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ORIGINAL ARTICLE

Iclaprim activity against wild-type and corresponding thymidine kinase–deficient *Staphylococcus aureus* in a mouse protection model

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Abstract

The in vitro and in vivo antimicrobial activities of dihydrofolate reductase (DHFR) inhibitors are inhibited in the presence of free thymidine in the growth milieu and in rodent efficacy models. However, for thymidine kinase (TK) deficient mutant bacteria, the presence of free thymidine does not impact the activity of DHFR inhibitors, and these mutants were used to assess the in vivo efficacy of the DHFR inhibitor, iclaprim. The efficacies of iclaprim, trimethoprim, and vancomycin were evaluated in a systemic mouse infection model. Female CD-1 mice were infected intraperitoneally (IP) with wild-type *Staphylococcus aureus* ATCC 25923 (MSSA) or AW 6 (MRSA) or their corresponding isogenic TK-deficient mutant *S. aureus* strains AH 1246 and AH 1252. Iclaprim showed potent antibacterial activity against both the TK-deficient mutant *S. aureus* strains, with PD_{50} values of 1.8 and < 0.5 mg/kg, respectively, for strains AH 1246 and AH 1252. In contrast, poor antibacterial activity was observed against corresponding wild-type (TK competent) *S. aureus* strains, with PD_{50} values of 10.8 and 2.2 mg/kg, respectively, for strains ATCC 25923 and AW 6. This study confirms that thymidine plays an important antagonistic role when determining the efficacy of DHFR inhibitors in vivo. This is the first study to show that iclaprim is active against TK-deficient *S. aureus* strains in a systemic mouse infection model, and that TK-deficient mutants may be used to evaluate iclaprim's activity in rodent models in vivo.

Keywords Iclaprim · Thymidine · Rodent

Introduction

Iclaprim, a bacterial dihydrofolate reductase (DHFR) inhibitor, has potent in vitro activity against Gram-positive antibiotic-sensitive and -resistant bacteria [1]. However, like other members within this class of antimicrobial agents, both in vitro and in vivo activities are impacted by the presence of free thymidine, which can bypass the requirement for DHFR in the nucleotide biosynthetic pathway, thereby decreasing the susceptibility of bacteria to DHFR inhibitors. Commercially available media for in vitro testing contain thymidine [2], and mouse plasma contains approximately 300-fold higher levels of thymidine than human plasma [3–5]. However, the utility of the conventional systemic mouse infection model to evaluate the efficacy of iclaprim is used because of convenience, costs, and practicality compared to the use of non-human primates, which have levels of thymidine more similar to human plasma. To offset the impact of these high levels of free thymidine in mice, thymidine kinase (TK)-deficient strains of *Staphylococcus aureus* were used to characterize the efficacy of iclaprim, compared with vancomycin and with the DHFR inhibitor trimethoprim, in a systemic mouse infection model.

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Materials and methods

Antimicrobial agents

Iclaprim powder (Motif BioSciences, Princeton, NJ), trimethoprim (Sigma-Aldrich, St. Louis, MO), and vancomycin

(Sigma-Aldrich, St. Louis, MO) were used in the in vivo studies after resuspension in sterile deionized water.

Bacterial growth media

Trypticase soy agar (TSA) plates (BBL, Franklin Lakes, NJ), brain heart infusion (BHI), broth (BBL, Franklin Lakes, NJ), and Type III hog gastric mucin (Sigma-Aldrich, St. Louis, MO) were used. The standard CLSI microdilution technique [6] was used to determine the MIC, done in triplicate, for the strains employed in these studies.

