In vitro activities of the novel bicyclolides modithromycin (EDP-420, EP-013420, S-013420) and EDP-322 against MDR clinical Neisseria gonorrhoeae isolates and international reference strains

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Objectives: New antimicrobials are essential to prevent gonorrhoea becoming an untreatable infection. Herein, the in vitro activities of the novel bicyclolides modithromycin (EDP-420, EP-013420, S-013420) and EDP-322 against Neisseria gonorrhoeae strains were investigated and compared with antimicrobials currently or previously recommended for treatment of gonorrhoea.

Methods: MICs (mg/L) were determined using an agar dilution method (modithromycin and EDP-322) or Etest (seven antimicrobials) for a large geographically, temporally and genetically diverse collection of clinical N. gonorrhoeae isolates (n=225) and international reference strains (n=29), including diverse MDR and XDR isolates.

Results: The MIC range, modal MIC, MIC50 and MIC90 of modithromycin and EDP-322 were 0.004–256, 0.25, 0.25 and 1 mg/L and 0.008–16, 0.5, 0.5 and 1 mg/L, respectively. The activities of modithromycin and EDP-322 were mainly superior to those of azithromycin and additional antimicrobials investigated. In general, there was no cross-resistance with other antimicrobials.

Conclusions: Modithromycin and EDP-322 exhibited high levels of in vitro activities against N. gonorrhoeae, including isolates resistant to azithromycin, cefixime, ceftriaxone, spectinomycin, ampicillin, tetracycline and ciprofloxacin. However, some cross-resistance with high-level azithromycin resistance (MIC = 4096 mg/L) was observed. Modithromycin and EDP-322 could be effective options for treatment of gonorrhoea, particularly for cases resistant to extended-spectrum cephalosporins and as a part of an antimicrobial combination therapy regimen. Nevertheless, it is important to detail the in vitro selection, in vivo emergence and mechanisms of resistance, pharmacokinetics/pharmacodynamics in humans and optimal dosing, and perform appropriate randomized controlled clinical trials.

Keywords: N. gonorrhoeae, gonorrhoea, treatment, antimicrobial resistance

Introduction

Neisseria gonorrhoeae is a common sexually transmitted pathogen, which causes serious public health problems globally. The WHO has estimated that there are 106 million new cases of gonorrhoea worldwide each year.1 Moreover, N. gonorrhoeae has developed antimicrobial resistance to all previous first-line antimicrobial therapies used over the past 70–80 years. Currently, the third-generation extended-spectrum cephalosporins (ESCs), the last available class that is sufficiently effective in antimicrobial monotherapy, are also threatened by the evolving resistance. Treatment failures with cefixime have been reported in Japan, Norway, the UK, Austria, France, Canada and South Africa2–5 and ceftriaxone failures in Japan, Sweden, Australia and Slovenia.2–8 Furthermore, the first XDR N. gonorrhoeae isolates with high-level resistance to all ESCs have been described in Japan, France and Spain.5,6,9 As a result of this developing situation, dual antimicrobial therapy with ceftriaxone and either azithromycin or doxycycline is now recommended in Europe and the USA.9,10,11 However, to prevent gonorrhoea becoming an untreatable infection, new antimicrobial treatment options are urgently needed.

Modithromycin (also known as EDP-420, EP-013420, S-013420) and EDP-322 are novel 6,11-bridged bicyclolides currently under clinical development. Modithromycin has a unique...
6,11-O-bridging moiety where the cladinose sugar is replaced by a 3-keto group. The bactericidal activity of modithromycin involves inhibition of protein synthesis by binding near the entrance to the peptide exit tunnel in the 50S ribosomal subunit and forming interactions with both domain V and domain II of 23S rRNA. The additional binding motif of modithromycin to the 50S ribosomal subunit (i.e. a second binding site along the 23S rRNA hairpin loop 35 of domain II) provided by the 6,11-O-bridging moiety, resulting in an improved structure–activity relationship, is designed to overcome macrolide, lincosamide and streptogramin B resistance. Modithromycin has shown potent in vitro activity against a number of pathogens especially causing respiratory tract infections, including anaerobic and atypical pathogens. Modithromycin was also more potent than azithromycin against a small collection of \( N. \) gonorrhoeae isolates. However, only 41 gonococcal isolates were tested, none of which displayed an MIC of azithromycin of \( >2 \) mg/L or high-level resistance to other currently used antimicrobials. The in vitro activity of EDP-322 against \( N. \) gonorrhoeae isolates has not been previously evaluated.

The aim of the present study was to detail the in vitro activity of the novel bicyclolides modithromycin and EDP-322 against a large geographically, temporally and genetically diverse collection of clinical \( N. \) gonorrhoeae isolates and international reference strains \( (n=254) \). The collection included all described types of high-level in vitro and clinical resistance to antimicrobials currently or previously recommended for treatment of gonorrhoea, as well as numerous MDR and XDR gonococcal isolates.

