Brief reports

Tentative interpretive criteria and quality control parameters for in-vitro susceptibility testing of Neisseria gonorrhoeae to two fluoroquinolones (PD 131628 and grepafloxacin (OPC 17116))


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The susceptibility of Neisseria gonorrhoeae to PD 131628 and grepafloxacin (OPC 17116) was evaluated by agar dilution and disc diffusion methods. A tentative susceptibility category for both fluoroquinolones included strains for which the MICs are ≤0.06 mg/L and the zones of inhibition are ≥38 mm for PD 131628 and ≥37 mm for grepafloxacin. Quality control studies with N. gonorrhoeae ATCC 49226 suggested that agar dilution MIC limits were 0.002–0.008 mg/L and 0.004–0.03 mg/L for PD 131628 and grepafloxacin, respectively. The zone size limits were 50–58 mm for PD 131628 and 44–52 mm for grepafloxacin.

Introduction

Because of the increasing prevalence of antimicrobial resistance among gonococci there is a need for alternative therapeutic agents which are effective and easy to administer. PD 131628 and grepafloxacin (OPC 17116) are new oral fluoroquinolone antimicrobial agents with broad spectrum in-vitro activity against Gram-positive bacteria, and Gram-negative bacteria including Neisseria gonorrhoeae (Barrett et al., 1992; Neu et al., 1992; Wise, Andrews & Brenwald, 1993; Zenilman et al., 1993).

In this report, we document their in-vitro activity against gonococci, establish interpretive criteria for disc diffusion tests, and determine quality control limits with the standard control strain of N. gonorrhoeae (ATCC 49226) following the protocol outlined by the National Committee for Clinical Laboratory Standards (NCCLS, 1994a).
Materials and methods

Bacterial strains

The comparison between zone diameters and MICs was performed at the Clinical Microbiology Institute (Tualatin, Oregon) using 150 *N. gonorrhoeae* stock isolates which included 23 laboratory-selected strains with relative resistance to the fluoroquinolones (Barry et al., 1993). The 127 remaining gonococci included 23 β-lactamase-producing strains, 27 penicillin-resistant β-lactamase non-producing isolates, and 77 penicillin-susceptible strains. Agar dilution and disc diffusion quality control studies were performed in 5 independent laboratories with *N. gonorrhoeae* ATCC 49226.

Antibiotics

PD 131628 and grepafloxacin were provided as powders of known potency by Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan, USA and Otsuka America Pharmaceutical, Inc., Rockville, Maryland, USA, respectively. The 5 μg grepafloxacin and PD 131628 discs were prepared by Becton-Dickinson Microbiology Systems (BDMS), Cockeysville, MD, USA. For quality control studies, additional discs were prepared by Remel Microbiology Products, Kansas, USA (grepafloxacin); and by Difco Laboratories, Michigan, USA (PD 131628). The 5 μg ciprofloxacin discs were obtained from BDMS.

Susceptibility testing

Agar dilution (MIC range 0.002–1.0 mg/L) and disc diffusion susceptibility tests were performed in accordance with NCCLS procedures (NCCLS, 1993a; b). All tests with *N. gonorrhoeae* isolates were performed on cysteine-free GC agar containing an XV supplement (PML Microbiologicals, Tualatin, Oregon, USA), incubated at 35°C in an atmosphere of 5 to 7% CO₂ and read after 20–24 h. For control purposes, 5 μg ciprofloxacin discs were evaluated in parallel with the study drugs.

Both the disc diffusion and the agar dilution quality control studies were performed in the five laboratories represented by the coauthors, each using a different lot of GC medium from one of three manufacturers and a sixth lot that was common to all laboratories. In the disc diffusion study, two lots of 5 μg PD 131628 and grepafloxacin discs were tested with 35 separate inocula by each investigator. Equivalent results were recorded with the different lots of discs and medium. For each study drug, 350 zone diameters were produced. Control limits were calculated from the median (Gavan et al., 1981) and the mean (Barry et al., 1993) for results from all five laboratories.

In the agar dilution study 30 separate inocula were tested on each of the lots of medium used in the disc study. The media did not differ in their performance and thus all 300 agar dilution MICs were combined. Ciprofloxacin was tested at the same time as a control drug.

