 97015; and Microbiology Laboratory, St. Francis Hospital, Wichita, Kansas 67214

Disk susceptibility tests with two structurally related aminoglycosides (amikacin and Sch 21420) were evaluated. Tests with 10- and 30-μg amikacin disks confirmed previous recommendations for interpretive zone standards; 30-μg disks are preferred. Tests with 10-, 20-, and 30-μg Sch 21420 disks led to similar conclusions. The 30-μg Sch 21420 disks are recommended, with zone standards of ≤14 mm for the resistant category (minimal inhibitory concentration, ≥32 μg/ml) and ≥17 mm for the susceptible category (minimal inhibitory concentration, ≤16 μg/ml). If a minimal inhibitory concentration breakpoint of ≤8 μg/ml is preferred for defining the susceptible category, somewhat different zone standards may be used (≤15 mm and ≥19 mm). Further evaluation documented the fact that tests with 30-μg amikacin disks predicted resistance or susceptibility to Sch 21420 almost as well as did a 30-μg Sch 21420 disk. Thus, the class concept of disk testing was judged to be applicable, and routine testing with Sch 21420 may not be required.

Sch 21420 is a semisynthetic aminoglycoside which is produced from gentamicin B by a process similar to that used to produce amikacin from kanamycin A (9). The in vitro activity of Sch 21420 is similar to that of amikacin (2, 11-13, 16), but Sch 21420 has the potential for diminished nephrotoxicity (6). The present report summarizes our efforts to establish interpretive zone standards for Sch 21420 disk susceptibility tests using 10-, 20-, and 30-μg disks. Furthermore, efforts were made to determine whether tests with amikacin disks could be used to predict susceptibility to Sch 21420.

Amikacin susceptibility tests were initially standardized by using 10-μg disks and zone size breakpoints of ≤11 mm for the resistant category and ≥14 mm for the susceptible category. Moeller et al. (8) found that these disk test standards were unacceptable; i.e., nearly half of the microorganisms which were found to be resistant by the disk technique were actually found to be susceptible by a dilution procedure. Washington et al. (15) reported a collaborative study designed to reevaluate disk susceptibility test standards for 10- and 30-μg amikacin disks. They recommended zone size breakpoints of ≤9 mm and ≥12 mm for 10-μg disks, but they preferred tests with 30-μg disks and zone standards of ≤14 mm and ≥17 mm. In 1979, the National Committee for Clinical Laboratory Standards (NCCLS) subcommittee on antimicrobial susceptibility tests reviewed the above references and data from other unpublished studies. This led to a recommendation that 30-μg amikacin disks should be used, with appropriate zone size breakpoints (10). The present report includes confirmatory data which support the current NCCLS recommendations for amikacin disk tests.

Like previous authors (8, 15), we initially utilized minimal inhibitory concentration (MIC) size breakpoints of ≥32 μg/ml for the resistant category and ≤16 μg/ml for the susceptible category. Farchione and Chudzik (2) have suggested that therapy with amikacin may be successful even when the peak blood level only slightly exceeds the MIC for the pathogen being treated. Peak blood levels of both amikacin and Sch 21420 rarely exceed 25 to 30 μg/ml. Consequently, strains with amikacin MICs of 16 μg/ml should not be considered resistant, and those with MICs of ≤8 μg/ml are clearly susceptible. Sufficient clinical experience with Sch 21420 has not yet been gathered to determine whether strains which are inhibited by 16 μg/ml will be responsive to therapy. With both drugs, a conservative categorization would include all strains with MICs of ≤8 μg/ml in the susceptible category and those inhibited by 16 μg/ml in an intermediate category, i.e., neither resistant nor une-
quievocally susceptible. This alternative approach to categorization was considered in analyzing the data in the present report.

MATERIALS AND METHODS

Tests were performed at the Center for Disease Control, Atlanta, Ga., and at the University of California (Davis) Medical Center, Sacramento. Data collected in these two centers were combined after comparable results were demonstrated by previously described (1, 4) control procedures.

Both laboratories performed the disk diffusion technique as outlined by the NCCLS (10). Tests included the use of disks containing 10, 20, or 30 µg of Sch 21420 (prepared by one of the investigators) or 10 or 30 µg of amikacin (BBL Microbiology Systems, Cockeysville, Md.). Gentamicin and tobramycin disks (BBL) were also tested against the control strains. All controls performed satisfactorily; i.e., zones were within the NCCLS control limits (10).

A previously described (1, 3, 13) microdilution procedure was used to determine the MICs of Sch 21420 (Schering Corp., Bloomfield, N.J.) and amikacin (Bristol Laboratories, Syracuse, N.Y.). Both drugs are known to be affected by divalent cations in the test medium (7, 13, 14). For monitoring the performance of the broth medium that was used, gentamicin and tobramycin were also included in tests with the control strains. Performance of the cation-supplemented Mueller-Hinton broth used in these studies was measured by testing the control strain of Pseudomonas aeruginosa (ATCC 27853); MICs recorded on five separate occasions during the study were 4 or 8 µg of amikacin per ml, 8 µg of Sch 21420 per ml, 4 µg of gentamicin per ml, and 1 or 2 µg of tobramycin per ml. Control tests with Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923), and Streptococcus faecalis (ATCC 29212) also produced MICs in the expected ranges.

