In vitro activities of sparfloxacin, ceftriaxone, penicillin, tetracycline and doxycycline against Chlamydia trachomatis and Neisseria gonorrhoeae

Hazel Talbot, BSc, RT, Barbara Romanowski, MD, FRCP

Hazel Talbot, B. Romanowski. In vitro activities of sparfloxacin, ceftriaxone, penicillin, tetracycline and doxycycline against Chlamydia trachomatis and Neisseria gonorrhoeae. Can J Infect Dis 1992;3(3):114-117. In vitro sparfloxacin was highly active against 223 penicillin-susceptible isolates of Neisseria gonorrhoeae with a 90% minimal inhibitory concentration (MIC90) of 0.004 μg/mL. Resistant strains of N. gonorrhoeae totalled 55:32 were penicillinase-producing and 23 chromosomally resistant. The MIC90 for these isolates was 0.004 μg/mL and 0.008 μg/mL, respectively. Chlamydia trachomatis was also very susceptible with an MIC50 of 0.063 μg/mL and a 50% minimal bactericidal concentration of 0.032 μg/mL for 11 isolates.

Key Words: Chlamydia trachomatis, In vitro sparfloxacin, Neisseria gonorrhoeae

Sparfloxacin is one of the new fluoroquinolone antibiotics. It is hoped that this agent will demonstrate both in vitro and in vivo activity against Neisseria gonorrhoeae and Chlamydia trachomatis. Quinolones such as norfloxacin, enoxacin, ofloxacin and ciprofloxacin have been documented to be effective in vitro and in vivo against penicillin-susceptible and -resistant strains of N. gonorrhoeae (1). However, these agents have been disappointing in their activities against C. trachomatis, a major pathogen in both cervicitis and urethritis. With dramatic increases in the incidence of both chlamydial infections and resistant strains of...
gonorrhea, new agents are required to facilitate management of these conditions.

In this study, the in vitro activity of sparfloxacin was compared with activities of ceftriaxone, penicillin, tetracycline and doxycycline against both N gonorrhoeae and C trachomatis.

PATIENTS AND METHODS

The antimicrobial agents tested were sparfloxacin (Parke-Davis, Michigan), ceftriaxone (Hoffmann-La Roche Ltd), penicillin G potassium (Wyeth Ltd), and tetracycline hydrochloride and doxycycline hydrochloride (Pfizer Canada). They were obtained in powder form and all except sparfloxacin were solubilized in sterile distilled water. Sparfloxacin was solubilized according to the manufacturer's directions in 95% ethanol, sterile distilled water and 1 N sodium hydroxide. Powders were stored dessicated at 4°C. Stock solutions of the drugs and test media containing serial twofold dilutions of the agent were prepared the day of use.

Clinical isolates of C trachomatis were obtained from 1988 to 1990 from patients attending the Edmonton Sexually Transmitted Disease Clinic with nongonococcal urethritis or mucopurulent cervicitis. Standard isolation methods were used (2,3). Eleven isolates of C trachomatis were passed an average of 14 times (range eight to 21) in cell culture medium (4) with 1 µg/mL cycloheximide and no antibiotics. All cell culture maintenance was done in medium containing no antibiotics.

In vitro sensitivity testing was done using McCoy cells grown in 96-well plates. Isolates retrieved from -70°C were diluted to yield 1x10³ to 5x10³ inclusions per well. Duplicate trays were inoculated and centrifuged at 1000 g for 1 h at 34°C. The inoculum was immediately removed and the monolayers overlayed with cell culture medium containing 1 µg/mL cycloheximide and serial twofold dilutions of the test antibiotic. Cultures were incubated for 48 h at 37°C in 0.3% carbon dioxide, and one tray was stained with fluorescein-conjugated monoclonal antibody (Kallestad Canada) to determine the minimal inhibitory concentration (MIC). The MIC was defined as the lowest concentration at which at least 99.9% inhibition of inclusions occurred, i.e., one or two inclusions were considered an endpoint in some cases. The companion tray was used to determine the minimal bactericidal concentration (MBC) as follows. The antibiotic medium was removed, the monolayer rinsed twice, and 0.2 mL of cell culture medium was added. The tray was then frozen at -70°C, thawed and passed to new monolayers with incubation in antibiotic-free medium. MBC endpoints were defined in the same manner as the MIC.

RESULTS

Table 1 summarizes drug activities against the 11 isolates of C trachomatis. MBC was usually lower than MIC by one dilution, reflecting the appearance of abnormal inclusions near the MIC which were not infectious. The abnormal inclusions made endpoints difficult to interpret. The MBC was therefore considered a better method of analyzing drug activities in this study. Sparfloxacin had an MBC50 value of 0.032 µg/mL and demonstrated activity comparable to tetracycline and doxycycline.

