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In vitro growth of multidrug-resistant *Neisseria gonorrhoeae* isolates is inhibited by ETX0914, a novel spiropyrimidinetrione ☆

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Abstract

Antimicrobial resistance in *Neisseria gonorrhoeae* has severely limited the number of treatment options, and the emergence of extended-spectrum cephalosporin resistance threatens the effectiveness of the last remaining recommended treatment regimen. This study assessed the in vitro susceptibility of *N. gonorrhoeae* to ETX0914, a novel spiropyrimidinetrione that inhibits DNA biosynthesis. In vitro activity was determined by agar dilution against 100 *N. gonorrhoeae* isolates collected from men presenting with urethritis in the USA during 2012–2013 through the Gonococcal Isolate Surveillance Project. The minimum inhibitory concentration (MIC) that inhibited growth in 50% (MIC_{50}) and 90% (MIC_{90}) of isolates was calculated for each antimicrobial agent. ETX0914 demonstrated a high level of antimicrobial activity against *N. gonorrhoeae*, including isolates with decreased susceptibility or resistance to currently available agents. The ability of ETX0914 to inhibit the growth of *N. gonorrhoeae* was similar to ceftriaxone, which is currently recommended in combination with azithromycin to treat gonorrhoea. The data presented in this study strongly suggest that ETX0914 should be evaluated in a clinical trial for the treatment of *N. gonorrhoeae*.

Keywords

Neisseria gonorrhoeae; Gonorrhoea; Antimicrobial susceptibility; Novel drug

1. Introduction

Gonorrhoea is a global health concern highlighted by the World Health Organization (WHO) and the US Centers for Disease Control and Prevention (CDC) as a bacterial infection with

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few remaining treatment options [1,2]. The development of antimicrobial resistance among *Neisseria gonorrhoeae* has required frequent changes in therapeutic management from the early use of sulfonamides to third-generation cephalosporins [3]. Most countries currently recommend a combination of ceftriaxone and azithromycin to treat *N. gonorrhoeae* infections [4–6]. Combination drug therapy involving two distinct antimicrobial classes with different mechanisms of action has been recommended in an attempt to slow down the progression of resistance to cephalosporins [4]. However, there continues to be an upward trend in the minimum inhibitory concentrations (MICs) of cephalosporins, particularly cefixime, among *N. gonorrhoeae* isolates collected and tested in countries with established antimicrobial resistance surveillance systems [7,8]. The likelihood that chemotherapeutic options currently recommended and used to treat gonorrhoea will become less effective over time has heightened the necessity to identify new antimicrobial agents for treatment.

Existing antimicrobial agents that inhibit the growth of *N. gonorrhoeae* impair bacterial DNA replication, protein synthesis or bacterial cell wall development. Ciprofloxacin had been recommended for the treatment of *N. gonorrhoeae* in the USA from 1993 to 2004, but the gradual emergence and spread of resistant strains facilitated its removal as a primary therapeutic option [9]. Strains acquired resistance to ciprofloxacin through mutations in the *gyrA* and *parC* genes that encode DNA gyrase and DNA topoisomerase, respectively, which are enzymes critical for DNA replication and decatenation [10]. A novel spiropyrimidinetrione, ETX0914 (Entasis Therapeutics, Waltham, MA), previously referred to as AZD0914, inhibits bacterial DNA biosynthesis through inhibition of type II topoisomerases, which includes DNA gyrase and topoisomerase IV[11]. The compound stabilises the cleaved covalent of DNA gyrase and prevents religation of DNA bound to the topoisomerase tetramer [12]. Although ETX0914 and ciprofloxacin both target DNA gyrase, the distinct binding modes are functionally sufficient for ETX0914 to inhibit the growth of ciprofloxacin-resistant strains [11,13].

In this study, the in vitro activity of ETX0914 against a well characterised panel of *N. gonorrhoeae* isolates collected from contemporary clinical infections in the USA was examined.

2. Materials and methods

2.1. Neisseria gonorrhoeae *isolates*

The antimicrobial susceptibility test panel consisted of 100 *N. gonorrhoeae* isolates collected between 2012 and 2013 for the Gonococcal Isolate Surveillance Project (GISP) in the USA. Briefly, GISP is a US-based sentinel surveillance system used to monitor trends in *N. gonorrhoeae* antimicrobial susceptibility and to inform national treatment recommendations [14]. Isolates are collected from consecutive visits of men presenting with urethral discharge at 1 of 25–30 sexually transmitted disease (STD) clinics across the USA. Antimicrobial susceptibility testing using agar plate dilution is performed at five CDC-funded reference laboratories, and isolates determined to be resistant or less susceptible to azithromycin, cefixime, ceftriaxone or ciprofloxacin are shipped to the CDC for confirmation of the reference laboratory results and for archival storage at −70 °C in trypticase soy broth with 20% glycerol. The quality of the MIC data was assured using *N. gonorrhoeae* quality

