Preliminary Disk Diffusion Susceptibility Testing Criteria for Cefdaloxime (RU29246, HR-916 Metabolite), a New Orally Administered Cephalosporin

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Cefdaloxime (formerly RU29246; Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.) is a new active component of the HR-916 ester, was tested by dilution and two disk (10- and 30-µg) diffusion susceptibility tests against 391 clinical isolates. Interpretive criteria were proposed for three potential MIC breakpoints of ≤1, ≤2, and ≤4 µg/ml. Analyses by regression line and error rate bounding method minimized (very major) errors and produced ≥90% absolute interpretive agreement between susceptibility test methods. The ≤2-µg/ml breakpoint seemed optimal when 10-µg disks and the available human pharmacokinetics were used. The following inhibition zone diameter criteria were proposed: susceptible, ≥19 mm; resistant, ≤15 mm. These recommendations for clinical trials should remain tentative until additional information about cefdaloxime formulations, pharmacokinetics, and patient outcomes can be correlated with in vitro susceptibility test results.

Cefdaloxime (compound RU29246; Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.) is the active metabolite of the orally administered HR-916 ester. Previously reported antimicrobial susceptibility data indicate that cefdaloxime has a broad spectrum of activity against pathogens that infect outpatients, such as the majority of members of the family Enterobacteriaceae, staphylococci, Streptococcus spp., Neisseria gonorrhoeae, Moraxella catarrhalis, Haemophilus influenzae, and some Acinetobacter spp. strains (7). This spectrum and potency make cefdaloxime generally superior to most currently available oral cephalosporins (3) and most similar to other investigational ester cephalosporin compounds such as BMY-28271, MEI207, cefpodoxime (CS-807), cefetamet (Ro15-8074), and cefteram (Ro19-5247) (3–6, 11). However, the spectra of activity of many of these compounds differ sufficiently to require individual susceptibility tests and applications of breakpoint susceptibility criteria based on the bioavailability of each drug (3). This report summarizes the disk diffusion test (10 and 30 µg) development for cefdaloxime against rapidly growing aerobic bacteria by using the methods and guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (9, 10).

Cefdaloxime was provided by Hoechst-Roussel Pharmaceuticals. Disks containing 10 and 30 µg of cefdaloxime were obtained from Becton-Dickinson Microbiology Systems (Cockeysville, Md.). The MIC tests were performed by following the procedures of NCCLS (9, 10) in cation-adjusted Mueller-Hinton medium, and all disk tests were performed on Mueller-Hinton agar plates. Two disk contents (10 and 30 µg) were used against 391 recent clinical isolates, most of which were originally derived from patient blood cultures at the University of Iowa Hospitals and Clinics. Only organisms that do not require special growth supplements were processed. Neisseria gonorrhoeae, fastidious streptococci, and Haemophilus influenzae strains require specialized procedures and media (unpublished data). Only the enterococci were tested on 5% sheep blood-containing agar to observe a possible enhanced effect of the medium on the potency of cefdaloxime (1). The general strains of groups tested were members of the family Enterobacteriaceae (250 strains, 22 species), Pseudomonas aeruginosa (30 strains), Xanthomonas maltophilia (10 strains), Acinetobacter spp. (10 strains), Enterococcus faecalis (16 strains), and Staphylococcus spp. (75 strains; 13 organisms were oxacillin resistant). Statistical processing of MIC and inhibition zone diameter results were analyzed by the method of least squares and application of the error rate bounding method that minimizes false-susceptible (very major) errors to ≤1% of all tests that are compared (8).

Table 1 summarizes the regression statistics and error rate results after applying three possible cefdaloxime susceptibility MIC breakpoints (≤1, ≤2, and ≤4 µg/ml). Preliminary human pharmacokinetics indicate that any of these options could be applied, depending on the selected clinical dose level (indication directed), frequency of oral use, and interactions with food (2). Therefore, three options covering the range of potential doses, each having acceptable error rate statistics and correlation coefficients (r = 0.89 to 0.91), were presented. For the 10-µg cefdaloxime disk test, the absolute agreement ranged from 88.8% to 91.9%, and 100% of false-resistant (very major) errors were acceptable at ≤1.0%.Figure 1 shows the scattergram and interpretive criteria for the ≤2-µg/ml breakpoint. The false-susceptible errors were contributed by three groups of organisms (Enterobacter cloacae, Serratia marcescens, and oxacillin-resistant staphylococci) in various numbers, with all errors for the ≤4-µg/ml breakpoint being Serratia marcescens (three strains, 0.8% error). A wide variety of bacteria caused the false-resistant errors (error range, 0.3 to 1.3%), including Enterobacter aerogenes, Enterobacter cloacae, Morganella morgani, Proteus vulgaris, Providencia stuartii, Shigella sonnei, and Staphylococcus epidermidis.

