High In Vitro Activity of the Novel Spiropyrimidinetrione AZD0914, a DNA Gyrase Inhibitor, against Multidrug-Resistant Neisseria gonorrhoeae Isolates Suggests a New Effective Option for Oral Treatment of Gonorrhea

Susanne Jacobsson, a Daniel Golparian, a Richard A. Alm, b Michael Huband, b John Mueller, b Jorgen Skov Jensen, a Makoto Ohnishi, d Magnus Unemo a

WHO Collaborating Centre for Gonorrhoea and other Sexually Transmitted Infections, National Reference Laboratory for Pathogenic Neisseria, Department of Laboratory Medicine, Clinical Microbiology, Örebro University Hospital, Örebro, Sweden; aInfection iMed, AstraZeneca R&D Boston, Waltham, Massachusetts, USA; bSwedish Institute of Infectious Disease Control, Stockholm, Sweden; cNational Institute of Infectious Diseases, Tokyo, Japan

We evaluated the activity of the novel spiropyrimidinetrione AZD0914 (DNA gyrase inhibitor) against clinical gonococcal isolates and international reference strains (n = 250), including strains with diverse multidrug resistance and extensive drug resistance. The AZD0914 MICs were substantially lower than those of most of those most currently or previously recommended antimicrobials. AZD0914 should be further evaluated, including in vitro selection, in vivo emergence and mechanisms of resistance, pharmacokinetics/pharmacodynamics in humans, optimal dosing, and performance, in appropriate randomized and controlled clinical trials.

Gonorrhea is a significant public health problem globally (1). Neisseria gonorrhoeae has developed antimicrobial resistance (AMR) to all drugs previously used as first-line treatments. In the past decade, resistance to the last options for empirical first-line antimicrobial monotherapy, i.e., the extended-spectrum cephalosporins (ESCs) cefixime and ceftriaxone, has emerged (2–18). Alarming, extensively drug-resistant (XDR) (3) gonococcal isolates with high-level ESC resistance were recently described (9, 13, 19).

The World Health Organization (WHO) (20), Centers for Disease Control and Prevention (CDC) (21), and European Centre for Disease Prevention and Control (ECDC) (22) have published action plans aiming to control the spread of AMR gonorrhea. One of the few new antimicrobial classes incorporates a novel spiropyrimidinetrione that operates via a novel DNA gyrase/topoisomerase IV mode-of-inhibition (23). DNA gyrase (GyrA 2 and GyrB 2) and topoisomerase IV (ParC 2 and ParE 2) are type II DNA topoisomerases, which catalyze changes in the topology of DNA and their function is vital for DNA replication, repair, and decatenation (24–27). AZD0914 (Fig. 1) is a novel spiropyrimidinetrione that inhibits DNA biosynthesis, causing accumulations of double-strand cleavages, that was recently shown to have potent in vitro activity against many different bacterial species (23).

We detailed the in vitro activity of the novel spiropyrimidinetrione AZD0914 against a large geographically (global representativeness), temporally (obtained from 1991 to 2013), and genetically diverse collection of clinical gonococcal isolates and international reference strains (n = 250). The quinolone resistance-determining region (QRDR) of the gyrA gene and an AZD0914 resistance-determining region of gyrB (28) were sequenced to verify the lack of cross-resistance to fluoroquinolones and AZD0914 resistance mutations, respectively.

The strains comprised 29 international gonococcal reference strains, including the 2008 WHO reference strains (29), 100 consecutive clinical Swedish gonococcal isolates obtained in 2013, and 121 isolates selected for their resistance phenotype. The collection included all of the currently described XDR gonococcal strains (9, 13, 19), additional isolates with in vitro resistance (8–11, 13, 15, 16), different types of ciprofloxacin resistance, and other high-level clinical resistance and multidrug resistance (MDR) to other antimicrobials previously used for treatment. The MICs of AZD0914 (AstraZeneca Pharmaceuticals LP) were determined by the agar dilution technique according to current CLSI guidelines (30). The MICs of ceftriaxone, spectinomycin, cefixime, ampicillin, azithromycin, ciprofloxacin, and tetracycline were determined by the Etest method (AB bioMérieux), according to the manufacturer’s instructions, and mainly interpreted according to the CLSI breakpoints (Table 1). Selected regions of gyrA and gyrB were sequenced using primers described in Table S1 in the supplemental material.

