Combination therapy for gonorrhoea: in vitro synergy testing

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Objectives: Antimicrobial resistance in Neisseria gonorrhoeae is an increasing problem worldwide and combinations of antimicrobial agents have been recommended to delay the onset of treatment failures. The objective of this study was to obtain in vitro data on the activity of current (ceftriaxone or cefixime plus azithromycin) and alternative (gentamicin plus azithromycin) regimens.

Methods: A panel of 64 gonococcal isolates displaying various cefixime MICs was selected for inclusion in the study. Determination of the activities of the antimicrobial combinations of ceftriaxone, cefixime or gentamicin with azithromycin was performed using the agar dilution method and subsequent calculation of the fractional inhibitory concentration index (FICI) values.

Results: No antagonism for any of the antimicrobial combinations was detected among the 64 gonococcal isolates. When cefixime or ceftriaxone was combined with azithromycin all isolates showed additivity/indifference with a mean FICI of 2.0. All gonococcal isolates also showed additivity/indifference with the antimicrobial combination of gentamicin with azithromycin, but with a lower mean FICI of 1.7. No significant difference in the mean FICI between isolates fully susceptible to cefixime and isolates with decreased susceptibility to cefixime was observed.

Conclusions: The results obtained support the gonorrhoea treatment currently recommended in the UK national guidelines and suggest that gentamicin with azithromycin could be a future treatment option. The in vivo activity and efficacy of these combinations remain unknown and prospective clinical studies should be addressed.

Keywords: azithromycin, cefixime, ceftriaxone, gentamicin, Neisseria gonorrhoeae

Introduction

Neisseria gonorrhoeae has developed various resistance mechanisms to previous and current therapeutic agents, including high-level resistance to the extended-spectrum cephalosporins (ESCs) cefixime and ceftriaxone. Associated with this, treatment failures with ESCs have recently been observed in the UK and other European countries.1–3 In response to both these treatment failures and also increases in the proportion of isolates showing raised cefixime and ceftriaxone MICs in England and Wales5 the UK national guidelines for gonorrhoea treatment were recently updated.5 Now a dual therapy of 500 mg of ceftriaxone (increased from 250 mg) intramuscularly plus 1 g of azithromycin orally is recommended for the treatment of gonorrhoea. Cefixime with azithromycin is only administered as part of a second-line regimen. The inclusion of azithromycin is for co-treatment of chlamydia, to improve treatment effectiveness against pharyngeal infection and to possibly delay ESC resistance due to a broader spectrum of antimicrobial activity and possible antimicrobial synergy.5

In the face of dwindling treatment options, the use of gentamicin remains a current possibility, either singly or in combination with azithromycin.6 Gentamicin is currently not a recommended therapy in the UK, but an evaluation of gentamicin susceptibility in Europe demonstrated that this antimicrobial may be an alternative treatment option,7 although MIC breakpoints and more clinical trial data are still required.

The aim of this study was to provide in vitro susceptibility data using azithromycin in combination with cefixime or ceftriaxone, to support the current dual therapy treatment regimen, and to investigate the effect of using gentamicin with azithromycin. A panel of recent clinical N. gonorrhoeae isolates, displaying various cefixime MICs, was used to ascertain whether synergistic or antagonistic activity is observed.

Materials and methods

Bacterial isolates

N. gonorrhoeae isolates (n = 56) were obtained by the Sexually Transmitted Bacteria Reference Laboratory, HPA, London, UK, from 2007 to 2010.
Eight reference strains (WHO F, G, K, L, M, N, O and P) were included. The 64 isolates displayed various MICs of cefixime (≥0.125 mg/L (n=19), 0.03–0.06 mg/L (n=22) and ≤0.015 mg/L (n=23)).

**Antimicrobial susceptibility testing**

Isolates were confirmed as *N. gonorrhoeae* and susceptibility testing was performed as previously described using the following antimicrobial concentration ranges: gentamicin, 1–16 mg/L; azithromycin, 0.03–2 mg/L; cefixime, 0.002–0.25 mg/L; and ceftriaxone, 0.002–0.125 mg/L.

