In-vitro activity of iclaprim and comparison agents tested against *Neisseria gonorrhoeae* including growth supplement effects

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ABSTRACT

Background: Iclaprim (ICL) is a novel diaminopyrimidine which is currently in Phase III development. ICL acts as a dihydrofolate reductase inhibitor with a similar mode of action as trimethoprim (TMP). This study was conducted to determine the activity of ICL compared to TMP and other commonly prescribed drug classes against *Neisseria gonorrhoeae* (NG). The effect of growth supplements on ICL and TMP was determined on a subset of gonococci.

Methods: A global collection of 156 NG isolates, including strains with various penicillin (PEN) and ciprofloxacin (CIP) susceptibility (S) patterns, was tested by CLSI agar dilution methods using GC agar with the defined growth supplement. A subset of strains was tested without supplement to evaluate the effects of cysteine and thiamine HCL on antimicrobial activity of ICL and TMP.

Results: The activity of ICL and comparison agents is listed in the table.

Antimicrobial agent	MIC (µg/mL)		% Susceptibility[a]
	50%	90%	
ICL	4	8	-[b]
TMP	64	>64	-
Azithromycin	0.25	0.5	-
Ceftriaxone	0.015	0.03	100.0
Ciprofloxacin	0.25	>4	37.8
Levofloxacin	0.25	4	56.4
Penicillin	1	>4	7.7
Tetracycline	1	2	14.1

[a]Susceptibility as defined by the CLSI (2006). For levofloxacin, the ofloxacin breakpoints were applied.
[b]- = no criteria have been published.

ICL was at least 16-fold more active than TMP overall, and the S patterns of PEN, CIP or the geographic origin of the strain had no effect on the potency of ICL or TMP. The effects of cysteine and thymidine in the medium supplement was more pronounced for testing TMP compared to ICL, typically increasing MIC values by two-fold.

Conclusions: ICL (MIC_{50}/MIC_{90}, 4/8 µg/mL) was more potent than TMP (MIC_{50}/MIC_{90} 64/>64 µg/mL) against NG isolates. Growth supplement did not significantly affect the MIC values with ICL, but did show a more pronounced affect on the MIC values with TMP. The lack of cross resistance between ICL and other commonly used anti-gonococcal agents is promising. ICL may provide an alternative treatment option for the emerging quinolone-resistant NG.

INTRODUCTION

Iclaprim is a novel diaminopyrimidine antibiotic with a mode of action similar to that of trimethoprim, but with greater potency against their common target, microbial dihydrofolate reductase (DHFR), an enzyme essential in folate biosynthesis. This increased potency of iclaprim justifies the finding that iclaprim has retained activity against strains bearing trimethoprim-resistant DHFRs. The antibacterial spectrum of iclaprim, which is currently in Phase III clinical development for complicated skin and skin structure infections (cSSSI), has been studied extensively *in vitro*.

Differently from trimethoprim, iclaprim has been found to be active against chlamydiae and interestingly against *Chlamydia trachomatis*, a pathogen involved in sexually transmitted diseases (STDs). The activity of iclaprim against another important STD causative pathogen, *N. gonorrhoeae*, has not been evaluated before. This study aimed to assess the activity of iclaprim, trimethoprim and other comparison agents against a large collection of *Neisseria gonorrhoeae* isolates with different resistant phenotypes. Iclaprim and trimethoprim were also evaluated against a small subset of *N. gonorrhoeae* for possible effects of growth supplements commonly used for the neisseria susceptibility testing.

METHODS

Recent clinical isolates of *N. gonorrhoeae* were collected from numerous sites in the United States (USA), where resistance to commonly prescribed anti-gonococcal agents is known to be endemic. These sites were located in New York (11 isolates), California (five isolates), Washington (34 isolates), Oregon (12 isolates), Hawaii (23 isolates) and Ohio (10 isolates). The remaining strains were from older stock cultures collected from medical centers located outside the USA (including Japan and the Netherlands). The overall collection consisted of 92.3% penicillin non-susceptible strains (17.4% β-lactamase positive) and 62.2% ciprofloxacin non-susceptible strains. Several of the ciprofloxacin-resistant isolates had documented amino acid mutations within the quinolone resistance-determining region.

