

***In vitro* activity of the novel oral antimicrobial SMT-571, with a new mechanism of action, against MDR and XDR *Neisseria gonorrhoeae*: future treatment option for gonorrhoea?**

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Background: Lack of effective treatment of gonorrhoea due to increasing antimicrobial resistance in *Neisseria gonorrhoeae* is a serious threat to the management and control of the infection. Novel antimicrobials are required to prevent the infection becoming untreatable.

Objectives: Herein, we investigated the *in vitro* activity of a novel small-molecule antimicrobial with a new mechanism of action, SMT-571, against a large collection of clinical *N. gonorrhoeae* isolates ($n = 228$) and international gonococcal reference strains ($n = 34$), including numerous MDR and XDR gonococcal isolates.

Methods: MICs of SMT-571 were determined by agar dilution and MICs of ceftriaxone, cefixime, azithromycin, ciprofloxacin, ampicillin, spectinomycin and tetracycline were determined by Etest.

Results: SMT-571 showed potent *in vitro* activity against all the tested *N. gonorrhoeae* isolates ($n = 262$). The MICs ranged from 0.064 to 0.125 mg/L and the MIC₅₀, MIC₉₀ and modal MIC were all 0.125 mg/L. No cross-resistance or correlation between the MICs of SMT-571 and comparator agents was seen.

Conclusions: SMT-571 demonstrated potent *in vitro* activity against all tested gonococcal isolates and no cross-resistance to previously and currently used antimicrobials was seen. With its promising supplementary *in vitro* and *in vivo* preclinical data, including high levels of oral bioavailability, SMT-571 could be an effective option for the oral treatment of gonorrhoea. Randomized controlled clinical trials for gonorrhoea that examine the treatment efficacy, pharmacokinetics/pharmacodynamics, toxicity and safety of SMT-571, and include urogenital and extragenital (rectal and pharyngeal) samples, are crucial.

Introduction

Lack of effective treatment of gonorrhoea due to increasing antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* is a serious threat to the management and control of the infection. *N. gonorrhoeae* is estimated to cause 78 million new infections among adults globally each year¹ and gonorrhoea resulted in 225 400 years lived with disability per year and 313 900 disability-adjusted life years according to the global burden of disease study in 2013.^{2,3} The mainstay in controlling gonorrhoea is effective antimicrobial treatment together with appropriate prevention, sensitive and specific diagnostics, contact tracing followed by treatment, and follow-up of patients including test of cure. *N. gonorrhoeae* has

developed AMR to all formerly used gonorrhoea therapeutic agents.⁴ The susceptibility to the last remaining class for effective empirical first-line monotherapy, the extended-spectrum cephalosporins (ESCs; ceftriaxone and cefixime), has also substantially decreased during the past decade.^{4–6} Previously, only sporadic cases of ceftriaxone-resistant isolates have been identified across many countries.^{7–11} However, in 2015–18 the first international spread of a ceftriaxone-resistant strain (FC428, initially cultured in 2015 in Japan¹²) was documented, with cases reported from Japan, Australia, Canada, Denmark, Ireland and France,^{12–17} and in 2018 the first cases with ceftriaxone resistance plus high-level azithromycin resistance were described in the UK and Australia.^{18,19} Consequently, enhanced AMR

surveillance for *N. gonorrhoeae*, but also novel antimicrobials for effective treatment of gonorrhoea, are essential.^{4,20}

SMT-571 is a novel, orally available, small-molecule antimicrobial under development as a therapy specifically for urogenital, rectal and pharyngeal *N. gonorrhoeae* infections, whose discovery and development have been enabled by the Discuva platform.^{21,22} Briefly, the Discuva platform allows screening of novel bacterial targets, elucidation of mechanism(s) of action and AMR profiles, and includes proprietary high-density transposon libraries (transposons inserted every 5–10 bp) generated in defined bacterial strains.^{21,22} SMT-571 has shown bactericidal activities against *N. gonorrhoeae* and novel mechanism(s) of action associated with cell division.^{21,22} Previously,²³ the susceptibility of *N. gonorrhoeae* to SMT-571 had only been evaluated for a small panel of gonococcal isolates, which demonstrated relatively low MICs of currently used antimicrobials such as ESCs and azithromycin.

