In Vitro Activity of Ro 15-8074, Ro 19-5247, A-56268, and Roxithromycin (RU 28965) against Neisseria gonorrhoeae and Chlamydia trachomatis

WILLIAM R. BOWIE,1* CAROL E. SHAW,2 DAVID G. W. CHAN,2 and WILLIAM A. BLACK2,3

The Provincial Health Laboratory, British Columbia Ministry of Health,2 and the Divisions of Infectious Diseases1 and Medical Microbiology,3 Faculty of Medicine, University of British Columbia, Vancouver, British Columbia V5Z 1M9, Canada

Received 22 August 1986/Accepted 5 December 1986

In vitro Ro 15-8074 and Ro 19-5247 (T2525), two new oral cephalosporins, were active against 410 penicillin-susceptible and -resistant isolates of Neisseria gonorrhoeae. Two new macrolides, A-56268 and to a lesser extent roxithromycin (RU 28965), were active against Chlamydia trachomatis. A-56268 had activity against N. gonorrhoeae similar to that of erythromycin.

Management of sexually transmitted syndromes is facilitated by having antimicrobial agents active against all of the major genital pathogens. Currently marketed macrolides are useful for treatment of Chlamydia trachomatis infection, but there is laboratory (10) and clinical evidence that erythromycins are not always effective (1) and macrolides in higher doses are often poorly tolerated (3). Macrolides are not advocated for use in the treatment of infections due to Neisseria gonorrhoeae (6). Development of newer macrolides with increased in vitro activity against both of these organisms or better tolerance or activity in vivo could be a useful advance. Like older cephalosporins, newer ones are not anticipated to be active against C. trachomatis, but newer cephalosporins have high activity against N. gonorrhoeae (4), and ceftriaxone has assumed major importance in the management of gonococcal infections (6). Oral cephalosporins with high activity against N. gonorrhoeae in vivo would be highly useful because they would circumvent the need for an injection and related equipment. In this study, the in vitro activity of the newer macrolides, A-56268 and roxithromycin (RU 28965), and the cephalosporins, Ro 15-8074 and Ro 19-5247 (T2525), were evaluated for in vitro activity against either or both N. gonorrhoeae and C. trachomatis.

Most non-beta-lactamase-producing isolates of N. gonorrhoeae were obtained from men and women presenting to the Sexually Transmitted Disease Clinic in the Vancouver Provincial Health Building from June 1982 to June 1984. Also studied were additional isolates of N. gonorrhoeae obtained since 1982 from all over British Columbia that were penicillinase producing or showed decreased zones of inhibition around a 10-U penicillin disk applied to chocolate agar (GC medium base, 1% hemoglobin, 1% defined GC supplement) (Alkhem-Western Ltd.). Before April 1985, a zone size of ≤19 mm was used to screen for isolates with decreased susceptibility to penicillin, but since April 1985, a zone size of <26 mm has been used. Ninety-nine isolates with a penicillin MIC of <1 μg/ml were obtained in January 1986. Clinical specimens were directly inoculated on Thayer-Martin medium (chocolate agar containing colistin [7.5 μg/ml], vancomycin [3.0 μg/ml], and nystatin [12.5 μg/ml]) and were incubated in 5% CO2 at 35°C within 30 min.

Oxidase-positive colonies showing typical colonial morphology and containing gram-negative diplococci were identified as N. gonorrhoeae by fluorescent-antibody staining or sugar fermentation or both. Ten or more colonies were subcultured to chocolate agar, and a modification of the iodometric paper strip was used to screen for beta-lactamase activity (9). The presence of beta-lactamase activity in isolates positive by the iodometric test was confirmed with chromogenic cephalosporin (nitrocefin) (Gloxy Pharmaceuticals, Ltd., Greenford, England) (7). Isolates were stored at −70°C in glycerol citrate. The study included 235 isolates for which the MICs of penicillin were <1 μg/ml, 44 chromosomally mediated resistant N. gonorrhoeae isolates that were not beta-lactamase producing and for which the MICs of penicillin were 1 to 4 μg/ml, and 131 beta-lactamase-producing strains of N. gonorrhoeae. Isolates were tested by standard agar dilution techniques (7) for in vitro susceptibility to penicillin G, tetracycline, erythromycin, A-56268 (Abbott International Ltd., North Chicago, Ill.), ceftriaxone (Hoffmann-La Roche Ltd., Greenford, Ontario, Canada), the cephalosporins Ro 15-8074/005 and Ro 19-5247/003 (Hoffmann-La Roche Ltd., and spectinomycin. The susceptibility test medium was GC agar base (Difco Laboratories, Detroit, Mich.) with 1% Kellogg defined supplement. The inoculum was suspended in glycerol-lactate-salt suspending medium (7) and adjusted to a 0.5 McFarland standard to obtain an inoculum which delivered approximately 105 CFU with a Steers replicator. All tests were performed at least in duplicate.