reference strains ATCC 49226, F-28, P681E, CDC 10328, CDC 10329, SPL-4 and SPJ-15 with each antimicrobial susceptibility test run. MIC results were only accepted if the values of the seven quality control strains were within range [14]. Selection of *N. gonorrhoeae* isolates for testing against ETX0914 was based on those that were considered to be resistant or had reduced susceptibility to one or more of these antimicrobial agents. For ETX0914 testing, quality control strains WHO F, WHO G and WHO K were included to ensure data accuracy.

2.2. Agar dilution for minimum inhibitory concentration determination

The procedures set forth by the Clinical and Laboratory Standards Institute (CLSI) for agar plate dilution were followed to determine the MICs for all isolates [15]. Briefly, a bacterial suspension equivalent to a 0.5 McFarland standard was prepared following an overnight culture on chocolate agar and was used to inoculate doubling dilutions of antimicrobial agents incorporated into GC II agar supplemented with 1% IsoVitaleX™. Following overnight incubation at 37 °C with 5% CO_2, the MIC was recorded. The antimicrobial susceptibility of each isolate to azithromycin, cefixime, ceftriaxone, ciprofloxacin, penicillin and tetracycline was determined first at a GISP reference laboratory and was confirmed at the CDC. For ETX0914, all susceptibility testing was performed at the CDC with a dilution range from 0.002 to 32 μg/mL. Ciprofloxacin susceptibility was repeated at the CDC using the range of0.002–32 μg/mL during ETX0914 testing to assess the reproducibility of the test and to ensure quality. *Neisseria gonorrhoeae* strains WHO F, WHO G and WHO K were selected for ETX0914 and repeat ciprofloxacin susceptibility testing. The MICs that inhibited 50% (MIC_{50}) and 90% (MIC_{90}) of isolates were calculated for each antimicrobial agent. Isolates were also grouped by low or high MICs or by CLSI interpretative criteria where applicable. The modal MICs for isolates within each grouping were calculated and were compared with the ETX0914 modal value from that grouping.

3. Results

All isolates tested had relatively low MICs to ETX0914 and a wide range of values to other antimicrobial agents (Table 1). The highest ETX0914 MIC was 0.25 μg/mL, which was less than the highest recorded for ceftriaxone and ciprofloxacin. Eleven isolates had ceftriaxone MICs of 0.125 μg/mL and all had an ETX0914 MIC of0.06 μg/mL. One isolate with a ceftriaxone MIC of 0.5 μg/mL had a corresponding ETX0914 MIC of 0.008 μg/mL. The MIC_{90} value for ETX0914 was 0.125 μg/mL, which was comparable with ceftriaxone and cefixime at 0.125 μg/mL and 0.25 μg/mL, respectively. In contrast, the MIC_{90} values for azithromycin, ciprofloxacin, penicillin and tetracycline were considerably higher. The MIC_{50} and MIC_{90} values for azithromycin were 1 μg/mL and 8 μg/mL, respectively. One isolate with an azithromycin MIC of 256 μg/mL had an ETX0914 MIC of0.06 μg/mL. For ciprofloxacin, both the MIC_{50} and MIC_{90} values were 16 μg/mL. All quality control strains were within range for ciprofloxacin and had reproducible results.

Comparing the modal MIC values between isolates of various MIC groupings (i.e. resistant versus susceptible, and high versus low MIC) revealed a consistent modal MIC of 0.06 μg/mL for ETX0914 regardless of the modal MIC for any other antimicrobial agent (Table

2). The ETX0914 modal value was one dilution lower than the ceftriaxone modal of 0.125 μg/mL for the high ceftriaxone MIC grouping. There were 70 ciprofloxacin-resistant isolates (MIC ≥ 1.0 μg/mL) (ciprofloxacin-resistant MIC range, 2–32 μg/mL; modal MIC 16 μg/mL); the ETX0914 modal value was 0.06 μg/mL when tested with these ciprofloxacin-resistant isolates.

4. Discussion

The continual progress towards antimicrobial resistance among *N. gonorrhoeae* challenges healthcare providers faced with treating this highly prevalent global STD [1,2]. A total of 350,062 gonorrhoea cases in the USA were reported to the CDC in 2014 [16]. The rate of reported cases in the USA increased from 105.3 per 100,000 in 2013 to 110.7 per 100,000 in 2014. Currently recommended combination therapy with ceftriaxone and azithromycin remains effective in treating gonorrhoea, although MICs have increased slightly [17]. Furthermore, sporadic cases of failed therapy have been reported in various countries [18]. If untreated, *N. gonorrhoeae* infections can progress to serious sequelae such as pelvic inflammatory disease, urethritis and disseminated gonococcal infection.