Table 2 outlines the MIC values for N gonorrhoeae. Against penicillin-susceptible (penicillin MIC less than 1 mg/mL), penicillinase-producing and chromosomally mediated resistant strains of N gonorrhoeae the MIC90 of sparfloxacin was 0.004, 0.004 and 0.008 µg/mL, respectively. All N gonorrhoeae isolates with chromosomally mediated resistance to penicillin had tetracycline MICs less than or equal to 2 µg/mL. The
**TABLE 1**
In vitro activity against 11 isolates of *Chlamydia trachomatis*

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Minimal inhibitory concentration (µg/mL)</th>
<th>Minimal bactericidal concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range 50% 90%</td>
<td>Range 50% 90%</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>0.032 to greater than 0.125 0.063</td>
<td>0.016 to 0.063 0.032</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>Greater than 32 Greater than 32</td>
<td>16 16</td>
</tr>
<tr>
<td>Penicillin</td>
<td>Greater than 0.5 Greater than 0.5</td>
<td>0.063 to 0.5 0.125</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.125 to 0.25 0.125</td>
<td>0.032 to 0.063 0.063</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.063 to 0.125 0.063</td>
<td>0.016 to 0.063 0.063</td>
</tr>
</tbody>
</table>

**TABLE 2**
In vitro activity against 278 isolates of *Neisseria gonorrhoeae*

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Penicillin MIC&lt;1 µg/mL (n=223)</th>
<th>PPNG (n=32)</th>
<th>CMRNG (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range 50% 90%</td>
<td>Range 50% 90%</td>
<td>Range 50% 90%</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>≤0.00025 to 0.004 0.002 0.004</td>
<td>0.004 0.004</td>
<td>0.004 0.004</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≤0.00025 to 0.008 0.002 0.004</td>
<td>&gt;256 &gt;256</td>
<td>1 to 2 1 to 2</td>
</tr>
<tr>
<td>Penicillin</td>
<td>≤0.032 to 0.5 0.063 0.25</td>
<td>2 2</td>
<td>2 2</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.125 to 16' 0.25 0.5 0.5 to 16</td>
<td>2 2</td>
<td>2 2</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.063 to 2 0.25 0.5 0.5 to 8</td>
<td>2 2</td>
<td>2 2</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Sparfloxacin demonstrated excellent in vitro activity against *C. trachomatis* and penicillin-susceptible and -resistant strains of *N. gonorrhoeae*. Similar results against *C. trachomatis* were reported by Nagayama and Kitajima (9), Oh and Bowie (10), Wise and Andrews (11), Wong and Stamm (12), and Nakamura (13). Methods of *C. trachomatis* antibiotic susceptibility testing have not been standardized. The method used in this study has been used by others (14). Variations in methods for chlamydial in vitro testing have been discussed in other papers (15-17).

In the present study, the test antibiotic was added immediately after centrifugation and the MBC defined as the point of inhibition after one passage of the antibiotic-treated culture in antibiotic-free medium. Ninety-six-well plates were used instead of vials and fluorescein-conjugated monoclonal antibody stain in place of iodine. The present results are similar to those reported in other studies for the tetracyclines, ceftriaxone and penicillin (17-19). Although not used clinically in the treatment of chlamydial infections, ceftriaxone and penicillin are important antibiotics in the treatment of other sexually transmitted diseases and were included for control purposes. Both agents had some activity with MBC values of 0.125 and 16 mg/mL for penicillin and ceftriaxone, respectively.

When difficulties arose determining the MIC, due to the appearance of abnormal inclusions, the MBC clarified the MIC. It was evident that aberrant inclusions were not infectious and therefore MBC values were generally lower than MIC values for each antibiotic. Using the definition of the endpoint as the lowest concentration at which at least 99.9% inhibition of inclusions occurred allowed the authors to assign definite endpoints (as opposed to the ‘greater than’ values used in a previous study [4] when inclusions persisted past the obvious dropoff point). Again, in some cases one or two inclusions persisted past the 99.9% inhibition point and in these instances ‘greater than’ values would have been assigned had absolute endpoints been defined. Defining the endpoint as the complete absence of inclusions would not have facilitated a concise comparison of antibiotics. The bodies that persisted may have been identifiable although aberrant inclusions or antigenic material (20).

Recent research has shown that if a small number of organisms in the population has antibiotic resistance, they may be detected when large inocula are used (21). The inoculum in this study ranged from 1000 to 5000 inclusion-forming units per well. Speculatively, the latter inoculum may have been high enough that resistant organisms were manifest.

Sparfloxacin demonstrated excellent activity against...
all strains of N gonorrhoeae tested. For penicillin-susceptible strains, these results were comparable to those of ceftriaxone but for resistant strains, sparfloxacin achieved results one to two dilutions lower than ceftriaxone. Similar results have been reported by Gransden and King (22) as well as Kojima et al (23) and Nakamura (13).

Clinical trials of the new quinolone agents have so far resulted in varied outcomes. None of the currently available quinolone agents is effective in a single dose for eradication of C trachomatis infection even though in vitro activity of some quinolones (ciprofloxacin and ofloxacin) is relatively high. Trials using ciprofloxacin, ofloxacin, fleroxacin and norfloxacin for seven to 10 days demonstrated failure rates of 0 to 70% (24). Further research is needed on this group of antimicrobial agents before a recommendation can be made for their general use in the treatment of chlamydial infections.

Based on the findings of the present study, the future of sparfloxacin is promising. The incidence of resistant N gonorrhoeae is increasing and sparfloxacin shows excellent in vitro activity against these strains. In vitro results against C trachomatis are comparable to those of the tetracyclines. Ultimately, the place of sparfloxacin in vivo will depend upon tolerability and pharmacokinetic characteristics.

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REFERENCES