### Materials and methods

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

The strain collection examined included 29 international \( N. \) gonorrhoeae reference strains (including the 2008 WHO reference strains), 100 consecutive clinical gonococcal isolates obtained in 2013 in Sweden and 125 isolates selected for their resistance phenotype (including a high proportion of azithromycin-resistant isolates) (Table S1, available as Supplementary data at JAC Online). Furthermore, the collection included all the currently described XDR gonococcal strains, additional isolates with in vitro and clinical resistance to ESCs, and different levels and types of azithromycin resistance as well as other high-level clinical resistance and MDR to other antimicrobials previously used for treatment of gonorrhoea. Accordingly, the strain panel represented a large geographically (mainly global representativeness), temporally (obtained from 1991 to 2013), phenotypically and genetically diverse selection.

The MICs (mg/L) of modithromycin and EDP-322 (Enanta Pharmaceuticals, Inc., Watertown, MA, USA) were determined by an agar dilution technique, according to CLSI guidelines (www.clsi.org). The MICs (mg/L) of ceftriaxone, azithromycin, spectinomycin, cefixime, ciprofloxacin, ampicillin and tetracycline were determined by the Etest method (AB bioMérieux, Solna, Sweden), according to the manufacturer’s instructions. All MICs were interpreted according to EUCAST breakpoints (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_4.0.pdf; Table 1). Only whole MIC dilutions are reported in the present manuscript.

### Table 1. MIC range, MIC\(_{50}\), MIC\(_{90}\) and modal MIC of modithromycin (EDP-420, EP-013420, S-013420) and EDP-322 for all \( N. \) gonorrhoeae isolates \( (n=254) \), consecutive isolates \( (n=100) \), selected isolates \( (n=125) \) and reference strains \( (n=29) \), and proportion of all isolates \( (n=254) \) categorized as susceptible, intermediately susceptible and resistant to antimicrobials currently or previously recommended for treatment of gonorrhoea.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC(_{50}) (mg/L)</th>
<th>MIC(_{90}) (mg/L)</th>
<th>Modal MIC (mg/L)</th>
<th>S/I/R (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Modithromycin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all isolates</td>
<td>0.004 – 256</td>
<td>0.25</td>
<td>1</td>
<td>0.25</td>
</tr>
<tr>
<td>consecutive isolates</td>
<td>0.004 – 2</td>
<td>0.25</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>selected isolates</td>
<td>0.016 – 256</td>
<td>0.25</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>reference strains</td>
<td>0.008 – 2</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>EDP-322</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all isolates</td>
<td>0.008 – 16</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>consecutive isolates</td>
<td>0.008 – 2</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>selected isolates</td>
<td>0.032 – 16</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>reference strains</td>
<td>0.064 – 4</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Ceftriaxone (S(_{\leq 0.12}))</td>
<td>&lt;0.002 – 4</td>
<td>0.016</td>
<td>0.125</td>
<td>0.008</td>
</tr>
<tr>
<td>Spectinomycin (S(_{\leq 64}))</td>
<td>4 to 1024</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Cefixime (S(_{\leq 0.12}))</td>
<td>&lt;0.016 – 8</td>
<td>&lt;0.016</td>
<td>0.25</td>
<td>&lt;0.016</td>
</tr>
<tr>
<td>Ampicillin (S(_{\leq 0.06, R&gt;1}))</td>
<td>&lt;0.016 to &gt;256</td>
<td>1</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Azithromycin (S(_{\leq 0.25, R&gt;0.5}))</td>
<td>0.016 – 4096</td>
<td>0.5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Ciprofloxacin (S(_{\leq 0.03, R&gt;0.06}))</td>
<td>&lt;0.002 to &gt;32</td>
<td>8</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Tetracycline (S(_{\leq 0.5, R&gt;1}))</td>
<td>0.125 – 256</td>
<td>2</td>
<td>32</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup>MIC was determined using an agar dilution technique for modithromycin and EDP-322 and with the Etest method for the additional antimicrobials.

<sup>b</sup>S, susceptible; I, intermediately susceptible; R, resistant. The EUCAST breakpoints (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_4.0.pdf) were applied for all antimicrobials.

<sup>c</sup>Not determined due to lack of interpretative criteria.
Results

The susceptibility results of modithromycin, EDP-322 and seven currently or previously recommended antimicrobials are summarized in Table 1. The material was divided into different groups, i.e. all isolates, consecutive isolates, selected isolates and international reference strains.

