JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1984, p. 409–412
0095-1137/84/090409-04$02.00/0

Vol. 20, No. 3

Disk Diffusion Testing, Quality Control Guidelines, and Antimicrobial Spectrum of HR810, a Fourth-Generation Cephalosporin, in Clinical Microbiology Laboratories

R. N. JONES,[1]* C. THORNSBERRY,[2] A. L. BARRY,[3] L. AYERS,[4] S. BROWN,[5] J. DANIEL,[1] P. C. FUCHS,[6] T. L. GAVAN,[7] E. H. GERLACH,[8] J. M. MATSEN,[9] L. B. RELLER,[10] AND H. M. SOMMERS[11]

Kaiser-Permanente Regional Laboratory, Clackamas, Oregon 97015[1]; Centers for Disease Control, Atlanta, Georgia 30333[2]; The Clinical Microbiology Institute, Tualatin, Oregon 97062[3]; Department of Pathology, Ohio State University, Columbus, Ohio 43210[4]; Good Samaritan Hospital, Portland, Oregon 97210[5]; St. Vincent Hospital and Medical Center, Portland, Oregon 97225[6]; The Cleveland Clinic Foundation, Cleveland, Ohio 44106[7]; St. Francis Hospital, Wichita, Kansas 67214[8]; Department of Pathology, University of Utah, Salt Lake City, Utah 84132[9]; Department of Medicine, University of Colorado, Denver, Colorado 80220[10]; and Department of Pathology, Northwestern University, Chicago, Illinois 60611[11]

Received 2 April 1984/Accepted 5 June 1984

HR810 is a new, very broad-spectrum cephalosporin with significant activity against members of the family *Enterobacteriaceae*, pseudomonads, gram-positive cocci, and anaerobes that is generally greater than the third-generation cephalosporins (99.6% of 4,128 clinical facultative enteric isolates were inhibited by ≤8.0 μg of HR810 per ml). Tests and statistical methods to establish in vitro antimicrobial susceptibility test criteria favor tentative breakpoints of ≥18 mm (≤8.0 μg/ml) as susceptible and ≤14 mm (≥32 μg/ml) as resistant. This provides a 93.7 to 98.3% absolute interpretive accuracy. Several preliminary ranges for zone sizes obtained with quality control organisms are proposed for the 30-μg HR810 disk diffusion test used during the clinical trials.

HR810 or 1-[[(6R,7R)-7-[2-amino-4-thiazolyl)-glyoxyl-amido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-en-3-yl] methyl]-6,7-dihydro-5H-cyclopenta-[b]pyridinium hydroxide, 7-(Z)-(O-methyl-oxim) sulfate is a new 3′ pyridium-substrated cefotaxime-like cephalosporin (3, 4, 9, 10, 13–15). Its reported antimicrobial spectrum includes virtually all gram-negative bacilli, *Staphylococcus* spp., *Streptococcus* spp., *Neisseria* spp., and *Haemophilus influenzae*. It also has moderate or marginal activity against anaerobes, enterococci, and methicillin-resistant *Staphylococcus aureus* (3, 4, 9, 10, 13–15). In this report we expand an earlier study (9) to include: (i) data on the HR810 spectrum against 7,768 routine clinical isolates with reference in vitro methods, (ii) disk diffusion test interpretive criteria for the 30-μg HR810 disk, and (iii) the disk diffusion test quality control guidelines with three lots of commercially prepared disks.

MATERIALS AND METHODS

Antimicrobial agents. HR810 was supplied as a sulfate laboratory standard powder by Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J. The other comparative drugs were kindly provided by their respective manufacturers. The microorganisms in the first phase of this three-part evaluation came from four institutions. All strain isolates were processed by routine reference susceptibility tests over a 30- to 60-day interval. Agar dilution tests were performed at Northwestern Memorial Hospital, Chicago, Ill. (H. M. Sommers) and St. Francis Hospital, Wichita, Kans. (E. H. Gerlach); broth microdilution tests were performed at the Ohio State University Medical Center, Columbus, Ohio (L. W. Ayers) and The Cleveland Clinic Foundation, Cleveland, Ohio (T. L. Gavan).

