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In Vitro Activity of Iclaprim against Isolates in Two Phase 3 Clinical Trials (REVIVE-1 and -2) for Acute Bacterial Skin and Skin Structure Infections

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ABSTRACT Iclaprim, a selective bacterial dihydrofolate reductase inhibitor, and other antibiotics were tested against Gram-positive isolates from two phase 3 studies of acute bacterial skin and skin structure infections (ABSSSIs) (REVIVE-1 and -2). Seven hundred ninety baseline isolates, including *Staphylococcus aureus*, β-hemolytic streptococci, and *Streptococcus anginosus* group, underwent antibacterial susceptibility testing. Iclaprim had an MIC_{90} of 0.12 μg/ml for *S. aureus* (0.12 μg/ml for methicillin susceptible, 0.25 μg/ml for methicillin resistant), 0.25 μg/ml for β-hemolytic streptococci, and 0.008 μg/ml for *S. anginosus* group. Iclaprim demonstrated potent activity against these Gram-positive ABSSSI isolates.

KEYWORDS Gram-positive bacteria, iclaprim, *in vitro* activity, skin infections

Skin and soft tissue infections (SSTIs) are among the most common causes of infection in patients of all ages (1). Many of these infections result in hospital admissions or prolonged hospital stays (1, 2). The etiology of >80% of acute bacterial skin and skin structure infections (ABSSSIs) is Gram-positive bacteria (3); *Staphylococcus aureus* is the most common pathogen of wound infections, abscesses, and cellulitis (2). Currently, many antibiotics are approved for the treatment of SSTIs, although several have issues related to safety and/or resistance (4–8). Therefore, there still remains a medical need for well-tolerated antimicrobial agents active against antibiotic-resistant bacteria.

Iclaprim is a selective bacterial dihydrofolate reductase (DHFR) inhibitor, designed to have increased potency compared with trimethoprim and to be active against some isolates with trimethoprim resistance (9–11). Iclaprim has demonstrated *in vitro* and *in vivo* activity against Gram-positive pathogens, including methicillin-resistant *S. aureus* (MRSA), linezolid-resistant *S. aureus*, daptomycin-nonsusceptible *S. aureus*, and vancomycin-resistant *S. aureus* (12). Iclaprim does not need to be combined with a sulfonamide, which is commonly associated with adverse events, including renal toxicity, hepatotoxicity, blood dyscrasias, anaphylaxis, and hypersensitivity reactions (13).

In two phase 3 clinical trials (REVIVE-1 and -2), iclaprim showed early response rates comparable with those of vancomycin among patients treated for ABSSSI (14–16). This evaluation reports the *in vitro* activities of iclaprim against baseline pathogens isolated from ABSSSIs in these trials.

Overall, 1,198 patients were included in the intent-to-treat population in the REVIVE-1 ($n = 598$) and REVIVE-2 ($n = 600$) trials, as previously described (14, 15). Both studies were 600-patient, double-blind, randomized (1:1), active-control trials among patients with ABSSSIs that compared iclaprim 80 mg fixed dose with vancomycin

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TABLE 1 MIC_{50} and MIC_{90} values for iclaprim and comparators against 790 isolates from patients in phase 3 ABSSSI clinical trials REVIVE-1 and -2

Pathogen	*n*	MIC (μg/ml) for[a]:											
		Iclaprim		TMP		TMP-SMX[b]		VAN		Linezolid		DAP	
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
S. aureus	594	0.06	0.12	1	2	≤0.12	≤0.12	1	1	2	2	0.5	0.5
MRSA	272	0.03	0.25	0.5	4	≤0.12	≤0.12	1	1	2	2	0.5	0.5
MSSA	322	0.06	0.12	1	2	≤0.12	≤0.12	1	1	2	2	0.5	0.5
S. pyogenes	52	0.015	0.12	0.25	0.5	0.12	0.25	0.5	0.5	1	1	0.06	0.06
S. agalactiae	11	0.25	0.5	2	4	0.25	0.25	0.5	0.5	1	2	0.25	0.25
S. dysgalactiae	20	0.06	0.12	1	1	0.12	0.25	0.25	0.5	1	2	0.06	0.12
S. anginosus group	113	≤0.004	0.008	≤0.12	≤0.12	≤0.06	0.06	1	1	1	2	0.5	0.5

