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In Vitro Bactericidal Activity of Iclaprim in Human Plasma[▽]

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This study evaluated the effect of human plasma on the in vitro bactericidal activity of the novel diaminopyrimidine iclaprim against methicillin (meticillin)-susceptible and -resistant *Staphylococcus aureus* strains. MICs and minimal bactericidal concentrations (MBCs) of iclaprim, with ~93% protein binding, were similar in the absence and in the presence of 50% human plasma; MICs and MBCs ranged from 0.06 to 0.125 μg/ml. Furthermore, the activity of iclaprim was not affected by plasma, with ≥99.9% reduction in CFU after 5.0 to 7.6 h.

Iclaprim is a novel dihydrofolate reductase inhibitor antibiotic that is under development for the treatment of hospitalized patients with severe infections caused by gram-positive pathogens, including *Staphylococcus aureus*, methicillin (meticillin)-resistant *S. aureus* (MRSA), and β-hemolytic streptococci (10, 11). Iclaprim has recently completed two pivotal phase III trials for the treatment of complicated skin and skin structure infections, including infections caused by MRSA. It has been shown to exhibit a rapid in vitro bactericidal activity against MRSA and vancomycin-nonsusceptible strains (11).

Plasma protein binding is often, though not always, associated with a certain loss in in vitro microbiological activity. Despite the observation that iclaprim is ~93% plasma protein bound, we have recently reported that the in vitro MIC of iclaprim is not affected by the presence of 50% human plasma (7). In contrast, the microbiological activity of fusidic acid (97 to 98% plasma protein bound) has been reported to be significantly affected by the addition of serum or blood to the test medium (4). The aim of this study was to determine the effect of human plasma on the bactericidal activity of iclaprim against *S. aureus* in comparison to the activity of teicoplanin and fusidic acid drugs with similar or higher protein binding (>90% and 97 to 98%, respectively) and vancomycin and linezolid with low reported protein binding (55% and 31%, respectively) (2).

Ten methicillin-susceptible *S. aureus* (MSSA) and 10 MRSA strains were tested, including clinical isolates from different countries in Europe and North America and two type strains. MICs against iclaprim, teicoplanin, vancomycin, fusidic acid, and linezolid were determined by broth microdilutions in cation-adjusted Mueller-Hinton broth (CAMHB; Oxoid, Basingstoke, United Kingdom) with and without 50% pooled human plasma (PAA Laboratories GmbH, Pasching, Austria) following the standard CLSI protocol (3). Minimal bactericidal concentrations (MBCs) were determined via plating of aliquots on Mueller-Hinton agar taken from wells with no visual growth at 24 h, according to the CLSI guideline (9). To avoid the impact of potential drug carryover, cells grown with fusidic acid were washed twice with phosphate-buffered saline before plating (6). Washing did not affect the CFU counts. The rates of the bactericidal activities of iclaprim and vancomycin in the presence of 50% human plasma were assessed for one MSSA and four MRSA strains by time-kill methodology (9). The bacteria (~1×10^6 CFU/ml) were grown in 24-well plates containing 2 ml of CAMHB either with or without 50% human plasma and either containing no antibiotic (control) or containing iclaprim or vancomycin at 4× MIC, and the CFU/ml were determined. Samples were taken at 0, 2, 4, 6, 8, and 24 h, and appropriate dilutions were plated onto Mueller-Hinton agar to determine the CFU. Bactericidal activity was defined as a ≥3-$\log_{10}$ CFU/ml reduction in bacterial density (i.e., ≥99.9% kill) compared with the level in the initial inoculum (9). Bacteria growing in the presence of plasma were sonicated briefly to disaggregate cellular clumps that can form in the presence of human plasma (Branson Sonifier 250; 30 s at 40% duty cycle and 40% capacity), which can result in an underestimation of CFU. The gentle sonication did not affect the viability of the cells.

The activity of iclaprim was not affected by the presence of 50% human plasma, with MICs ranging from 0.06 to 0.125 μg/ml with and without 50% human plasma, in agreement with recently published data (7). Moreover, the MBCs of iclaprim were also not affected by the presence of human plasma. The MBC/MIC ratios ranged from 1 to 2 both in CAMHB and in the presence of 50% human plasma (Table 1). Therefore, iclaprim exhibited similar bactericidal activity irrespective of the presence or absence of 50% plasma according to MBC determinations. The activities of vancomycin and linezolid were also generally unaffected by the presence of human plasma, with similar MICs and MBCs in the presence and absence of 50% plasma (Table 1). Vancomycin was bactericidal and the MBC/MIC ratios ranged from 1 to 2, while linezolid was bacteriostatic (Table 1), which is in agreement with reported data (1, 12). The presence of plasma had a minimal effect on the activity of teicoplanin (Table 1). These

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TABLE 1. Antibacterial activities of iclaprim and comparators against 20 isolates of *S. aureus*[a]