* Corresponding author.

Organisms tested. In the second phase of this study, 508 recent clinical isolates, representative of various bacterial pathogens, were collected from six geographically separate medical centers, and additional isolates were contributed by the Centers for Disease Control, Atlanta, Ga. Other contributors included The Cleveland Clinic Foundation; Northwestern Memorial Hospital; University of California, Davis, Medical Center, Sacramento, Calif.; St. Francis Hospital; Kaiser-Permanente Regional Laboratory, Clackamas, Oreg.; and St. Vincent Hospital and Medical Center, Portland, Oreg. These strains included 73 *Staphylococcus aureus* (29 resistant to methicillin), 17 *Staphylococcus epidermidis* (4 resistant to methicillin), 18 *Streptococcus faecalis*, 20 *Streptococcus pyogenes*, 20 *Streptococcus agalactiae*, 20 *Streptococcus pneumoniae* (1 strain with a penicillin minimal inhibitory concentration [MIC] of 0.5 μg/ml), 15 *Acinetobacter calcoaceticus* subspecies, 50 *Pseudomonas aeruginosa*, 31 other *Pseudomonas* spp., and 244 *Enterobacteriaceae* from 16 species and 10 genera.

QC study. The third phase was a disk diffusion quality control (QC) evaluation. The study design and applied statistical methods have been reported before (1, 5). Participants in this phase included A. L. Barry, S. Brown (Good Samaritan Hospital, Portland, Oreg.), P. C. Fuchs (St. Vincent Hospital and Medical Center), T. L. Gavan, E. H. Gerlach, J. M. Matsen (University of Utah, Salt Lake City, Utah), and L. B. Reller (University of Colorado, Denver, Colo.).

Susceptibility testing. All agar and broth microdilution MIC susceptibility tests were performed as outlined by the National Committee for Clinical Laboratory Standards (NCCLS) (11). In the first phase of the study, each clinical laboratory tested HR810, ceftazidime, and cefotaxime (at 0.12, 0.5, 1.0., 2.0, 4.0, 8.0, and 32 μg/ml). In the disk-regression evaluations, HR810 was tested in two-fold dilu-

tions ranging from 256 to 0.0005 μg/ml, diluted in cation-supplemented Mueller-Hinton broth, and then dispensed (Prepared Media Laboratory, Tualatin, Oreg.) into microdilution trays along with dilutions of other control drugs, cefotaxime and ceftazidime. Disk diffusion tests were performed by the method of Bauer et al. (2) as recently modified by the NCCLS (12). Disks containing 30 μg of HR810 were prepared in our laboratories and 30-μg cefotaxime disks were obtained from BBL Microbiology Systems, Cockeysville, Md. Three lots of commercially prepared HR810 disks (Difco Laboratories, Detroit, Mich.) were used in the QC phase of this study.

RESULTS AND DISCUSSION

The in vitro antimicrobial spectrum of HR810 is summarized in Table 1, which includes the results of testing 7,768

TABLE 1. Cumulative percentage of routine clinical bacterial isolates inhibited by increasing concentrations of HR810