Bacterial strains and bacteria culture

Two wild-type *S. aureus* strains (*S. aureus* ATCC 25923 [MSSA] and *S. aureus* AW 6 [MRSA]) and their respective TK-deficient *S. aureus* strains (*S. aureus* AH 1246 and *S. aureus* AH 1252) were supplied by Motif BioSciences, Princeton, NJ. TK mutants were derived as described by Haldimann et al. [7] and biologically stable [8]. Virulence studies determined that fitness of the TK knock outs was not affected. *S. aureus* was grown in a shaking overnight culture in BHI at 37 °C to stationary phase and diluted 1:10 in fresh media for an additional 5 h of incubation. The 5-h culture was subsequently diluted into 8% hog gastric mucin, pH 7.5 to achieve a bacterial inoculum that would result in 0% survival in infected animals 24 h after infection. Bacterial counts were performed to determine inoculum size. For consistency of infection, both pairs of wild type and mutants were prepared at the same inoculum concentration.

Animals

CD-1 female mice (weighing 18 to 22 g) from Charles River Laboratories (Wilmington, MA) were acclimated for 5 days prior to the first study day. All studies were performed under approved IACUC protocols and conform to OLAW standards as set forth by the National Research Council's Guide for the Care and Use of Laboratory Animals [9]. Animals had free access to food and water throughout the study.

Infection studies

Bacterial strains utilized for these studies were first assessed for virulence over a range of inoculum concentrations to determine the proper infection input which would result in lethality within 48 h from infection. Bacteria were prepared for infection from a 5 h (log phase) growth in liquid culture and diluted in 8% hog gastric mucin pH 7.4 (Sigma-Aldrich) to the infection concentration. Mice ($n = 5$ per group) were infected with a bacterial inoculum of 2×10^6 to 5.55×10^6 CFU/mouse. For the MSSA isolate and corresponding thymidine knock out, the inoculum input was 2.8×10^6 and 2.0×10^6 CFU per mouse, respectively. For the MRSA isolate and corresponding thymidine knockout strain, the inoculum input was 5.55×10^6 and 5.45×10^6 CFU/mouse, respectively. Bacteria were delivered via intraperitoneal injection (0.5-mL injection volume). Two groups of mice ($n = 5$ per group) received 10-fold titrations of infection inoculum for QC purposes. At 60 min post-infection, a group of five mice was treated with the drug via a subcutaneous route (0.2-mL injection volume) in doubling concentrations from 0.25 to 40 mg/kg of iclaprim, trimethoprim, or vancomycin. The survival of the mice was assessed at 24-h post-infection.

Data analysis

The percent survival of each group of animals at 24 h was calculated. Probit analyses were conducted to fit the sigmoid dose response curves of: (1) dose effecting 50% survival and the protective dose 50% (PD_{50}) along with their corresponding 95% confidence intervals using Graphpad Prism (GraphPad Software).

Results and discussion

The growth rates between the wild-type *S. aureus* and its corresponding TK mutant were similar. The MIC for iclaprim against the *S. aureus* ATCC 25923 and its corresponding TK mutant used in these studies was 0.12 μg/mL and against the AW 6 (MRSA) and its corresponding TK mutant was 0.06 μg/mL. Based on worldwide surveillance studies, these MIC values are within the natural distribution of MICs in the

Table 1 Minimum inhibitory concentration (μg/mL) in broth

Compound	*S. aureus* AH 1246 (TK^-)	*S. aureus* ATCC 25923 (WT)	*S. aureus* AH 1252 (TK^-)	*S. aureus* AW 6 (WT)
Iclaprim	0.12	0.12	0.06	0.06
Vancomycin	1	1	1	0.5
Trimethoprim	2	2	0.5	1

WT, wild-type; TK^-, thymidine kinase mutant strains

S. aureus population (Iclaprim MIC_{50}/MIC_{90} of 0.06/0.12 μg/mL) [10]. Clinical studies have shown that patients achieve iclaprim mean concentrations nearly 6× or more the MIC_{90} of *S. aureus* [11]. The MIC values for trimethoprim and vancomycin against the *S. aureus* strains are shown in Table 1. MIC values for each compound were within twofold of each other for the wild-type vs. TK mutant strains of each strain.