As control strains, three strains of N. gonorrhoeae recommended by the World Health Organization (WHO III, WHO V, and WHO VII) as reference strains for penicillin susceptibility testing were obtained from the Antimicrobials and Molecular Biology Division, Laboratory Centre for Disease Control, Ottawa, Ontario, Canada. Two additional isolates from our own laboratory, including a beta-lactamase producer and an isolate showing intermediate resistance to penicillin (MIC, 4 μg/ml), were also selected as controls. The range and MICs for 50 and 90% of strains of individual antimicrobial agents were calculated by standard techniques.

In vitro susceptibility testing was performed on 10 clinical isolates of C. trachomatis by our previous methodology (2). The MICs and MBCs were determined for each strain in
Antimicrobial agent | MIC (μg/ml) for strain group\(^a\) | Penicillin MIC <1 μg/ml | PPNG | CMRNG | \(\geq 500\) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>8.0–&gt;8.0 &gt;8.0 &gt;8.0</td>
<td>1.0–4.0 2.0 4.0</td>
<td>≤0.008–0.5 0.25 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.5–4.0 2.0 4.0</td>
<td>1.0–4.0 2.0 2.0</td>
<td>0.063–16.0 (^b) 1.0 1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.032–4.0 2.0 2.0</td>
<td>1.0–8.0 2.0 4.0</td>
<td>0.032–4.0 0.5 2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-5626</td>
<td>0.016–2.0 2.0 2.0</td>
<td>0.25–4.0 2.0 2.0</td>
<td>0.016–2.0 0.25 1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≤0.001–0.016 0.004 0.008</td>
<td>0.004–0.032 0.008 0.032</td>
<td>≤0.001–0.008 0.002 0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ro 15-2247/003</td>
<td>≤0.001–0.125 0.032 0.063</td>
<td>0.032–0.25 0.063 0.25</td>
<td>≤0.001–0.25 0.008 0.032</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ro 15-8074/005</td>
<td>0.002–0.125 0.016 0.032</td>
<td>0.016–0.125 0.032 0.125</td>
<td>≤0.001–0.25 0.016 0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>8.0–32.0 16.0 16.0</td>
<td>16.0 16.0 16.0</td>
<td>15.0 15.0 15.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) PPNG, beta-lactamase positive N. gonorrhoeae (n = 131); CMRNG, chromosomally mediated resistant N. gonorrhoeae (beta-lactamase negative with penicillin MIC of ≥1.0 μg/ml) (n = 44). Penicillin MIC, <1 μg/ml (n = 235), 50% and 90%, MICs for 50 and 90% of isolates, respectively.

\(^b\) Tetracycline range = 0.063 to 1.0 μg/ml, except for three resistant isolates (MIC = 16.0 μg/ml).

The results indicate that A-5626, with in vitro activity similar to that of tetracycline, has considerable promise as an antimicrobial agent for use in treatment of chlamydial infections. In vitro data suggest that it would not be an improvement over erythromycin for gonococcal infections. Erythromycin had similar activity to that of erythromycin against C. trachomatis and could be effective for nongonococcal urethritis and nongonococcal cervicitis. The studies of others indicate that it also has activity against N. gonorrhoeae similar to erythromycin activity (8). The cephalosporins Ro 15-8074 and Ro 19-5247 had very promising in vitro activity against N. gonorrhoeae. Ro 15-8074 is the active cephalosporanic acid derivative of its orally absorbable pivaloyloxymethylene Ro 15-8075. Ro 19-5247 is the active derivative of the orally absorbable ester derivative Ro 19-5248. The ultimate usefulness of these antimicrobial agents in vivo will depend upon tolerability and pharmacokinetic characteristics.

This research was funded by Abbott International Ltd., Hoffmann-La Roche Ltd., and Roussel Canada Inc.

**LITERATURE CITED**