Organism (no. tested)	Cumulative % inhibited by HR810 concn (μg/ml) of:						
	≤0.12	0.5	1.0	2.0	4.0	8.0	32
Citrobacter diversus (58)	89.7	100.0					
Citrobacter freundii (160)	73.1	83.1	93.1	99.4	100.0		
Enterobacter aerogenes (178)	75.3	96.6	97.8	98.3	98.9	100.0	
Enterobacter agglomerans (47)	80.9	82.7	91.5	97.9	100.0		
Enterobacter cloacae (354)	78.2	87.0	90.7	94.4	96.3	97.3	100.0
Escherichia coli (1,587)	97.7	98.8	99.2	99.5	99.6	99.8	99.9
Klebsiella spp.[b] (825)	94.4	98.3	99.3	99.9	100.0		
Morganella morganii (132)	85.6	95.5		98.2			99.2
Proteus mirabilis (389)	98.5	99.5				99.7	100.0
Proteus vulgaris (40)	70.0	90.0	100.0				
Providencia spp.[c] (44)	72.7	88.6	93.2	97.7			
Salmonella spp. (24)	95.8			100.0			
Serratia marcescens (240)	83.3	93.3	95.8	98.8		99.2	99.6
Other enteric bacilli[d] (50)	88.0	94.0			96.0	100.0	
Acinetobacter antiratus (75)		10.7	32.0	72.0	88.0	94.7	96.0
Acinetobacter lwoffii (15)	13.3	60.0	80.0	100.0			
Aeromonas hydrophila (15)	93.3	100.0					
Pseudomonas aeruginosa (972)	1.6	3.6	9.2	33.8	62.1	80.8	95.2
Pseudomonas maltophila (31)							9.7
Pseudomonas spp.[e] (21)	9.5	14.3	23.8	61.9	71.4	81.0	85.7
Miscellaneous nonenteric bacilli							
Group I[f] (29)	75.9	89.7	96.6		100.0		
Group II[g] (18)	5.6					22.2	50.0
Staphylococcus aureus (856)	0.4	52.5	98.1	99.3	99.9		100.0
Coagulase-negative *Staphylococcus* spp.[h] (576)	14.1	51.4	70.0	82.6	89.9	93.9	96.4
Streptococcus faecalis (895)	1.0	1.6	2.6	4.9	24.6	65.3	95.0
Streptococcus bovis (30)	76.7	80.0	83.3			90.0	96.7
Other serogroup D *Streptococcus* spp. (46)	2.2	4.3	8.7	13.0	26.1	34.8	58.7
Alpha-hemolytic *Streptococcus* spp. (54)	83.3	94.4	98.1		100.0		
Listeria monocytogenes (7)			14.3	28.6	57.1	85.7	100.0

[a] A total of 7,768 strains from four medical centers were tested over 30 to 60 days.
[b] Includes 131 *Klebsiella oxytoca* and 694 *Klebsiella pneumonia*.
[c] Includes 13 *Providencia rettgeri* and 31 *Providencia stuartii*.
[d] Includes 11 additional species.
[e] Includes eight *Pseudomonas fluorescens*, three *Pseudomonas cepacia*, two *Pseudomonas paucimobilis*, and one strain each of *Pseudomonas stutzeri* and two *Pseudomonas* spp. NOS.
[f] Includes 1 *Branhamella catarrhalis*, 1 *Campylobacter fetus* ssp. *jejuni*, 4 *Eikenella corrodens*, 1 *Kingella denitrificans*, 4 *Moraxella* spp., 2 *Pasteurella multocida*, 3 *Pleisemonas shigelloides*, 1 Centers for Disease Control group II-F, 1 Centers for Disease Control group VE-2, 1 *Flavobacterium* spp., and 10 *Haemophilus* spp.
[g] Includes HR810-resistant (majority of organisms in each species with MICs of ≥32 μg/ml) organisms from the *Achromobacter* and *Alkaligenes* genera.
[h] The tabulated coagulase-negative species include 526 *Staphylococcus epidermidis* NOS, 21 *Staphylococcus warneri*, 10 *Staphylococcus hominis*, 9 *Staphylococcus saprophyticus*, 4 *Staphylococcus capitis*, 3 *Staphylococcus simulans*, 2 *Staphylococcus intermedius*, and 1 *Staphylococcus cohnii*. Note that 11 of 20 strains of *Staphylococcus haemolyticus* (untabulated) had HR810 MICs of >8.0 μg/ml.