Isolates were tested against iclaprim, trimethoprim, fluoroquinolones (ciprofloxacin and levofloxacin), β-lactams (penicillin and ceftriaxone), azithromycin and tetracycline. Susceptibility to the agents was determined using agar dilution methods according to CLSI guidelines by GC agar base with 1% defined growth supplement. Susceptibility percentages were based upon CLSI interpretive criteria (M100-S17, 2007), and levofloxacin susceptibility was defined using ofloxacin breakpoints. A subset of 15 *N. gonorrhoeae* isolates were tested on GC agar without the addition of cysteine and thiamine HCL in the growth supplement to determine if there were alterations in the test results. *N. gonorrhoeae* ATCC 49226 and *Escherichia coli* ATCC 25922 were utilized as quality control (QC) strains; all QC results were within QC ranges as published by the CLSI.

RESULTS

Table 1. Activity of iclaprim and several comparison agents tested against a collection of 156 *N. gonorrhoeae* isolates

Antimicrobial agent	MIC (µg/mL)			% Susceptibility[a]
	50%	90%	Range	
Iclaprim	4	8	2->16	-[b]
Trimethoprim	64	>64	8->64	-
Azithromycin	0.25	0.5	0.03-2	-
Ceftriaxone	0.015	0.03	≤0.002-0.12	100.0
Ciprofloxacin	0.25	>4	≤0.008->4	37.8
Levofloxacin	0.25	4	≤0.008->4	56.4
Penicillin	1	>4	0.015->4	7.7
Tetracycline	1	2	0.06->4	14.1

[a]Susceptibility as defined by the CLSI (M100-S17, 2007). For levofloxacin, the ofloxacin breakpoints were applied.
[b]- = no criteria have been published.

- Iclaprim (MIC_{50}; 4µg/mL) was approximately 16-fold more active than trimethoprim (MIC_{50}; 64 µg/mL) against the 156 *N. gonorrhoeae* isolates tested in this study (Table 1). Only two strains (1.3%) had an iclaprim MIC value >16 µg/mL compared to 96.2% of strains tested against trimethoprim.
- Ceftriaxone was the most potent agent (MIC_{90}; 0.03 µg/mL) followed by azithromycin (MIC_{90}; 0.5 µg/mL; Table 1). Levofloxacin was more active than ciprofloxacin with susceptibility rates of 56.4% and 37.8%, respectively, while penicillin and tetracycline showed limited activity against this collection of isolates (only 7–14% susceptibility).

Table 2. Comparative antimicrobial activity of iclaprim and trimethoprim tested against 156 isolates of *N. gonorrhoeae* by CLSI methods.

Resistance subsets (no. tested)	Iclaprim MIC (µg/mL)		Trimethoprim MIC (µg/mL)	
	50%	90%	50%	90%
Penicillin				
Susceptible (12)	4	8	64	64
Intermediate (85)	8	16	64	>64
Resistant (59)	4	8	32	>64
Ciprofloxacin				
Susceptible (59)	8	16	64	>64
Intermediate (47)	4	8	32	64
Resistant (50)	8	8	64	>64
Geographic origin				
USA (95)	8	8	64	>64
Rest of world (61)	4	8	32	64
All strains (156)	4	8	64	>64

- Co-resistance to other drug classes was not observed as the activity of iclaprim and trimethoprim was not affected by penicillin or ciprofloxacin resistances (Table 2).

Table 3. Effects of the cysteine and thiamine HCL containing supplement on the activity of iclaprim and trimethoprim (15 *N. gonorrhoeae* strains) using the CLSI agar dilution method (2007).

Antimicrobial	Ratio for MIC results (with/without supplement)					
	0.25	0.5	1	2	4	8
Iclaprim	0	4[a]	5[a]	5[a]	1	0
Trimethoprim	0	2[a]	5[a]	7[a,b]	0	1

[a]Within acceptable test variation = 93.3% of results at ± one log_2 dilution step.
[b]Modal value was skewed higher by one log_2 MIC dilution step.

- The MIC results of iclaprim were unaffected when utilizing GC agar with or without the L-cysteine or thiamine HCL in the defined growth supplement (Table 3). Trimethoprim MIC values were skewed higher with a one log_2 dilution shift in the modal value for strains tested with the supplement.

CONCLUSIONS

- **Iclaprim was demonstrated to possess superior potency (16-fold) when compared to trimethoprim against isolates of *N. gonorrhoeae* as well as strains resistant to other antimicrobial classes.**
- **Iclaprim was not significantly affected by the presence of growth supplements commonly used for the susceptibility testing of *N. gonorrhoeae*.**
- **These data warrant further investigational studies to evaluate the potential of iclaprim in STD therapies.**

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