The MIC range, modal MIC, MIC50, and MIC90 of AZD0914 were 0.004 to 0.25 μg/ml, 0.125 μg/ml, 0.125 μg/ml, and 0.25 μg/ml, respectively. With exception of the ESCs, the MIC50 of AZD0914 was lower than that of other antimicrobials substantially higher than those observed for AZD0914. A total of 165 (66%) of the isolates were resistant to the previously recommended fluoroquinolone ciprofloxacin, and 92 (37%) had a ciprofloxacin MIC of ≥32 μg/ml (AZD0914 MIC90, 0.125 μg/ml). For AZD0914, the highest MIC value (0.25 μg/ml) was found in 31 (12%) isolates. The MIC distributions for AZD0914 and ciprofloxacin and a comparison of the MIC values of AZD0914 and ciprofloxacin are
shown in Fig. S1A and S1B, respectively, in the supplemental material. No obvious cross-resistance between AZD0914 and ciprofloxacin or any other tested antimicrobial was identified (see Fig. S1 and Table S2 in the supplemental material). The consecutive Swedish isolates appeared to mainly represent a wild-type MIC distribution for AZD0914 (data not shown), indicating a general lack of AZD0914 resistance mutations. No nonsynonymous mutations in amino acid codons 91 and 95 in GyrA QRDR, resulting in fluoroquinolone resistance, or other nonsynonymous mutations in gyrB showed any correlations with the AZD0914 MIC values (see Table S2). Only seven polymorphic nucleotide positions, including five synonymous substitutions and two nonsynonymous substitutions resulting in S467N and M521I alterations, were found in the examined 480-bp region of gyrB that encodes the region of GyrB that surrounds the residues shown to confer resistance to AZD0914 (28) (see Table S2).

AZD0914 was also previously shown to be active against a small collection of N. gonorrhoeae isolates. However, few of these isolates were MDR or displayed resistance to, e.g., the currently recommended ESCs or high-level resistance to spectinomycin or azithromycin (31), the latter included in the introduced dual-antimicrobial treatment regimens (250 to 500 mg ceftriaxone together with 1 to 2 g azithromycin) (32, 33). The frequency of spontaneous resistance mutations to AZD0914 has also been shown to be low. Accordingly, when five gonococcal strains (four of which showed high-level resistance to ciprofloxacin) were exposed to AZD0914, no mutants could be isolated from any strain at 8× MIC (limit of detection, <3.3 × 10⁻⁸ to < 2.1 × 10⁻⁷), and mutants could be isolated from only two strains at lower AZD0914 concentrations. These mutants showed 16-fold to 32-fold increases in the MIC of AZD0914 (MIC, 1 to 2 μg/ml). Whole-genome sequencing of the mutants identified a single mutation (D429N or K450T) in the C terminus of GyrB that resulted in the increased AZD0914 MIC, which was also confirmed by transformation experiment (28). In the present study, the region of gyrB that contained these resistance-determining amino acid residues was highly conserved, and the in vitro-selected resistance mutations, D429N and K450T, in GyrB (28) were not found. Only two amino acid alterations in GyrB, S467N and M521I, were found among the 250 isolates, and the AZD0914 MICs of the corresponding isolates were 0.125 μg/ml and 0.06 μg/ml, respectively, which were within the AZD0914 wild-type MIC distribution. AZD0914 has good penetration into target tissues, good bioavailability, and sufficiently high safety and tolerability margins, as indicated from initial preclinical animal toxicity studies, to support further development.

The novel spiropyrimidinetrione AZD0914, a DNA gyrase inhibitor, was highly active against gonococci, and the results indicated a lack of cross-resistance to other antimicrobial classes. AZD0914 should be evaluated in additional studies, including in vitro selection and the in vivo emergence and mechanisms of AZD0914 resistance. Pharmacokinetics/phar-

---

**TABLE 1** MIC range, MIC₅₀, MIC₉₀, and modal MIC value of AZD0914 among all isolates, consecutive Swedish isolates, selected isolates, and reference strains and proportion of all isolates in different resistance categories for antimicrobials currently or previously recommended for treatment of gonorrhea