Tests were performed with 471 isolates selected to provide a significant proportion of strains relatively resistant to amikacin and Sch 21420. Because of a disk supply problem, only 345 of the strains were tested with 30-µg amikacin disks. The total number of isolates tested (and number tested with 30-µg amikacin disks) were as follows: E. coli, 29 (15); Enterobacter spp., 30 (15); Klebsiella spp., 30 (15); Proteus mirabilis, 30 (15); other Proteus spp., 42 (18); Providencia spp., 30 (15); Serratia sp., 33 (15); S. aureus, 60 (60); S. faecalis, 10 (10); Aeromonas hydrophila, 8 (8); Acinetobacter sp., 18 (9); P. aeruginosa, 131 (130); and other Pseudomonas spp., 20 (20). Five tests with 10-µg Sch 21420 disks and two tests with 30-µg Sch 21420 disks were considered unsatisfactory and were excluded from the calculations.

RESULTS AND DISCUSSION

Data accumulated during this study are summarized in Table 1. Tests with P. aeruginosa demonstrated no tendency to deviate from the composite results obtained from all strains that were tested. The method of least squares was utilized to calculate regression formulas, using all test results with zones >6 mm and with MICs ranging from 0.25 to 64 µg/ml (excluding only "off scale" endpoints). With 10-µg amikacin disks, the slope of the regression line essentially paralleled that obtained with 30-µg amikacin disks. However, the intercepts differed by about one MIC dilution interval. Changing disk potency from 10 to 30 µg increased the average zone diameter by about 3 mm. Increasing the potency of Sch 21420 disks from 10 to 20 µg also increased the average zone diameter by about 3 mm. Zones with 30-µg Sch 21420 disks were about 5 mm larger than those observed with 10-µg disks. The slopes of the regression lines calculated for tests with Sch 21420 were essentially the same as those calculated for amikacin, but the intercepts were different for each disk potency. Presumably, Sch 21420 diffuses through the agar gel somewhat more rapidly than does amikacin.

With the regression formulas listed in Table 1, MIC correlates can be calculated for any given zone diameter. The MIC correlating to a 6-mm zone (no inhibition around a 6-mm disk) is the theoretical maximum MIC level that could be detected with each disk potency. However, experience has demonstrated that zones less than 10 or 12 mm in diameter tend to yield relatively unreliable results. Thus, the MIC correlating to a 10-mm zone should serve as a more practical estimate of the maximal MIC level that can be detected with a given disk potency. Since resistance to amikacin and resistance to Sch 21420 are both defined as an MIC ≥32 µg/ml, the 10-mm zone correlate should exceed 32 µg/ml. A 30-µg amikacin disk is clearly preferable to a 10-µg disk, and a 20-µg Sch 21420 disk yielded MIC correlates similar to those calculated for tests with 30-µg amikacin disks.

Figure 1 summarizes the results of tests with Sch 21420 disks. By using the error rate-bounded method of Metzler and De Haan (5), we selected interpretive zone standards which yielded a minimum number of major (≤1%), very major (≤1%), and minor (≤5%) discrepancies with MIC categories (≤16 µg/ml for susceptible and ≥32 µg/ml for resistant). Zone size breakpoints of ≤9 and ≥12 mm for 10-µg disks, ≤12 and ≥15 mm for 20-µg disks, and ≤14 and ≥17 mm for 30-µg disks were selected. Such zone standards provided an acceptable error rate of ≤2% major and very major discrepancies combined. The regression formulas calculated for all three disk potencies also showed that the zone size breakpoints for the resistant category correlated with an MIC of ≥32 µg/ml. However, the zone size breakpoints for the susceptible category actually correlated with an MIC of ≤8 µg/ml, as extrap-
<table>
<thead>
<tr>
<th>Disk content (no. of tests)</th>
<th>Mean (minimum-maximum) zone diameter (mm) for MIC level (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 mm</td>
</tr>
<tr>
<td><strong>All tests (244)</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Table 1. Summary of disk diffusion susceptibility test results with amikacin and Sch 21(63) disks of various potencies.**

- *Y, MIC (μg/mL); X, zone diameter (mm) expressed as the MIC (μg/mL) + 9 μg/mL.
- Correlation coefficients varied from 0.8 to 0.91 for the different sets of data.
Strains with MICs of 16 µg/ml in the intermediate category. The width to the intermediate category could also be reduced by adding 1 mm to the breakpoint for the resistant category without adding a significant number of major interpretive discrepancies. Such changes would increase the number of minor discrepancies (intermediate versus susceptible or resistant). Modifications of interpretive criteria should be held as possible options that can be considered after more clinical experience with Sch 21420 has been gathered which specifically documents the response of patients infected with organisms that require 16 µg/ml for inhibition.