**Synergy testing**

The antimicrobial combinations of gentamicin, cefixime or ceftriaxone with azithromycin were investigated using the previously described agar dilution method, which determines the fractional inhibitory concentration index (FICI). The FICI was calculated as the sum of the FICs of the antimicrobials. The FIC of an antimicrobial was determined by establishing the MIC of the antimicrobial (A or B) in the combination (MICcomb) divided by the MIC of the antimicrobial acting alone (MICalone). The formula used to calculate the FICI is the following:

\[ \text{FICI} = \frac{\text{FIC}_A + \text{FIC}_B}{\text{MIC}_A/\text{MIC}_A + \text{MIC}_B/\text{MIC}_B} \]

An FICI ≤0.5 indicates synergy, an FICI >0.5 to 4 shows an additive or indifferent effect and an FICI >4 reflects an antagonistic interaction.

**Statistical analysis**

A χ² test was performed to determine differences for the groups of isolates in the antibiotic combinations. Observed differences were considered significant at *P*<0.05.

**Results**

The MICs for the control strains were all within the expected limits. The mean FICI for the three antimicrobial combinations was established as well as the corresponding standard deviation and FICI categorial interpretation (Table 1). The mean FICI ranged from 1.6 to 2.2 (Table 1). For each cefixime MIC category (≥0.125, 0.03–0.06 and ≤0.015 mg/L) the mean MIC of each antimicrobial when alone and in combination was additionally presented (Table 1).

**Cefixime or ceftriaxone plus azithromycin**

When cefixime or ceftriaxone was combined with azithromycin, all isolates showed additivity/indifference with a mean FICI of 2.0 for both combinations. When *N. gonorrhoeae* isolates were grouped according to the cefixime MIC, no significant difference in the mean FICI among the different groups of isolates was observed.

**Gentamicin plus azithromycin**

With the antimicrobial combination of gentamicin with azithromycin the gonococcal isolates showed a mean FICI of 1.7. No synergistic or antagonistic activity between these two antimicrobials was observed. Nonetheless, the antimicrobial activity of azithromycin was enhanced 2-fold in the presence of gentamicin in 72% of the isolates. When grouping according to the MIC of cefixime, no significant difference in the mean FICI among the different groups of isolates was observed.

**Discussion**

A previous study by Furuya et al. showed that the *in vitro* activities of cefixime and azithromycin were enhanced when combined, resulting in synergistic antimicrobial activity. Our study was performed to investigate if similar results would be obtained using recent clinical isolates from the UK and the same susceptibility testing methodology used in the national surveillance programme, which informs the national treatment guidelines. Additionally, we investigated the *in vitro* action of ceftriaxone and azithromycin, which is the current recommended treatment for gonorrhoea, and it is the first known study to carry out synergy testing using gentamicin with azithromycin.

Our study showed no *in vitro* synergy when cefixime or ceftriaxone was combined with azithromycin. Even though no synergy was observed, it is encouraging that no antagonistic activity was observed *in vitro*. However, previous *in vitro* studies have also shown that interaction between macrolides and cephalosporins can range from synergy to antagonism.

Gentamicin has potential for use in future treatment of gonococcal infections. Although the combination of gentamicin and azithromycin did not show synergy, enhancement of azithromycin activity in the presence of gentamicin was observed. As gentamicin and azithromycin have a similar target, but different binding sites (azithromycin binds to the 50S ribosomal subunit and gentamicin to the 30S subunit), and consequently both inhibit protein synthesis, it was predicted that antimicrobial synergistic activity may occur. Our data suggest that an aminoglycoside such as gentamicin can be seen as a potential integrant of a dual treatment, particularly with azithromycin. These data provide some laboratory evidence to support the ongoing clinical randomized trial in the USA that is investigating the use of azithromycin with gentamicin for gonorrhoea treatment (htp://clinicaltrials.gov; trial identifier NCT00926796) and will provide valuable clinical data.

It is important to mention that the determination of synergy is method-dependent and the interpretation of FICI values depends upon the calculation method used. For instance, Hsieh et al. calculated the FICI as the average of the total FICs, with an FIC for each well calculated along the growth–no growth surface. Using this method, lower FICI values for the antimicrobial combinations would be obtained for our data: 1.1 for azithromycin in combination with ceftriaxone and cefixime and 1.0 for gentamicin with azithromycin, although identical interpretation would be achieved for this study (all isolates showing indifference/additivity). Different methods of FICI calculation and methods of synergy testing make inter-laboratory comparison difficult. In addition, FICI interpretation depends upon the interpretation criteria, e.g. Furuya et al. interpreted FICI values between 0.5 and 1 as partial synergy. In this study, if partial synergy was considered no alteration in the results would be shown. However, if the Hsieh et al. method of FICI calculation was used, partial synergy would be observed in 63% of the isolates with gentamicin plus azithromycin and in 38% and 16% of the isolates with azithromycin plus cefixime and ceftriaxone, respectively. A defined *in vitro* test FICI calculation and interpretive categories are crucial to compare gonococcal combination therapy *in vitro* data, particularly when combination therapy is one of the few options for future gonorrhoea treatment.