<table>
<thead>
<tr>
<th>Agent and isolates or strains (n)</th>
<th>MIC (μg/ml)ᵃ</th>
<th>Range</th>
<th>50%</th>
<th>90%</th>
<th>Modal value</th>
<th>S/I/R (%)ᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZD0914</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All isolates (250)</td>
<td>0.004 to 0.25</td>
<td>0.125</td>
<td>0.25</td>
<td>0.125</td>
<td></td>
<td>NDᵇ</td>
</tr>
<tr>
<td>Consecutive isolates (100)</td>
<td>0.004 to 0.25</td>
<td>0.125</td>
<td>0.25</td>
<td>0.125</td>
<td></td>
<td>NDᵇ</td>
</tr>
<tr>
<td>Selected isolates (121)</td>
<td>0.004 to 0.25</td>
<td>0.125</td>
<td>0.25</td>
<td>0.125</td>
<td></td>
<td>NDᵇ</td>
</tr>
<tr>
<td>Reference strains (29)</td>
<td>0.008 to 0.25</td>
<td>0.125</td>
<td>0.25</td>
<td>0.125</td>
<td></td>
<td>NDᵇ</td>
</tr>
<tr>
<td>Ciprofloxacin-resistant strains (165)</td>
<td>0.004 to 0.25</td>
<td>0.125</td>
<td>0.25</td>
<td>0.125</td>
<td></td>
<td>NDᵇ</td>
</tr>
<tr>
<td>Other agents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone (250)</td>
<td>&lt;0.002 to 4</td>
<td>0.016</td>
<td>0.125</td>
<td>0.008</td>
<td></td>
<td>98.8/1.2ᵈ</td>
</tr>
<tr>
<td>Spectinomycin (250)</td>
<td>4 to &gt;1,024</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td></td>
<td>97.6/4/2.0</td>
</tr>
<tr>
<td>Cefixime (250)</td>
<td>&lt;0.016 to 8</td>
<td>&lt;0.016</td>
<td>0.25</td>
<td>&lt;0.016</td>
<td>&lt;0.016</td>
<td>95.6/4/4ᵈ</td>
</tr>
<tr>
<td>Ampicillin (250)</td>
<td>&lt;0.016 to &gt;256</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>18.0/53.2/28.8</td>
<td></td>
</tr>
<tr>
<td>Azithromycin (250)</td>
<td>0.016 to &gt;256</td>
<td>0.5</td>
<td>4</td>
<td>1</td>
<td>32.4/22.4/45.2</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin (250)</td>
<td>&lt;0.002 to &gt;32</td>
<td>8 &gt;32&gt;32</td>
<td>32.6/4.4/66.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline (250)</td>
<td>0.125 to 256</td>
<td>2</td>
<td>32</td>
<td>2</td>
<td>0/28.4/71.6</td>
<td></td>
</tr>
</tbody>
</table>

ᵃ The MIC was determined using the agar dilution technique for AZD0914 and the Etest method for the additional antimicrobials. (Only whole MIC dilutions are reported in this article.) 50% and 90%, MIC₅₀ and MIC₉₀, respectively.
ᵇ S, susceptible; I, intermediate susceptible; R, resistant. The CLSI breakpoints (M100–S24 [30]) were used for all antimicrobials, with exception of azithromycin, for which the breakpoints from the European Committee on Antimicrobial Susceptibility Testing (34) were applied. The susceptibility categories for ampicillin were inferred from the penicillin G breakpoints stated by the CLSI (30).
ᶜ ND, not determined due to lack of interpretative criteria.
ᵈ Decreased susceptibility or resistance.
macodynamic properties should be further studied in humans, and appropriate randomized and strictly controlled clinical trials, including patients with both genital and extragenital (especially pharyngeal) gonorrhea, should be performed while evaluating such parameters as optimal dosing, tolerability, efficacy, cost, and safety.

ACKNOWLEDGMENTS

We are grateful to the whole AstraZeneca team involved in the discovery and development of AZD0914.

The present study was supported by the Örebro County Research Committee, the Foundation for Medical Research at Örebro University Hospital, Sweden, and Infection iMed, AstraZeneca Pharmaceuticals LP, Waltham, MA.

The work was performed at the WHO Collaborating Centre for Gonorrhea and other Sexually Transmitted Infections, National Reference Laboratory for Pathogenic Neisseria, Department of Laboratory Medicine, Microbiology, Örebro University Hospital, Örebro, Sweden.

REFERENCES


