In vitro activity of iclaprim, a novel diaminopyrimidine, tested against β-hemolytic streptococci from the USA and Europe: results from the International Study of Iclaprim Susceptibility (ISIS)

R.N. Jones[1], T.R. Fritsche[1], S. Hawser[2], K. Islam[2], H.S. Sader[1].

[1]*JMI Laboratories, North Liberty, Iowa, USA,* [2]*Arpida AG, Reinach, Switzerland*

***H.S. Sader**, MD, PhD,*
Director, International Surveillance Programs
JMI Laboratories, 345 Beaver Kreek Centre, Suite A, North Liberty, Iowa, 52317, USA
Phone: +1 319 665-3370 Fax: +1 319 665-3371 e-mail: helio-sader@jmilabs.ca

ABSTRACT

Background: Group A *Streptococcus* (GAS) is an important cause of cutaneous infections, while group B *Streptococcus* (GBS) is the predominant cause of neonatal sepsis. Iclaprim (ICL) is a novel antimicrobial agent that selectively inhibits bacterial dihydrofolate reductase with a similar mode of action as trimethoprim (TMP). We evaluated the activity of ICL and various comparators against a large collection of recent clinical strains of β-hemolytic streptococci (βHS).

Methods: 808 strains of βHS (604 GAS and 204 GBS) were susceptibility (S) tested by CLSI broth microdilution method against ICL, TMP, co-trimoxazole (TMP/SMX) and comparators. MBC values for ICL, TMP and vancomycin (VAN) was assessed for a subset of 40 strains (20 GAS and 20 GBS) by plating broth from MIC wells onto appropriate agar media.

Results: ICL was 16-fold more potent than TMP and 4-fold more potent than TMP/SMX against GAS (see Table). GBS showed slightly higher ICL and TMP MIC values compared to GAS. No significant difference was observed between strains from the USA and EU regarding the activity of these 3 compounds. The highest ICL MIC values were 0.12 µg/mL for GAS and 0.5 µg/mL for GBS. Nine GAS (45% of strains tested) had MBC/MIC ratios ranging from 1 to 8, while 11 strains (55.0%) showed MBC/MIC ratio ≥32. Among GBS, MBC/MIC ratio ≤4 was most often (65.0%) observed for ICL. By contrast, all βHS tested for MBC showed VAN bacteriostatic activity (MBC/MIC, ≥32).

Antimicrobial susceptibility of β-hemolytic streptococci

Antimicrobials	MIC_{50} (µg/mL) / MIC_{90} (µg/mL) /%S	
	Group A (n = 604)	Group B (n = 204)
ICL	0.015/0.03/-[a]	0.12/0.25/-
TMP	0.25/0.5/-	1/4/-
TMP/SMX	0.06/0.12/-	0.06/0.12/-
Erythromycin	≤0.12/>4/83	≤0.12/>4/73
Clindamycin	≤0.12/≤0.12/96	≤0.12/>4/89
Tetracycline	≤0.5/≤0.5/90	>16/>16/22
Levofloxacin	0.5/1/100	0.1/1/99.5

[a]- = no breakpoint has been established by CLSI.

Conclusions: ICL was highly active against recent βHS collected in the USA and EU, and should be further evaluated for the treatment of infections caused by GAS and GBS.

INTRODUCTION

Group A *Streptococcus (S. pyogenes)* is the most frequent cause of bacterial pharyngitis and an important cause of a variety of cutaneous and systemic infections. On the other hand, group B *Streptococcus (S. agalactiae)* has become the predominant cause of septicemia and meningitis in neonates and children younger than 3 months.

Iclaprim is a novel dihydrofolate reductase (DFHR) inhibitor belonging to the diaminopyrimidine class of antibiotics for which trimethoprim (TMP) is the most well-known representative. TMP is frequently used in combination with sulfamethoxazole (SMX) and this combination has been used in clinical practice for almost five decades. SMX is highly synergistic with TMP as a result of inhibition of two sequential enzymes in the folate pathway, thereby enhancing the potency and bactericidal activity as well as reducing the potential for resistance development. However, SMX is often associated with allergic reactions. In contrast, iclaprim by itself shows a potent activity against a variety of pathogens and exhibits a potent bactericidal action against *Staphylococcus aureus* and *Streptococcus pneumoniae*.