With appropriate zone size interpretive standards, Sch 21420 tests with 10-, 20- and 30-µg disks were all reasonably accurate. However, the breakpoints for 10-µg disks involve relatively small zones of inhibition; consequently, more potent disks are preferred (8, 15). The regression formulas demonstrate that the 20-µg Sch 21420 disks give zones about the same size as those given by 30-µg amikacin disks; thus, the same interpretive standards should apply to either disk. However, the assumption was not supported by the data in Fig. 1. Because of the broad distribution of endpoints on either side of the regression line, interpretive zone standards were not based upon the regression analysis alone. When the error rate-bounded method was used to minimize interpretive discrepancies, zone standards for 30-µg Sch 21420 disks were the same as those for 30-µg amikacin disks.

Amikacin disk test data are summarized in Fig. 2. The interpretive zone standards of Washington et al. (15) appear to be appropriate. Regression analysis of the 30-µg disk data suggested that the zone standards of Washington et al. (15) should be reduced by 1 mm (to ±13 mm and ±16 mm). However, such a minor change would not significantly improve the correlation with dilution tests. Consequently, modification of the current zone standards (10, 15) cannot be justified. The vast majority of strains that produced zones of ≥17 mm had MICs of ≤8 µg/ml, and 13 of 25 strains with MICs of 16 µg/ml gave intermediate zones of 15 or 16 mm. All strains that were susceptible (zones of ≥12 mm) with the 10-µg disk test were inhibited by 16 µg/ml or less. The 30-µg disk would be preferred, especially if susceptibility were defined as an MIC of ≤8 µg/ml. Neither disk accurately categorized all of the strains which required 16 µg/ml for inhibition.

To determine whether separate disk tests are necessary for Sch 21420, we first compared the activity of Sch 21420 with that of amikacin. A direct comparison of 471 pairs of MIC values

**FIG. 1.** Sch 21420 microdilution MICs versus zone diameters with disks containing 10, 20, or 30 µg of Sch 21420. Numbers represent the number of strains in each category. Interpretive breakpoints and regression lines are superimposed. Regression analysis included all data with strains giving MICs ranging from 0.25 to 64 µg/ml and zones of inhibition >6 mm in diameter.

olated from the regression lines. Examination of the scattergrams (Fig. 1) demonstrates that the majority of strains with MICs of 16 µg/ml were actually susceptible by disk methods, in spite of the calculated MIC correlates. MICs of 16 µg/ml were obtained with only 11% of our isolates (45 P. aeruginosa, 5 Proteus sp., 3 Providencia sp., and 1 Serratia sp.). Only 7% of the isolates had amikacin MICs of 16 µg/ml (21 P. aerugina

osa and 4 Proteus sp.).

Future clinical experience with Sch 21420 might suggest that the susceptible category should be limited to strains with MICs of ≤8 µg/ml and that strains with MICs of 16 µg/ml are more appropriately placed in an intermediate category. In that event, the zone size standards described above could be modified by adding 2 mm to the breakpoint for the susceptible category. That would include a larger proportion of

Vol. 18, 1980

Sch 21420 AND AMIKACIN DISK TESTS

619
revealed a correlation coefficient of 0.91, with 95% of the strains giving MICs which were the same or within one doubling dilution interval and 99.6% of the MICs being within ±2 dilution intervals. However, interpretive discrepancies were observed (Table 2), especially with those strains which required 16 μg/ml for inhibition. Amikacin is somewhat more active than Sch 21420; thus, most strains which were inhibited by 16 μg of amikacin per ml were resistant (MIC, ≥32 μg/ml) to Sch 21420. The vast majority of strains included in this study were clearly resistant or susceptible to both drugs. Both antimicrobial agents have been found to be resistant to most of the known aminoglycoside-inactivating enzymes produced by some resistant variants (3, 6, 11, 13).

Interpretive zone standards for 30-μg amikacin disks are the same as those for 30-μg Sch 21420 disks. Since the two drugs have comparable spectra of activity, tests with disks containing one drug might be expected to be capable of predicting susceptibility to the other. Figure 2 includes an estimate of how well zones with 30-μg amikacin disks predict susceptibility to Sch 21420: such results may be compared with the 30-μg Sch 21420 disk test results in Fig. 1. Sch 21420 susceptibility was predicted with 30-μg amikacin disks almost as well as with 30-μg Sch 21420 disks. Consequently, the class concept of disk testing is clearly applicable and routine tests with Sch 21420 disks may not be needed. However, tests with Sch 21420 disks might be appropriate under special circumstances, i.e., during future clinical trials. For those purposes, 30-μg Sch 21420 disks are recommended with zone standards of ≤14 mm for the resistant category (MIC >32 μg/ml) and ≤17 mm for the susceptible category (MIC ≤16 μg/ml).

**LITERATURE CITED**


7. Minshaw, B. H., H. M. Pollock, F. D. Schoenknicht, and J. C. Sherris. 1977. Emergence in a burn center of populations of bacteria resistant to gentamicin, tobramycin and amikacin: evidence for the need for changes.