Our aim was to comprehensively investigate the *in vitro* activity of SMT-571, which acts through a new mechanism of action, against a large collection of clinical *N. gonorrhoeae* isolates ($n = 228$) and international gonococcal reference strains ($n = 34$), including numerous MDR and XDR gonococcal isolates. MDR and XDR *N. gonorrhoeae* were defined based on an updated version of the definitions published by Tapsall et al.,²⁴ i.e. MDR (XDR) isolates were resistant to one (two) or more of the antimicrobials in category 1 (antimicrobials currently generally recommended for gonorrhoea treatment: ceftriaxone, cefixime and azithromycin) and resistant to two (three) or more of the antimicrobials listed in category 2 (antimicrobials now less frequently used for gonorrhoea treatment: spectinomycin, ciprofloxacin, ampicillin and tetracycline).

Materials and methods

The work was performed at the WHO Collaborating Centre for Gonorrhoea and Other Sexually Transmitted Infections, Department of Laboratory Medicine, Microbiology, Örebro University Hospital, SE-701 85 Örebro, Sweden.

N. gonorrhoeae isolates

The collection of 262 *N. gonorrhoeae* isolates investigated in the present study represented a large geographically (mainly global representativeness), temporally (obtained from 1991 to 2018), phenotypically and genetically diverse selection. The collection consisted of 34 international gonococcal reference strains, 100 consecutive clinical Swedish gonococcal isolates collected in 2016 and 128 gonococcal clinical isolates selected for their AMR phenotype. The international gonococcal reference strains included the 2016 WHO reference strains ($n = 14$),^{25,26} WHO A-E, WHO I, WHO J, CCUG 41810–41813, A02, A17, A25, G07-700, A04, G07-672, G06-1153, FA1090 and MS11. The selected isolates included isolates with *in vitro* or clinical resistance to ESCs (12 ceftriaxone-resistant isolates and 25 cefixime-resistant isolates), high-level clinical resistance to other antimicrobials previously used for treatment of gonorrhoea and a large number of MDR ($n = 57$) and XDR ($n = 14$) gonococcal isolates.

Antimicrobial susceptibility testing

The MICs (mg/L) of SMT-571 (Summit Therapeutics, Cambridge, UK) were determined using an agar dilution technique, according to current CLSI guidelines (www.clsi.org). The MICs (mg/L) of ceftriaxone, cefixime, azithromycin, ciprofloxacin, spectinomycin, tetracycline and ampicillin were determined using the Etest method (AB bioMérieux, Marcy-l'Étoile, France), in accordance with the manufacturer's instructions. Only whole MIC dilutions

are reported in the present study. With the exception of SMT-571, for which no interpretative criteria currently exist, all MICs were interpreted as susceptible, intermediately susceptible or resistant according to the clinical breakpoints stated by EUCAST (www.eucast.org).

Results

The susceptibility results for SMT-571 and seven antimicrobials currently or previously recommended for the treatment of gonorrhoea are summarized in Table 1. The isolates are divided into different groups, i.e. all isolates, consecutive isolates, selected isolates and international reference strains (Table 1).

SMT-571 displayed potent *in vitro* activity against all the tested *N. gonorrhoeae* isolates ($n = 262$). The MIC ranged from 0.064 to 0.125 mg/L and the MIC₅₀, MIC₉₀ and modal MIC were all 0.125 mg/L. With the exception of the ESCs (ceftriaxone and cefixime), the modal MIC, MIC₅₀ and MIC₉₀ of the additional antimicrobials tested were all substantially higher than those observed for SMT-571. No cross-resistance or correlation between the MICs of SMT-571 and the MICs of any of the other tested currently or previously used antimicrobials was observed, with the Spearman's rank correlation coefficient ranging from 0.024 to 0.261 when comparing the MICs of SMT-571 and the MICs of the additional antimicrobials (data not shown).