Results and discussion

Scattergrams that compare the zones of inhibition around 5 μg PD 131628 or grepafloxacin discs to the appropriate agar dilution MICs are presented in the Figure. Zone diameters for both drugs ranged from 16 to 56 mm. The very large zone sizes
recorded for both antimicrobial agents suggest that less potent discs should be used, though it may be difficult to justify the manufacture of a separate disc for gonococci. Interpretive breakpoints were selected to distinguish the susceptible isolates from those less susceptible. With the exception of one isolate tested against grepafloxacin, this method correctly categorized the susceptibility of all clinical isolates to both fluoroquinolones. For the susceptible clinical strains of gonococci, MICs of both PD 131628 and grepafloxacin were \( \leq 0.06 \) mg/L and zones of inhibition were \( \geq 38 \) mm and \( \geq 37 \) mm in diameter respectively. For one of the laboratory-selected resistant strains, the MIC of grepafloxacin was \( 0.06 \) mg/L with a zone diameter of \( 36 \) mm; all of the other MICs being \( \geq 0.12 \) mg/L. In the absence of resistant clinical isolates to either antimicrobial agent, these tentative interpretive breakpoints do not identify any categories other than susceptible as with other quinolones (NCCLS, 1994b).

For \( N.\ gonorrhoeae \) ATCC 49226, all 300 agar dilution MICs for both study drugs fell into a 3- or 4-dilution range \((0.002–0.008 \) mg/L for PD 131628 and \( 0.004–0.03 \) mg/L for grepafloxacin). The proposed control limits for PD 131628 \((0.002–0.008 \) mg/L) and the published control limits for grepafloxacin \((0.004–0.03 \) mg/L) (NCCLS, 1994b) would include all 300 MICs for both drugs. Ciprofloxacin MICs were \( 0.004 \) or \( 0.008 \) mg/L, all being within the control limits of \( 0.001–0.008 \) mg/L (NCCLS, 1993b).

The results of the disc diffusion susceptibility test for \( N.\ gonorrhoeae \) ATCC 49226 are shown in the Table. Data from all five laboratories were analyzed using the median and the mean \( \pm \) twice the average standard deviation. Both statistical approaches produced identical results. Based on these data, we propose tentative limits of 50–58 mm for PD 131628 and 44–52 mm for grepafloxacin. The 9 mm range suggested for both

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![Figure](image-url) **Figure.** Agar dilution MICs for \( N.\ gonorrhoeae \) compared with zones of inhibition around 5 \( \mu \)g grepafloxacin (a) or 5 \( \mu \)g PD 131628 (b) discs. Vertical and horizontal lines represent proposed interpretive breakpoints for identifying susceptible categories: a resistant category has not been defined.
Table. Results of fluoroquinolone disc tests with *N. gonorrhoeae* ATCC 49226, in five laboratories

<table>
<thead>
<tr>
<th>Zone diameters (mm) around 5 µg discs</th>
<th>PD 131628</th>
<th>grepafloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab A</td>
<td>median</td>
<td>range</td>
</tr>
<tr>
<td>Lab B</td>
<td>54</td>
<td>6</td>
</tr>
<tr>
<td>Lab C</td>
<td>52</td>
<td>9</td>
</tr>
<tr>
<td>Lab D</td>
<td>54</td>
<td>8</td>
</tr>
<tr>
<td>Lab E</td>
<td>57</td>
<td>13</td>
</tr>
<tr>
<td>Target</td>
<td>55</td>
<td>6</td>
</tr>
<tr>
<td>Variability*</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Calculated QC limits*</td>
<td>50 to 58</td>
<td>50 to 58</td>
</tr>
</tbody>
</table>

*Target zone diameters were defined as the all-laboratory median and all-laboratory mean zone diameter.

*The extent of acceptable variability was ±1/2 the median of 5 ranges or twice the average of five standard deviations.

*Calculated zone-size limits are rounded out to whole numbers.

The efficacy of PD 131628 and grepafloxacin for the treatment of gonococcal infections needs to be established in clinical trials.

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