Iclaprim, trimethoprim, and vancomycin were further evaluated in a systemic mouse infection model using wild-type and TK-deficient *S. aureus* strains. The exposure response for each of the four strains is shown in Table 2. Iclaprim demonstrated a sixfold increase in the potency at 24-h post-infection when comparing TK mutant strain AH 1246 to its wild-type counterpart, ATCC

Table 2 Protective dose (PD_{50}) and exposure response of iclaprim, trimethoprim, and vancomycin vs. *S. aureus* in a systemic infection mouse protection model

Strain	Genotype	Treatment	Dose (mg/kg)	Survival/total tested	PD_{50} (95% CI)
AH1246	TK-	Iclaprim	0.5	2/5	1.8 (0.5–3.1)
			2	3/5	
			5	5/5	
			10	5/5	
		Trimethoprim	2	0/5	4.5 (4.5–4.9)
			5	3/5	
			10	5/5	
			40	5/5	
		Vancomycin	0.25	1/5	1.1 (0.6–1.5)
			0.5	1/5	
			1	2/5	
			2	5/5	
ATCC 25923	WT	Iclaprim	0.5	1/5	10.8 (1.7–17.0)
			2	1/5	
			5	2/5	
			10	4/5	
		Trimethoprim	2	1/5	> 40 (> 40)
			5	1/5	
			10	2/5	
			40	2/5	
		Vancomycin	0.25	0/5	1.1 (0.6–1.5)
			0.5	0/5	
			1	2/5	
			2	5/5	
AH 1252	TK-	Iclaprim	0.5	3/5	< 0.5 (< 0.5)
			2	4/5	
			5	5/5	
			10	5/5	
		Trimethoprim	2	2/5	2.2 (2.1–2.3)
			5	5/5	
			10	5/5	
			40	5/5	
		Vancomycin	0.25	1/5	0.89 (0.5–1.3)
			0.5	1/5	
			1	3/5	
			2	4/5	
AW6	WT	Iclaprim	0.5	0/5	2.2 (1.2–3.3)
			2	5/5	
			5	5/5	
			10	5/5	
		Trimethoprim	2	1/5	4.7 (0.06–9.4)
			5	3/5	
			10	3/5	
			40	3/5	
		Vancomycin	0.25	0/5	0.58 (0.4–0.7)
			0.5	2/5	
			1	4/5	
			2	5/5	
Positive control		Bacteria administered, but no treatment	–	5/40	–
Negative control		No bacteria administered	–	38/40	–

Compounds were dosed by SC injection 1 h after infection and PD_{50} measured at 24-h post-infection

WT, wild-type; *TK⁻*, thymidine kinase mutant strains, *95% CI*, confidence intervals

25923. A similar increase in potency for iclaprim (> 4.5-fold) was observed when comparing TK mutant strain AH 1252 to its wild-type counterpart, AW6 (MRSA). Trimethoprim, although less potent as a DHFR inhibitor than iclaprim, displayed a similar difference in potency in the TK mutant compared to wild-type strains. In contrast, vancomycin, demonstrated a similar potency against both mutant and wild-type strains (Table 2), suggesting that the potency was unaffected by free thymidine levels. Though PD_{50} values increased with the wild-type strain, the efficacy of iclaprim was better than that for trimethoprim.

In summary, this is the first in vivo rodent model to demonstrate in vivo the potent efficacy of iclaprim compared with the DHFR inhibitor trimethoprim and with vancomycin, a standard of care for the treatment of bacteremia caused by Gram-positive bacteria, including methicillin-resistant *S. aureus*. Furthermore, these studies demonstrate the usefulness of TK-deficient mutants, compared to wild-type strains, for evaluating the efficacy of DHFR inhibitors in rodent models of infection. Additional TK-deficient mutants with corresponding wild-type strains and complemented mutants in this in vivo rodent model are needed to confirm these findings.

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Compliance with ethical standards

Conflict of interest DBH is an employee of Motif BioSciences. TM is an employee of NeoSome Life Sciences.

Ethical approval This research involved animals. All procedures in this research were in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare.

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