Discussion

This study is the first broad evaluation of the *in vitro* activities of the promising new-mechanism novel small-molecule antimicrobial SMT-571 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, MDR and XDR gonococcal isolates. The activity of SMT-571 was also compared with the activities of seven antimicrobials that are currently or were previously recommended for gonorrhoea treatment, i.e. ceftriaxone, cefixime, azithromycin, spectinomycin, ciprofloxacin, ampicillin and tetracycline. SMT-571 displayed potent activity, with MICs of 0.064 to 0.125 mg/L for all the tested *N. gonorrhoeae* isolates, and no cross-resistance with any antimicrobials previously used for treatment of gonorrhoea.

Our results correspond well with the limited unpublished data presented at international conferences,^{23,27} where potent *in vitro* activity of SMT-571 was shown against the 2008 WHO reference strains ($n = 8$)²⁵ and WHO X²⁶ (SMT-571 MIC of 0.09 mg/L) and a clinical strain panel from NIAID preclinical services ($n = 91$; SMT-571 MICs range from 0.11 to 0.22 mg/L). Time-kill studies using the WHO M reference strain^{25,26} established a rapid bactericidal profile and low level of resistance frequency ($<8.2 \times 10^{-10}$) at $4 \times$ MIC with no resistant mutants identified after 144 h at $4 \times$ MIC.^{23,27} In addition to its favourable microbiological profile and oral pharmacokinetics, SMT-571 exhibits good *in vitro* and *in vivo* absorption, distribution, metabolism, excretion and toxicological (ADMET) profiles.^{23,27}

In conclusion, SMT-571 is a novel small-molecule antimicrobial, with a novel mechanism of action selectively targeting bacterial cell division,^{21,22} with high *in vitro* activity against a large collection of gonococcal international reference strains and clinical isolates, including numerous MDR and XDR isolates, and very low resistance mutation frequency. Together with additional promising *in vitro* and *in vivo* preclinical studies and its appropriate oral bioavailability,

Table 1. MIC range, MIC₅₀, MIC₉₀ and modal MIC values of SMT-571 and therapeutic antimicrobials currently or previously recommended for *N. gonorrhoeae* isolates

Antimicrobial, isolate group (n)	MIC range (mg/L)	MIC ₅₀ (mg/L)	MIC ₉₀ (mg/L)	Modal MIC (mg/L)	S/I/R ^a (%)
SMT-571					
all isolates (262)	0.064–0.125	0.125	0.125	0.125	ND
consecutive isolates (100)	0.064–0.125	0.064	0.125	0.064	ND
selected isolates (128)	0.064–0.125	0.125	0.125	0.125	ND
reference strains (34)	0.064–0.125	0.125	0.125	0.125	ND
Ceftriaxone (262)	<0.002–4	0.008	0.064	0.004	96.8/ND/3.2
Cefixime (262)	<0.016–8	<0.016	0.25	<0.016	88.9/ND/11.1
Azithromycin (262)	0.016 to >256	0.5	2	1	44.0/13.9/42.1
Spectinomycin (262)	4 to >1024	16	16	16	98.0/ND/2.0
Ciprofloxacin (262)	<0.002 to >32	2	>32	>32	39.7/0.0/60.3
Ampicillin (262)	<0.016 to >256	0.5	4	1	27.4/59.1/13.5
Tetracycline (262)	0.125–256	2	16	4	22.2/17.5/60.3

ND, not determined due to lack of interpretative criteria.

MICs were determined using an agar dilution technique for SMT-571 and using the Etest method for the additional antimicrobials.

^aS, susceptible; I, intermediately susceptible; R, resistant. EUCAST clinical breakpoints (www.eucast.org) were applied for all antimicrobials, with the exception of SMT-571.

SMT-571 could be an effective option for single-dose oral treatment of gonorrhoea. SMT-571 is currently undergoing Investigational New Drug (IND)-enabling studies ahead of a Phase 1 study, which will lead to randomized controlled clinical trials to explore patients with both urogenital and extragenital, especially pharyngeal, gonorrhoea.

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Transparency declarations

C. M., N. K. and P. M. are employed by Summit Therapeutics, Cambridge, UK. S. J. and M. U.: none to declare.

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