Iclaprim is being developed and administered as a stand-alone therapy for serious Gram-positive bacterial infections. The compound has been granted fast-track product designation and has recently completed two pivotal Phase III clinical studies in complicated skin and skin structures infections (cSSSI).

In this present study, we evaluated the comparative potency and bactericidal activity of iclaprim tested against groups A and B streptococcal strains collected from United States (USA) and European medical centers as part of the International Study on Iclaprim Susceptibility (ISIS) surveillance program.

METHODS

Bacterial isolates: A total of 808 strains, including 604 group A streptococci (301 from USA and 303 from European medical centers) and 204 group B streptococci (102 each from the USA and Europe) were evaluated in this surveillance trial. All organisms were collected between 2004 and 2006 and originated from various infection sources including bloodstream infections (46%) and skin and soft tissue infections (30%). MBC values for iclaprim and vancomycin were assessed for a randomly selected subset of 20 group A and 20 group B streptococcal strains.

Susceptibility testing: MIC values were evaluated by Clinical and Laboratory Standards Institute (CLSI) broth microdilution method per M7-A7 [2006]. Quality control (QC) ranges and interpretive criteria for comparator compounds as published in CLSI M100-S17 (2007). Similarly, QC ranges for iclaprim were those published in M100-S17 (2007).

Bactericidal activity: MBC values were assessed on 40 strains by plating the broth onto appropriate growth media from the microdilution tray wells at the MIC and at least five $\log_2$ dilutions above the MIC for each organism. Quantitative colony counts were per formed on the starting inoculum. The lowest concentration of antimicrobial agent that killed ≥99.9% of the starting test inoculum was defined as the MBC endpoint.

RESULTS

Table 1: Activity of iclaprim and comparator agents tested against β-hemolytic streptococci.

Antimicrobial agent	MIC_{50}	MIC_{90}	Range	% susceptible /resistant[a]
Group A				
USA (301)				
Iclaprim	0.015	0.03	≤0.004-0.12	- / -[b]
Trimethoprim	0.25	0.5	≤0.03-2	- / -
Trimethoprim/sulfamethoxazole	0.06	0.12	0.03-0.25	- / -
Penicillin	≤0.06	≤0.06	≤0.06	100.0 / -
Erythromycin	≤0.12	≤0.12	≤0.12->4	93.4 / 6.0
Clindamycin	≤0.12	≤0.12	≤0.12->4	99.3 / 0.7
Tetracycline	≤0.5	≤0.5	≤0.5->16	94.7 / 5.0
Levofloxacin	0.5	0.5	0.25-2	100.0 / 0.0
Vancomycin	≤0.5	≤0.5	≤0.5-1	100.0 / -
Europe (303)				
Iclaprim	0.015	0.03	≤0.004-0.12	- / -[b]
Trimethoprim	0.12	0.5	≤0.03-1	- / -
Trimethoprim/sulfamethoxazole	0.06	0.12	0.015-0.25	- / -
Penicillin	≤0.06	≤0.06	≤0.06	100.0 / -
Erythromycin	≤0.12	>4	≤0.12->4	73.3 / 26.1
Clindamycin	≤0.12	≤0.12	≤0.12->4	93.4 / 5.9
Tetracycline	≤0.5	>16	≤0.5->16	85.8 / 14.2
Levofloxacin	0.5	1	0.25-2	100.0 / 0.0
Vancomycin	≤0.5	≤0.5	≤0.5-1	100.0 / -
Group B				
USA (102)				
Iclaprim	0.12	0.25	0.015-0.5	- / -[b]
Trimethoprim	1	2	0.25-8	- / -
Trimethoprim/sulfamethoxazole	0.06	0.12	0.03-0.25	- / -
Penicillin	≤0.06	≤0.06	≤0.06	100.0 / -
Erythromycin	≤0.12	>4	≤0.12->4	60.8 / 39.2
Clindamycin	≤0.12	>4	≤0.12->4	83.3 / 14.7
Tetracycline	>16	>16	≤0.5->16	16.7 / 82.4
Levofloxacin	0.5	1	0.25-2	100.0 / 0.0
Vancomycin	≤0.5	≤0.5	≤0.5	100.0 / -
Europe (102)				
Iclaprim	0.12	0.25	0.06-0.5	- / -[b]
Trimethoprim	2	4	0.5-4	- / -
Trimethoprim/sulfamethoxazole	0.12	0.12	0.03-0.25	- / -
Penicillin	≤0.06	≤0.06	≤0.06	100.0 / -
Erythromycin	≤0.12	1	≤0.12->4	85.3 / 12.7
Clindamycin	≤0.12	≤0.12	≤0.12->4	93.1 / 5.9
Tetracycline	>16	>16	≤0.5->16	26.5 / 73.5
Levofloxacin	0.5	1	0.5->4	99.0 / 1.0
Vancomycin	≤0.5	≤0.5	≤0.5	100.0 / -