The MIC range, modal MIC, MIC of azithromycin, the MIC range, modal MIC, MIC of azithromycin for these isolates were 0.004 – 256, 0.25, 0.25 and 1 mg/L, respectively. For azithromycin, the MIC range (0.016 – 4096 mg/L) was wider and particularly the MIC of azithromycin (4 mg/L) was substantially higher (Table 1). Of all isolates, 113 (44.5%) were resistant to azithromycin (MIC of azithromycin ≤ 0.5 mg/L). However, only 33 (13.0%) of all isolates had an MIC of modithromycin of ≤ 0.5 mg/L and 91 (35.8%) an MIC of EDP-322 of ≤ 0.5 mg/L. Furthermore, only five (2.0%) of the isolates had an MIC of modithromycin of > 2 mg/L (4, 128, 256, 256 and 256 mg/L) and the corresponding MICs of azithromycin for these isolates were 2, 4096, 4096, 4096 and 4096 mg/L, respectively. Solely, four (1.6%) of the isolates had an MIC of EDP-322 of > 2 mg/L (4, 4, 4 and 16 mg/L) and the corresponding MICs of azithromycin for these isolates were 2, 4, 4096 and 4096 mg/L, respectively. The MICs of the currently recommended ESCs (ceftriaxone and cefixime) for all the isolates with an MIC of modithromycin and/or EDP-322 of > 2 mg/L were only ≤ 0.016 – 0.064 mg/L cefixime and 0.004 – 0.064 mg/L ceftriaxone. The MIC distributions of modithromycin, EDP-322 and azithromycin are shown in Figure 1(a). Comparisons of the MICs of modithromycin and EDP-322 and azithromycin are shown in Figure 1(b) and Figure 1(c), respectively.

Discussion

In the present study, we report the first comprehensive evaluation of the in vitro activities of the two novel bicyclolides modithromycin and EDP-322 against a large geographically, temporally and genetically diverse collection of clinical N. gonorrhoeae isolates and international reference strains, including various types of high-level resistant, XDR and MDR isolates. The activities of the bicyclolides were also compared with the activities of seven additional antimicrobials, i.e. ceftriaxone, spectinomycin, cefixime.
ampicillin, azithromycin, ciprofloxacin and tetracycline, currently or previously recommended for gonorrhoea treatment.

Both modithromycin and EDP-322 were highly active against most tested N. gonorrhoeae isolates and the MIC\textsubscript{50} and MIC\textsubscript{90} of both the bicyclolides were mainly superior to those of azithromycin, ciprofloxacin, ampicillin, spectinomycin and tetracycline. The MIC\textsubscript{90} of azithromycin was 4-fold higher than those of modithromycin and EDP-322. In general, no unambiguous cross-resistance between the bicyclolides and azithromycin was observed. However, isolates with high-level resistance to azithromycin (4096 mg/L; due to A2059G mutations in three to four alleles of the 23S rRNA gene\textsuperscript{18}) had MICs of modithromycin and EDP-322 of 128–256 and 1–16 mg/L, respectively. No other cross-resistance with the novel bicyclolides was found.

Modithromycin has been shown to be well tolerated, in doses up to 1.2 g, with no serious adverse events. Modithromycin is also readily absorbed after a single oral dose (in both suspension and capsule formulation), has a high systemic exposure compared with other macrolides and additional antimicrobials and has a long half-life ranging from 16 to 20 h with low clearance.\textsuperscript{19,20} With a projected susceptibility breakpoint of 2 mg/L, 98.0% of isolates tested in this study, a collection including a high proportion of azithromycin-resistant strains (44.5%), would be susceptible to modithromycin (98.4% for EDP-322). As macrolides, both modithromycin and EDP-322 are likely to also be active against additional bacterial sexually transmitted infections such as Chlamydia trachomatis infections and Mycoplasma genitalium infections. Further in vitro investigations for these pathogens and clinical trials for gonorrhoea are warranted.

In conclusion, both modithromycin and EDP-322 demonstrated high levels of in vitro activity against N. gonorrhoeae, including XDR and MDR strains. However, some cross-resistance with high-level azithromycin resistance (MIC = 4096 mg/L) was observed. Both these bicyclolides could be effective options for treatment of gonorrhoea, particularly for cases resistant to ESCs and as part of an antimicrobial combination therapy regimen. It is crucial to detail the in vitro selection, in vivo emergence and mechanisms of resistance (ideally both in gonococci and bystander organisms when treating gonorrhoea) and pharmacokinetics/pharmacodynamics in humans, focusing on sexually transmitted infections, and to perform appropriate randomized and strictly controlled clinical trials, including patients with both genital and extra-genital (especially pharyngeal) gonorrhoea and evaluating parameters such as optimal dosing, tolerability, efficacy, cost and safety.

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Supplementary data

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References