[a]DAP, daptomycin; TMP, trimethoprim; TMP-SMX, trimethoprim-sulfamethoxazole; VAN, vancomycin.
[b]TMP-SMX was tested at a fixed ratio of 1:19 (wt/wt). MIC value for trimethoprim shown.

15 mg/kg (adjusted for renal function), both administered intravenously every 12 h for 5 to 14 days.

At baseline, ABSSSIs were sampled for microbiological culture. In total, 790 baseline isolates of *S. aureus*, β-hemolytic streptococci, and *S. anginosus* group were collected. Of these, 594 were *S. aureus*, including 322 methicillin-susceptible *S. aureus* (MSSA) and 272 MRSA. Of the *Streptococcus* spp., 83 isolates were β-hemolytic streptococci (52 *Streptococcus pyogenes*, 11 *Streptococcus agalactiae*, and 20 *Streptococcus dysgalactiae*) and 113 isolates were *S. anginosus* group (including *S. anginosus*, *Streptococcus intermedius*, and *Streptococcus constellatus*). Isolates were collected in the United States (79.3%), Europe (20.4%), and Latin America (0.4%).

Isolates were analyzed at an independent reference laboratory (IHMA, Europe Sàrl, Monthey, Switzerland) for broth microdilution susceptibility testing conducted in accordance with CLSI M7 (17). Cation-adjusted Mueller-Hinton broth (CA-MHB) was used as the test medium, and CA-MHB was supplemented with 5% lysed horse blood for the testing of streptococci. Comparator antibiotic MIC results were within the CLSI published ranges against *S. aureus* ATCC 29213. Quality control ranges and interpretive criteria for comparator compounds were previously described (18). There are no published breakpoints for iclaprim. To identify plasmid-encoded and chromosomally encoded trimethoprim resistance genes, DNA extraction and PCR amplification were performed (19).

Iclaprim was active against *S. aureus* (MSSA and MRSA) and β-hemolytic streptococci, including *S. pyogenes*, *S. agalactiae*, and *S. dysgalactiae*, based on MIC_{50}/MIC_{90} values (Table 1). Based on the MIC_{90}, the activity of iclaprim was 4- to 16-fold more potent than that of trimethoprim alone and at least as active as trimethoprim-sulfamethoxazole against *S. aureus*, including MRSA and β-hemolytic streptococci. Six percent of isolates had an iclaprim MIC of ≥8 μg/ml; all were *S. aureus* except for one *S. anginosus* group isolate.

Figure 1 shows the activity of iclaprim against isolates from the United States and Europe. For MSSA and β-hemolytic streptococci isolates, MIC_{50}/MIC_{90} values were identical or within 2-fold in the United States and Europe, except for MRSA, for which the MIC_{50}/MIC_{90}s in Europe were elevated (0.12 and >8 μg/ml, respectively) compared with those in the United States (0.03 and 0.25 μg/ml, respectively). Because of the small number of MRSA isolates from Europe ($n = 11$), this difference was difficult to interpret. However, surveillance data from 2015 to 2016 of 160 MRSA isolates from Europe reported MIC_{50}/MIC_{90} values of 0.03 and 0.06 μg/ml, respectively, which were similar to those in North America (0.03 and 0.12 μg/ml, respectively; $n = 154$) (20).