Drug[b]	Median MIC	MIC range	Median MBC	MBC range	Median MBC/MIC range	MBC/MIC range
ICL	0.09 (0.11)	0.06–0.125 (0.06–0.125)	0.10 (0.11)	0.06–0.125 (0.06–0.125)	1.04 (1.08)	1–2 (1–2)
TEC	1.07 (1.23)	0.5–2 (0.5–8)	1.41 (1.68)	0.5–8 (0.5–8)	1.32 (1.37)	1–4 (1–4)
VAN	1.62 (1.46)	1–2 (1–4)	1.74 (1.52)	1–4 (1–4)	1.07 (1.04)	1–2 (1–2)
LZD	3.73 (2.55)	2–4 (2–4)	>32 (>32)	>32 (>32)	>16 (>16)	16–>16 (>16)
FUS	0.14 (6.73)	0.016–0.5 (2–16)	NC[c]	0.03–>8 (8–>128)	NC	2–>16 (4–>16)

[a] Data for 10 MSSA and 10 MRSA strains with respect to 50% human plasma are shown as follows: absence (presence). MICs and MBCs are in μg/ml. Data for MSSA and MRSA strains were similar, with MICs ranging from 0.06 to 0.125 μg/ml for both.
[b] ICL, iclaprim; TEC, teicoplanin; VAN, vancomycin; LZD, linezolid; FUS, fusidic acid.
[c] NC, not calculated.

data are in agreement with Dykhuizen et al., who reported similar MBCs of vancomycin and teicoplanin in the presence of 50% human serum (5). As expected from its high plasma protein binding, MICs for fusidic acid were 16- to 256-fold greater in the presence of plasma, which is in agreement with previously published data (4, 7). MBC ranges were 0.03 to >8 μg/ml in CAMHB and 8 to >128 μg/ml in the presence of 50% human plasma (Table 1).

Time-kill studies with one MSSA and four MRSA strains with iclaprim in 50% human plasma further support the MBC determinations. Importantly, iclaprim demonstrated a rapid bactericidal kill, resulting in ≥99.9% reduction in CFU of initial inocula within 5.0 to 7.6 h of exposure with 4× MIC of iclaprim in the presence of 50% human plasma, which was comparable to the data obtained in CAMHB (Table 2). As expected, vancomycin exhibited a slow bactericidal activity, with a ≥99.9% reduction in CFU after 12.0 to 22.5 h in CAMHB and 10.6 to 23.5 h in 50% human plasma.

Similar observations had been recently reported for telavancin, whose protein binding (93%) is comparable to that of iclaprim. Although, MICs of telavancin increased one- to fourfold in the presence of 50% heat-inactivated human serum, the bactericidal activity was maintained in the presence of serum against glycopeptide-nonsusceptible *S. aureus* isolates (8).

In conclusion, two different antibiotics with similar protein binding (iclaprim, ~93%; and teicoplanin, >90%) were compared with two antibiotics with lower protein binding (vancomycin, 55%; and linezolid, 31%) and one antibiotic with higher protein binding (fusidic acid, 97 to 98%). The presence of 50% human plasma did not significantly affect the antimicrobial activity, assessed by the MICs and MBCs of iclaprim, teicoplanin, vancomycin, and linezolid. Furthermore, the presence of 50% human plasma did not have an impact on the rate of bactericidal activity of iclaprim against one MSSA strain and four MRSA strains, which was also the case with vancomycin. In contrast, a significant reduction in the antibacterial activity of fusidic acid with human plasma was observed. Despite the observed protein binding of iclaprim, the addition of human plasma did not affect the antimicrobial properties of the drug. Furthermore, the fact that the MBC/MIC ratios for iclaprim were consistently 1 to 2 suggests that the bactericidal activity of the molecule remains important even in human plasma. Such bactericidal activity in humans could be important in human therapy, whereby bacterial burdens could be reduced earlier. Moreover, the rapid in vitro bactericidal activity was maintained in the presence of human plasma, probably indicative of the weak and rapidly reversible association of the drug with plasma proteins.

TABLE 2. Time to reach 99.9% killing at 4× MIC of iclaprim or 4× MIC vancomycin for five *S. aureus* strains in the absence and presence of 50% human plasma[a]

S. aureus strain	Time (h) for 99.9% kill			
	Iclaprim		Vancomycin	
	CAMHB[b]	CAMHB + 50% HuPl[c]	CAMHB	CAMHB + 50% HuPl
ATCC 25923	6.2	6.2	15.0	15.6
50478 MRSA	7.2	6.2	12.8	13.5
3817 MRSA	6.8	7.6	22.5	23.5
6 MRSA	6.0	5.5	12.0	10.6
20 MRSA	6.1	5.0	17.0	17.0

[a] Bactericidal activity was defined as time (h) to reach 99.9% reduction in initial inocula. Samples were diluted and plated for CFU determination.
[b] Cation-adjusted Mueller-Hinton broth.
[c] Cation-adjusted Mueller-Hinton broth containing 50% pooled human plasma.

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