TABLE 2. Quality control parameters for tests with 30-μg HR810 and cefotaxime disks[a]

Control strain and antimicrobial disk	No. of zones recorded	Central tendency		Overall range[b]	Control limits (% of tests within limits)		
		Mean	Median		Mean ± 2 SD[c]	Median ± 1/2 range	Proposed limits
Escherichia coli ATCC 25922							
HR810	1,332	32.0	32.0	26–38	29–35	29–35	29–35
Cefotaxime	443	32.2	32.0	26–38	29–36	29–35	29–35[d]
Staphylococcus aureus ATCC 25923 HR810	1,335	28.8	29.0	24–34	25–32	26–32	26–32
Pseudomonas aeruginosa ATCC 27853 HR810	1,335	26.8	26.0	21–36	23–31	23–29	23–29

[a] Results are a summary of a nine-laboratory collaborative study.
[b] Minimum to maximum.
[c] SD, Standard deviations; values rounded off to the nearest whole number.
[d] Identical to that published in Table 3 of reference 12.

consecutive clinical isolates in four clinical microbiology laboratories. The cumulative percentage of members of the *Enterobacteriaceae* inhibited at ≤8.0 μg/ml was 99.6%, a finding very similar to that reported earlier with a stock culture collection (9). These data are significantly better than the two comparison drugs, ceftazidime and cefotaxime, used in this phase of the study (data not shown). Only 18 *Enterobacteriaceae* strains from *Enterobacter cloacae*, *Escherichia coli*, *Morganella morganii*, *Proteus mirabilis*, *Providencia stuartii*, and *Serratia marcescens* were HR810-resistant (MICs of ≥32 μg/ml). Nine of the isolates were *Enterobacter cloacae*, with a prevalence of 2.5% HT810 resistance compared to 14.1% for ceftazidime and 16.5% for cefotaxime. HR810 MICs against the *Pseudomonas aeruginosa* isolates (50% MIC [MIC_{50}], 4.0 μg/ml; MIC_{90}, 32 μg/ml) were most comparable to those reported for cefoperazone (6, 7, 9, 14) and slightly higher than those published for ceftazidime (7, 9, 14) and aztreonam (9). The majority of *Pseudomonas* spp. that were not *Pseudomonas aeruginosa* were also susceptible (MIC_{50}, 2.0 μg/ml) to HR810, except *Pseudomonas maltophilia*. HR810 was comparable to first-generation cephalosporins against *Staphylococcus* spp. (3, 9, 10, 13, 14) and was the most potent cephalosporin tested by our group against all *Streptococcus* spp. (3, 9, 13, 14).

The HR810 activity against the enterococci (MIC_{50}, 8.0 μg/ml) and methicillin-resistant *Staphylococcus aureus* (MIC_{50}, 16 μg/ml) would contribute to susceptibility test interpretive errors if these organisms are routinely tested against these cephalosporins (3, 9, 13, 14). An unexpected finding was the 55% of *Streptococcus haemolyticus* coagulase-negative strains that had resistant HR810 MICs (>8.0 μg/ml).

The disk QC evaluation produced the results shown in Table 2. Cefotaxime was used as an internal control of procedures, and zones obtained were compared to established NCCLS guidelines. The HR810 disk zones from the nine contributing laboratories suggest several QC guidelines, depending on the statistical analysis employed. The mean ± two standard deviations could not be strictly applied to all data because the HR810 zones were abnormally distributed for the *Pseudomonas aeruginosa* QC strain tested. The medians technique reported by Gavan et al. (5) produced very similar and applicable ranges. The proposed limits were those in which the median zone diameter population statistics are used, e.g., for *Escherichia coli* ATCC 25922, 29 to 35 mm; for *Staphylococcus aureus* ATCC 25923, 26 to 32 mm; and for *Pseudomonas aeruginosa*, 23 to 29 mm. The cefotaxime 30-μg disk control produced statistics confirming the NCCLS published limits for *Escherichia coli* ATCC 25922 (28 to 35 mm) with 5.4 and 1.1% of observed zone above and below the established limits, respectively. The modal HR810 MICs for the commonly used dilution test QC strains were: *Escherichia coli* ATCC 25922, ≤0.12 μg/ml; *Staphylococcus aureus* ATCC 25923, 0.5 μg/ml; *Pseudomonas aeruginosa* ATCC 27853, 2.0 μg/ml; *Streptococcus faecalis* ATCC 29212, 4.0 μg/ml; and *Staphylococcus aureus* ATCC 29213, 0.5 to 1.0 μg/ml.