Iclaprim MIC_{90} values were 0.12 μg/ml for *S. aureus* in the REVIVE studies. The MIC_{90} values for trimethoprim were 2 μg/ml, and those for trimethoprim-sulfamethoxazole were ≤0.12 μg/ml. Iclaprim demonstrated similar activity against MSSA and MRSA, with MIC_{90} values of 0.12 μg/ml and 0.25 μg/ml, respectively. Iclaprim had increased po-

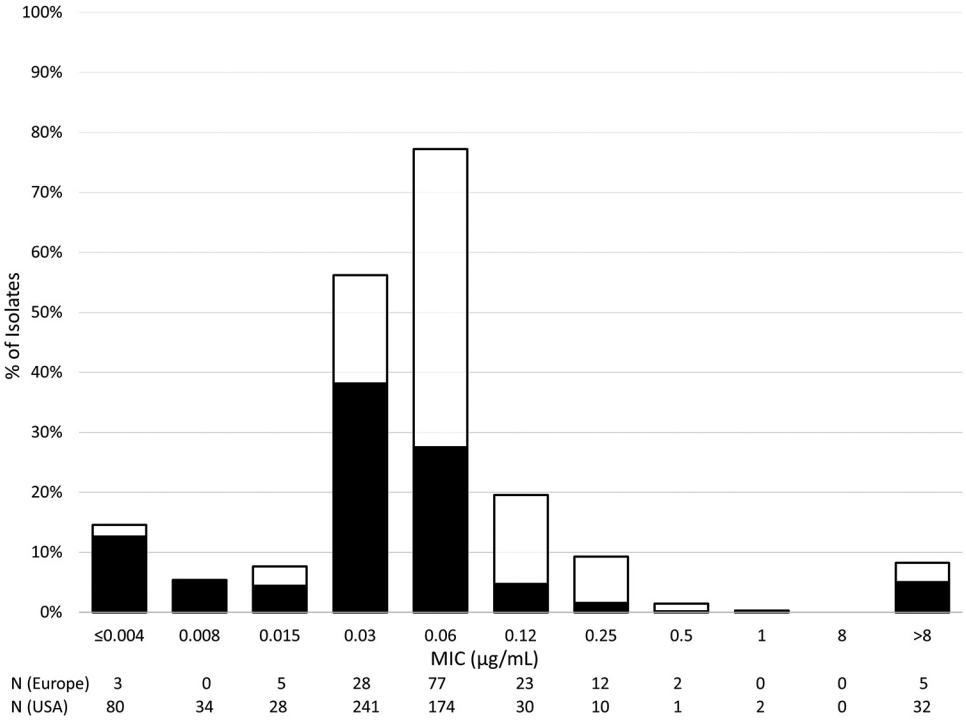

FIG 1 Iclaprim MIC distribution for Gram-positive pathogens in the United States (black bar; $n = 632$) and Europe (white bar; $n = 155$); 3 (0.4%) isolates from Latin America not included.

tency compared with trimethoprim (MIC_{90}, 2 and 4 μg/ml against MSSA and MRSA, respectively). For trimethoprim-sulfamethoxazole, the MIC_{90} was $\leq$0.12 μg/ml for both MSSA and MRSA isolates. Against MRSA, iclaprim had at least 2-fold greater potency than vancomycin (MIC_{90}, 1 μg/ml), linezolid (MIC_{90}, 2 μg/ml), and daptomycin (MIC_{90}, 0.5 μg/ml).

Increased potency of iclaprim relative to trimethoprim was consistent across the tested β-hemolytic streptococci. Based on MIC_{90}, iclaprim was 4-fold more potent than trimethoprim and 2-fold more potent than trimethoprim-sulfamethoxazole against *S. pyogenes*, with MIC_{90} values of 0.12 μg/ml. For *S. agalactiae* and *S. dysgalactiae* isolates, the MIC_{90} values for iclaprim were 0.5 and 0.12 μg/ml, respectively, whereas trimethoprim had MIC_{90} values of 4 and 1 μg/ml, respectively. Similar MIC_{90} values were noted with iclaprim and trimethoprim-sulfamethoxazole. Iclaprim had an MIC_{90} value of 0.008 μg/ml against *S. anginosus* group isolates, similar to that of trimethoprim and trimethoprim-sulfamethoxazole ($\leq$0.12 and 0.06 μg/ml, respectively).