The data in this report strongly suggest that ETX0914 is highly effective at inhibiting the in vitro growth of *N. gonorrhoeae*. The panel of isolates tested against ETX0914 was selected based on multidrug resistance and/or decreased susceptibility to contemporary therapeutic options. The MIC range for ETX0914 remained low irrespective of MICs for all other tested antimicrobial agents. Ceftriaxone, considered as one of the final treatment choices for gonorrhoea, had MICs that were similar to ETX0914. In addition to lower MICs, ETX0914 is administered orally, which compares favourably with the need for intramuscular injection of ceftriaxone. A previous study reported that ETX0914 was effective against *N. gonorrhoeae* growth using a panel of European isolates [19]. Collectively, ETX0914 may be an international option for the treatment of gonorrhoea and is currently in a phase 2 clinical trial.

The hallmark of resistance, genetic alteration through spontaneous mutations or acquired genetic material, implies that any organism capable of mutating will selectively adapt to render chemotherapeutic agents less effective. Recent recommendations for treating gonorrhoea with combination therapy involving antimicrobial agents with different mechanisms of action are based on the low likelihood that *N. gonorrhoeae* would develop simultaneous resistance [4]. The data in the current study demonstrated ETX0914 MICs that were similar to current therapeutic options for gonorrhoea. Although ETX0914 has a similar mechanism of action as ciprofloxacin in targeting DNA gyrase, the binding modes are sufficiently distinct [11] that ciprofloxacin-resistant isolates are highly susceptible to ETX0914. ETX0914 shows promise for treatment of gonococcal infections, including ciprofloxacin-resistant infections, and particularly as part of combination therapy with a second antimicrobial agent [19,20].

Research and discovery of new antimicrobial agents for the treatment of gonorrhoea are critical for the prevention of potentially life-altering disease. ETX0914 has excellent activity

against a multidrug-resistant panel of *N. gonorrhoeae* and is currently being further evaluated in a clinical trial for the treatment of gonorrhoea.

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Table 1

Comparison of the minimum inhibition concentrations (MICs) of 100 *Neisseria gonorrhoeae* isolates tested against various antimicrobial agents.

Antimicrobial agent	Concentration range tested (μg/mL)	MIC (μg/mL)		
		Range	MIC_{50}	MIC_{90}
ETX0914	0.002–32	0.008–0.25	0.06	0.125
Azithromycin	0.032–256	0.125–256	1	8
Ceftriaxone	0.001–4	0.004–0.5	0.06	0.125
Cefixime	0.001–4	0.015–1	0.25	0.25
Ciprofloxacin	0.002–32	0.004–32	16	16
Penicillin	0.004–64	0.25–8	2	4
Tetracycline	0.032–32	0.25–32	2	4

$MIC_{50/90}$, MIC that inhibited growth in 50% (MIC_{50}) and 90% (MIC_{90}) of isolates.

Table 2

Comparison of *Neisseria gonorrhoeae* modal minimum inhibition concentrations (MICs) between various antimicrobial agents and ETX0914.

Antimicrobial agent	MIC range (μg/mL)	MIC interpretation[a]	No. of isolates	Modal MIC (μg/mL)	Modal ETX0914 MIC(μg/mL)
Azithromycin	0.125–0.5	Susceptible	40	0.5	0.06
	1–256	Reduced susceptibility	60	2	0.06
Cefixime	0.015–0.125	Susceptible	39	0.015	0.06
	0.25–1	Reduced susceptibility	61	0.25	0.06
Ceftriaxone	0.004–0.06	Susceptible	88	0.06	0.06
	0.125–0.5	Reduced susceptibility	12	0.125	0.06
Ciprofloxacin	0.004–0.03	Susceptible	30	0.015	0.06
	2–32	Resistant	70	16	0.06
Penicillin	0.25–1	Susceptible	32	1	0.06
	2–8	Resistant	68	2	0.06
Tetracycline	0.25–1	Susceptible	19	1	0.06
	2–32	Resistant	81	4	0.06

[a]Interpretative criteria are based on Clinical and Laboratory Standards Institute (CLSI) breakpoints or Gonococcal Isolate Surveillance Project (GISP) alert values where applicable. For azithromycin, arbitrary breakpoints were set in the absence of CLSI breakpoints. For ceftriaxone and cefixime, GISP alert values were used since they are one dilution below the CLSI breakpoint and suggest a trend towards reduced susceptibility.