Figure 1 shows the scattergram (508 strains) of HR810 MICs determined by the NCCLS broth microdilution method plotted against HR810 30-μg concentration disk zone diameters determined by the NCCLS disk diffusion technique (11, 12). Only 16 isolates had HR810 MICs of ≥32 μg/ml, all having zone diameters of ≤14 mm. Twenty-eight bacteria had MICs of 16 μg/ml; 19 were methicillin-resistant staphylococci and *Streptococcus faecalis*. Because of the unusually high antimicrobial activity of HR810, nearly all clinically relevant strains had MICs of ≤8.0 μg/ml. Of the tested isolates, 260 (51.2%) had an HR810 MIC of ≤0.06 μg/ml. This fact limits the application of the regression analysis (equation for an MIC range of 0.004 to 256 μg/ml; $Y = 18.2 - 0.40X$, with a correlation coefficient of 0.85). The error-rate

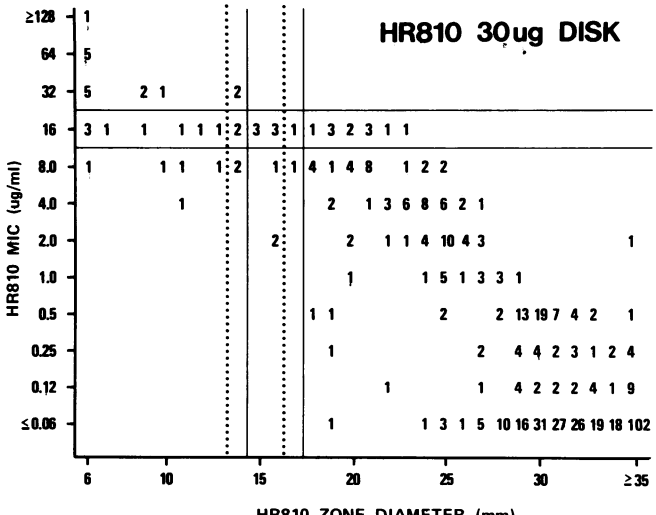

FIG. 1. Scattergram comparing the HR810 MICs determined by the NCCLS reference broth microdilution method (11) to the HR810 30-μg disk zone diameters, using the standardized disk diffusion procedure (2, 12). A total of 508 strains were plotted.

bounding technique also was slightly compromised; however, this method establishes a $Zs = \geq 21$ mm (all data) or $Zs = \geq 18$ mm (excluding methicillin-resistant staphylococci and enterococcal strains) by using a susceptible MIC breakpoint of ≤ 8.0 μg/ml. If the latter zone was utilized with a 3-mm intermediate category (resistant, ≤14 mm) the interpretive errors would be 0.0% very major (false susceptible), 1.4% major (false resistant), and 4.9% minor errors. By lowering each breakpoint by 1 mm, the rates become 0.0% very major, 1.0% major, and 5.3% minor errors, or a 94.7% absolute agreement. When the methicillin-resistant staphylococci and enterococcal strains are eliminated from the analysis, the error rates are markedly reduced to 0.0% combined major and very major and 2.1% minor errors for the ≥17-mm and ≤13-mm criteria and 0.0% very major, 0.2% major, and 1.5% minor errors for the ≥18-mm and ≤14-mm criteria. The absolute agreement for these data (98.7%) favors the use of ≥18 mm as susceptible and ≤14 mm as resistant, a result very similar to that reported for other cephalosporins and similar new β-lactams (7, 12).

In summary, we presented information concerning the HR810 antimicrobial susceptibility testing from clinical microbiology laboratories, including preliminary recommendation for in vitro disk and dilution testing and the QC of the disk diffusion method. We confirm that HR810 is an outstanding new β-lactam possessing a very wide spectrum of activity and particularly high potency against gram-positive cocci and nonenteric gram-negative bacilli (3, 4, 6–10, 13). We recommend tentative MIC breakpoints for standardized in vitro antimicrobial susceptibility tests (11, 12) of ≤ 8.0 μg/ml (≥18 mm) as susceptible and ≥32 μg/ml (≤14 mm) as resistant.