Outcomes by MIC for isolates from patients treated with iclaprim showed that most patients had low iclaprim MIC levels and achieved early clinical response (Table 2). Among the 37 isolates with MICs of $\geq$8 μg/ml to iclaprim, molecular characterizations for the presence of trimethoprim resistance genes *dfr*A, *dfr*C, *dfr*D, *dfr*F, *dfr*G, and *dfr*K and polymorphisms within the *DHFR* gene were evaluated. The isolates consisted of *S. aureus* ($n = 36$) and *S. anginosus* group ($n = 1$). Thirty-five isolates (all *S. aureus*) were positive for *dfr* and *DHFR*. Both remaining isolates (1 *S. aureus* and 1 *S. anginosus* group) were positive for the *DHFR* gene only. Plasmid-encoded *dfr* genes occurred in 9 *S. aureus* isolates for *dfr*A and 26 for *dfr*G. Gene sequencing revealed that the vast majority of *DHFR* genes had the wild-type sequence; the F98Y mutation was not identified in any isolates.

Iclaprim was developed as monotherapy for ABSSSI treatment and is in development for hospital-acquired bacterial pneumonia. Based on MIC_{90}, iclaprim showed potent *in vitro* activity against *S. aureus*, including MRSA, β-hemolytic

TABLE 2 Early clinical response in the iclaprim arm by iclaprim MIC (REVIVE-1 and -2)

Pathogen and MIC (μg/ml)	Early clinical response (*n*/*N* [%])
Staphylococcus aureus	
0.015	2/2 (100)
0.03	116/135 (86)
0.06	98/115 (85)
0.12	17/22 (77)
0.25	8/10 (80)
1	1/1 (100)
>8	10/16 (63)
Streptococcus anginosus group	
≤0.004	37/38 (97)
0.008	12/17 (71)
0.015	2/2 (100)
0.03	1/1 (100)
0.25	1/1 (100)
>8	1/1 (100)
Streptococcus pyogenes	
≤0.004	2/2 (100)
0.008	2/2 (100)
0.015	11/11 (100)
0.03	3/4 (75)
0.12	2/3 (67)
0.25	2/2 (100)
Streptococcus agalactiae	
0.12	3/4 (75)
0.25	2/2 (100)
0.5	1/1 (100)
Streptococcus dysgalactiae	
0.03	3/3 (100)
0.06	0/3 (0)
0.12	1/1 (100)

streptococci, and *S. anginosus* group from the phase 3 ABSSSI studies REVIVE-1 and -2. Iclaprim was more potent than trimethoprim alone and had similar activity to trimethoprim-sulfamethoxazole against the Gram-positive clinical isolates collected. Furthermore, iclaprim had greater potency against MRSA than vancomycin, linezolid, and daptomycin, which are standard-of-care Gram-positive therapies for the treatment of MRSA-suspected ABSSSI. In addition, based on MIC_{90}, iclaprim exhibited more potency than trimethoprim and similar potency to trimethoprim-sulfamethoxazole against *S. pyogenes*, similar to the other tested β-hemolytic streptococci.

In conclusion, based on MIC_{90}, iclaprim showed increased potency against Gram-positive pathogens relative to trimethoprim and similar potency to trimethoprim-sulfamethoxazole. Iclaprim has the potential to offer safety advantages and cost avoidance for treating patients with ABSSSIs and suspected/confirmed MRSA at risk of vancomycin acute kidney injury, including patients with preexisting moderate/severe renal impairment, obesity, and/or diabetes. Therefore, iclaprim may be an important new therapeutic option for the treatment of ABSSSIs caused by Gram-positive bacteria, including multidrug-resistant bacteria.

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