[a]Criteria as published by the CLSI [2006].
[b]- = No criteria have been established by the CLSI [20 07].

- Iclaprim was very active against group A streptococci (MIC_{50}, 0.015 µg/mL; MIC_{90}, 0.03 µg/mL, range ≤0.04–0.12 µg/mL) and was 8- to 16-fold more potent than TMP (MIC_{50} 0.12–0.25 µg/mL; MIC_{90} 0.5 µg/mL) and four-fold more potent than the TMP/SMX combination (MIC_{50} 0.06 µg/mL; MIC_{90} 0.12 µg/mL). The activity of iclaprim was not affected by resistance to other agents tested (Table 1).
- Iclaprim was also very active against group B streptococci (MIC_{50} 0.12 µg/mL; MIC_{90} 0.25 µg/mL) and was eight-fold more potent than TMP (MIC_{50} 1–2 µg/mL; MIC_{90} 2–4 µg/mL). Iclaprim exhibited similar activity to the TMP/SMX combination when tested against group B streptococci. The activity of iclaprim was not affected by resistance to other agents tested (Table 1).
- No significant difference was observed between strains from Europe and the USA regarding the activity of iclaprim against group A and group B streptococci. (Table 1).

Table 2: Iclaprim MIC and MBC results.

Organism (no. of isolates)	No. of isolates at MIC or MBC value (µg/mL) of:									
	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	>2
Group A (20)										
MIC	1	4	9	5	0	1	0	0	0	0
MBC	0	0	2	3	3	0	0	1	0	11
Group B (20)										
MIC	0	0	0	0	5	13	2	0	0	0
MBC	0	0	0	0	0	4	6	3	1	6

[a]MBC value was higher than the highest dilution tested indicating MBC/MIC >32.

- Iclaprim exhibited bactericidal activity against 9/20 of group A streptococci tested with MBC/MIC ratios ranging from 1 to 4. For the other 11 strains, iclaprim showed an MBC/MIC ratio ≥32 (Tables 2 and 3).

Table 3: MBC/MIC ratio results for iclaprim and vancomycin tested against a selected group of 40 organisms.

MBC/MIC ratio/organism (no.)	No. of isolates at MBC/MIC	
	Iclaprim	Vancomycin
Group A (20)		
1	1	0
2	6	0
4	2	0
8	0	0
16	0	0
≤32	11	18[a]
Group B (20)		
1	1	0
2	10	0
4	2	0
8	1	0
16	0	0
≤32	6	20

[a]MBC could not be evaluated in two strains because both MIC and MBC values were out of the dilution range tested (≤0.5 µg/mL).

- MBC/MIC ratio for vancomycin was ≥32 for 18 group A streptococci and could not be evaluated in the other two strains (Table 3).
- Iclaprim was also bactericidal against group B streptococci and exhibited MBC/MIC ratios ≤4 for 65% of the strains (Tables 2 and 3). In contrast, MBC/MIC ratio for vancomycin was ≥32 for all group B streptococci (Table 3).

CONCLUSIONS

- **Iclaprim was highly active against group A (MIC_{90} 0.03 µg/mL) and group B (MIC_{90} 0.25 µg/mL) β-hemolytic streptococci.**
- **The activity of iclaprim was not affected by resistance to other agents tested.**
- **Iclaprim activity was independent of the geographic region sampled (Europe or USA).**
- **Overall, iclaprim exhibited superior bactericidal activity as compared to vancomycin.**

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