The 30-µg cefdaloxime disk also performed well as a diagnostic reagent, having an absolute agreement between

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TABLE 1. Cefdaloxime interpretive criteria for 10- and 30-µg disks by using susceptible breakpoint criteria of ≤1, ≤2, and ≤4 µg/ml

<table>
<thead>
<tr>
<th>Disk content</th>
<th>Interpretive zone (mm) criteria (correlate MIC [µg/ml])</th>
<th>Error rates (%)</th>
<th>% Absolute agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Moderately susceptible</td>
<td>Resistant</td>
</tr>
<tr>
<td>10 µg</td>
<td>≥23 (≤1)&quot;f&quot;</td>
<td>20–22 (2)</td>
<td>≤19 (&gt;2)</td>
</tr>
<tr>
<td></td>
<td>≥18 (≤2)&quot;f&quot;</td>
<td>15–17 (4)</td>
<td>≤14 (&gt;4)</td>
</tr>
<tr>
<td></td>
<td>≥19 (≤2)&quot;f&quot;</td>
<td>16–19 (4)</td>
<td>≤15 (&gt;4)</td>
</tr>
<tr>
<td></td>
<td>≥16 (≤4)&quot;f&quot;</td>
<td>13–14 (8)</td>
<td>≤12 (&gt;8)</td>
</tr>
<tr>
<td></td>
<td>≥14 (≤4)&quot;f&quot;</td>
<td>11–13 (8)</td>
<td>≤10 (&gt;8)</td>
</tr>
<tr>
<td>30 µg</td>
<td>≥27 (≤1)&quot;f&quot;</td>
<td>24–26 (2)</td>
<td>≤23 (&gt;2)</td>
</tr>
<tr>
<td></td>
<td>≥26 (≤1)&quot;f&quot;</td>
<td>23–25 (2)</td>
<td>≤22 (&gt;2)</td>
</tr>
<tr>
<td></td>
<td>≥23 (≤2)&quot;f&quot;</td>
<td>20–22 (4)</td>
<td>≤19 (&gt;4)</td>
</tr>
<tr>
<td></td>
<td>≥20 (≤4)&quot;f&quot;</td>
<td>17–19 (8)</td>
<td>≤16 (&gt;8)</td>
</tr>
<tr>
<td></td>
<td>≥18 (≤4)&quot;f&quot;</td>
<td>15–17 (8)</td>
<td>≤14 (&gt;8)</td>
</tr>
</tbody>
</table>

"f" A total of 391 strains were tested with each disk.
"d" Very major, false-susceptible result by disk diffusion test; major, false-resistant result by disk diffusion test; minor, intermediate result by one of the methods being compared.
"e" Interpretive zone calculated by the error rate bounding method (8).
"f" Interpretive zone calculated by the regression formula y = 15.4 - 0.28x (r = 0.91).
"g" Interpretive zone calculated by the regression formula y = 15.5 - 0.25x (r = 0.89).  
  
methods of 86.7 to 92.1% (Table 1). The lowest agreement between methods was observed for the ≤1-µg/ml MIC breakpoint, and the best agreement between methods was for higher breakpoints of ≤2 or ≤4 µg/ml. Figure 2 shows the scattergram for the 30-µg disk zone diameters and MICs and the ≤2-µg/ml breakpoint criteria. The false-susceptible error rate was only 1.0%, with the discrepancies contributed by the same isolates that produced a similar error for the 10-µg disk test results. However, with the ≤4-µg/ml breakpoint (one option), very major errors were exclusively produced by Serratia marcescens (nine strains; 2.3% error [unacceptable]).

The final choice of the best cefdaloxime breakpoint for clinical use will ultimately depend on the selected drug formulation dose and the pharmacokinetics of the dose. Breakpoints of ≤1 or ≤2 µg/ml would be best served by a 10-µg disk diffusion test and the ≤4-µg/ml susceptibility breakpoint by the 10- or 30-µg disk diffusion test. For the lowest number of clinical strains tested, cefdaloxime MICs were 2 µg/ml (5.1% of the study collection), and cefdaloxime

FIG. 1. Scattergram comparing cefdaloxime (RU29246) MICs and inhibition zone diameters obtained with 10-µg disks. Criteria for the ≤2-µg/ml MIC breakpoint are plotted for the error rate (broken vertical lines) and regression line (solid vertical lines) calculations. Absolute agreement between method results was 90.8 to 92.4%.
MICs were 4 μg/ml for only 7.1% of strains tested. Cefdaloxime MICs of 2 or 4 μg/ml were most likely to be found for >10% of *Citrobacter freundii*, *Enterobacter* spp., *Morganella morganii*, *Proteus vulgaris*, acinetobacters, and some oxacillin-resistant *Staphylococcus* spp. (7). These facts indicate that these potential breakpoints are highly usable because of the reduced probability of occurrence of significant interpretive errors among the clinically indicated species (7).

Several investigator-prepared disks were also tested in replicate (five determinations) against NCCLS-recommended quality control strains. The results indicated that typical *Escherichia coli* and *Staphylococcus aureus* strains produced adequate zones (25 to 31 mm) around 5-, 10- and 30-μg disks. In contrast, the enterococci and *Pseudomonas aeruginosa* quality control strains produced zones of inhibition of ≤10 mm. However, when the *Enterococcus faecalis* ATCC 29212 strain was tested in the presence of 5% sheep blood cells, it had a significantly increased zone of inhibition, a finding consistent with other cephems with a similar structure (1).

Cefdaloxime appears to possess a good balance of activity against those gram-positive and -negative aerobic pathogens that cause infections of the respiratory, cutaneous, urinary, and genital tracts in outpatients (7). Among the oral cephalosporin ester compounds, only cepodoxime (3, 4), BMY-28232 (5), and ME1206 (11) offer a comparable wide spectrum. These proposed disk diffusion susceptibility testing criteria will await final selection of clinical formulations, publication of results more comprehensive human pharmacokinetic studies, and the ultimate choice between the 10- and 30-μg disk reagent for various areas of the world. Until this information is forthcoming, the criteria must be considered preliminary.

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REFERENCES