Although our data are preliminary, the combination of ceftriaxone or cefixime with azithromycin appears not to cause any
Table 1. Mean MICs (mg/L) of each antimicrobial (alone and in combination), FICIs and interpretations of different antimicrobial combinations against 64 gonococcal isolates divided into three groups according to the MIC of cefixime.

<table>
<thead>
<tr>
<th>Antimicrobial combination</th>
<th>MIC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of isolates for which the antimicrobial combination presented</th>
<th>FICI&lt;sup&gt;b&lt;/sup&gt;</th>
<th>synergy</th>
<th>additivity/indifference</th>
<th>antagonism</th>
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<tbody>
<tr>
<td></td>
<td>AZM</td>
<td>CFM</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AZM + CFM</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>MIC ≥0.125</td>
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<tr>
<td>MIC 0.03–0.06</td>
<td>0.447 ± 0.403</td>
<td>0.358 ± 0.421</td>
<td>0.132 ± 0.029</td>
<td>1.6 ± 0.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MIC ≤0.015</td>
<td>0.279 ± 0.422</td>
<td>0.207 ± 0.202</td>
<td>0.009 ± 0.005</td>
<td>2.1 ± 0.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>total</td>
<td>0.331 ± 0.34</td>
<td>0.266 ± 0.270</td>
<td>0.062 ± 0.053</td>
<td>2.0 ± 0.6</td>
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<tr>
<td>AZM + CRO</td>
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<tr>
<td>MIC ≥0.125</td>
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<tr>
<td>MIC 0.03–0.06</td>
<td>0.447 ± 0.403</td>
<td>0.421 ± 0.201</td>
<td>0.035 ± 0.023</td>
<td>2.1 ± 0.3</td>
<td>0</td>
<td>0</td>
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<tr>
<td>MIC ≤0.015</td>
<td>0.305 ± 0.456</td>
<td>0.191 ± 0.106</td>
<td>0.008 ± 0.004</td>
<td>1.9 ± 0.4</td>
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<td>0</td>
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<tr>
<td>total</td>
<td>0.341 ± 0.345</td>
<td>0.298 ± 0.175</td>
<td>0.024 ± 0.018</td>
<td>2.0 ± 0.3</td>
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<tr>
<td>AZM + GEN</td>
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<tr>
<td>MIC ≥0.125</td>
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<tr>
<td>MIC 0.03–0.06</td>
<td>0.447 ± 0.403</td>
<td>0.293 ± 0.419</td>
<td>7.474 ± 1.611</td>
<td>1.6 ± 0.3</td>
<td>0</td>
<td>0</td>
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<tr>
<td>MIC ≤0.015</td>
<td>0.272 ± 0.412</td>
<td>0.159 ± 0.199</td>
<td>4.8 ± 1.642</td>
<td>1.7 ± 0.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>total</td>
<td>0.332 ± 0.339</td>
<td>0.204 ± 0.267</td>
<td>5.967 ± 2.066</td>
<td>1.7 ± 0.5</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

AZM, azithromycin; CFM, cefixime; CRO, ceftriaxone; GEN, gentamicin.

<sup>a</sup>Each value represents mean ± SD.

<sup>b</sup>FICI calculated for isolates with both an alone and in-combination MIC, and not for those isolates that gave MICs greater than or less than the MIC range tested.
antagonistic activity and so seems to strengthen the present option for the use of ESCs with azithromycin for gonorrhoea treatment. The results obtained with gentamicin support a possible future option for gonorrhoea treatment. Nevertheless, we believe that further studies are warranted to determine the *in vitro* effect and, more essentially, clinical studies are needed to determine the *in vivo* effect of antibiotic combinations on *N. gonorrhoeae* infection to ultimately provide more treatment options to fight this infection.

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**Transparency declarations**
None to declare.

**References**