LITERATURE CITED

1. **Barry, A. L., T. L. Gavan, and R. N. Jones.** 1983. Quality control parameters for susceptibility tests with 30-μg netilmicin disks. J. Clin. Microbiol. **18**:1051–1054.
2. **Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disc method. Am J. Clin. Pathol. **45**:493–496.
3. **Bauernfeind, A.** 1983. Susceptibility of gram-positive aerobic cocci to the new cephalosporin HR810. Eur. J. Clin. Microbiol. **2**:354–355.
4. **Cullman, W., W. Opferkuch, and M. Stieglitz.** 1983. Relation between beta-lactamase production and antimicrobial activity: comparison of the new compound HR810 with cefotaxime. Eur. J. Clin. Microbiol. **2**:350–352.
5. **Gavan, T. L., R. N. Jones, A. L. Barry, P. C. Fuchs, E. H. Gerlach, J. M. Matsen, L. B. Reller, C. Thornsberry, and L. D. Thrupp.** 1981. Quality control limits for ampicillin, carbenicillin, mezlocillin, and piperacillin disk diffusion susceptibility tests: a collaborative study. J. Clin. Microbiol. **14**:67–72.
6. **Jones, R. N., and A. L. Barry.** 1983. Cefoperazone sodium: a review of the in vitro characteristics, antimicrobial spectrum, beta-lactamase stability and enzyme inhibition. Rev. Infect. Dis. **5**(Suppl.):S108–S126.
7. **Jones, R. N., A. L. Barry, C. Thornsberry, E. H. Gerlach, P. C. Fuchs, T. L. Gavan, and H. M. Sommers.** 1981. Ceftazidime, a pseudomonas-active cephalosporin: in vitro antimicrobial activity evaluation including recommendations for disk diffusion susceptibility tests. J. Antimicrob. Chemother. **8**(Suppl. B):187–211.
8. **Jones, R. N., and C. Thornsberry.** 1982. Cefotaxime (HR756): a review of the in vitro antimicrobial properties and spectrum of activity. Rev. Infect. Dis. **4**(Suppl.):S300–S315.
9. **Jones, R. N., C. Thornsberry, and A. L. Barry.** 1984. In vitro evaluation of HR810, a new wide-spectrum aminothiazolyl α-methoxyimino cephalosporin. Antimicrob. Agents Chemother. **25**:710–718.
10. **Machka, K., and I. Braveny.** 1983. In vitro activity of HR810, a new broad-spectrum cephalosporin. Eur. J. Clin. Microbiol. **2**:345–349.
11. **National Committee for Clinical Laboratory Standards.** 1982. Tentative standard M7-T. Standard methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
12. **National Committee for Clinical Laboratory Standards.** 1983. Tentative standard M2-T3. Performance standards for antimicrobic disk susceptibility tests, 3rd ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.
13. **Siebert, G., N. Klesel, M. Limbert, E. Schrinner, K. Seeger, I. Winkler, R. Lattrell, J. Blumback, W. Durckheimer, K. Fleischmann, R. Kirrstetter, B. Mencke, B. C. Ross, K.-H. Scheunemann, W. Schwab; and M. Wieduwilt.** 1983. HR810, a new parenteral cephalosporin with a broad antibacterial spectrum. Arzneim. Forsch. **33**:1084–1086.
14. **Siebert, G., M. Limbert, I. Winkler, and T. Dick.** 1983. The antibacterial activity in vitro and β-lactamase stability of the new cephalosporin HR810 in comparison with five cephalosporins and two aminoglycosides. Infection **11**:275–279.
15. **Tolxdorff-Neutzling, R. M., and B. Wiedemann.** 1983. HR810, a cephalosporin with low affinity for *Enterobacter cloacae* beta-lactamase. Eur. J. Clin. Microbiol. **2**:352–354.