



ABSTRACT

Background: CBR-2092 is a novel agent in development for treatment of serious *S. aureus* infections and is investigated here in a rabbit model of methicillin-resistant *S. aureus* (MRSA) IE.

Methods: Rabbit IE was induced with MRSA 67-0, following transcatheteric aortic valve indwelling catheterization. At 24h post-infection, animals received either: no therapy (Control); CBR-2092 10, 25 or 40 mg/kg, iv, bid; or Vancomycin (Vanco) 15 mg/kg, iv, bid. All treatments were for 3d. At 24h after the last dose, target tissues were quantitatively cultured. Relapse was investigated 3d after treatment was discontinued.

Results: MICs/MBCs of CBR-2092 and Vanco for MRSA 67-0 were 0.008/0.5 µg/mL, and 1/1 µg/mL, respectively. All regimens significantly decreased MRSA densities vs. Controls. CBR-2092 at 25 and 40 mg/kg exhibited the greatest efficacy and also prevented relapse.

Group (# animals)	Dose (mg/kg)	Log ₁₀ CFU/g. tissue ± SD (% Sterile)		
		Vegetation	Kidney	Spleen
Control (11)		8.4 ± 0.5 (0)	7.2 ± 1.1 (0)	6.3 ± 1.5 (0)
Vanco (7)	15	5.1 ± 1.4 (0)*	4.8 ± 1.6 (0)*	4.4 ± 1.4 (0)*
CBR-2092 (8)	10	5.8 ± 1.7 (0)*	4.0 ± 1.4 (0)*	4.1 ± 1.1 (0)*
CBR-2092 (8)	25	4.5 ± 1.4 (0)*	3.6 ± 1.1 (0)*	3.4 ± 0.6 (0)*
CBR-2092 (10)	40	1.9 ± 0.4 (80)**	1.8 ± 0.6 (60)**	2.1 ± 0.7 (30)**
Vanco relapse (6)	15	5.6 ± 1.9 (0)*	4.8 ± 1.7 (0)*	4.7 ± 1.2 (0)*
CBR-2092 relapse (5)	10	6.1 ± 3.2 (40)*	4.6 ± 3.1 (40)*	3.9 ± 2.3 (40)*
CBR-2092 relapse (5)	25	2.4 ± 0.3 (60)**	1.5 ± 0.3 (80)**	1.4 ± 0.2 (100)**
CBR-2092 relapse (9)	40	1.7 ± 0.1 (100)**	1.5 ± 0.3 (100)**	1.9 ± 0.7 (100)**

*P < 0.05 vs. Controls; ** P < 0.05 vs. Vanco and Controls.

Conclusion: These results show that CBR-2092 has significant efficacy in this severe MRSA infection (IE).

INTRODUCTION

S. aureus is a predominant cause of community-acquired and nosocomial infections. Despite the use of new antibiotics, morbidity and mortality of such infections remain high. One of the reasons is that *S. aureus* is adept at developing resistance to multiple antibiotics, including conventional drugs (e.g., vancomycin), and even the newest anti-staphylococcal agents (e.g., daptomycin). These observations raise the specter of systemic staphylococcal infections for which no effective antibiotic therapy exists, and the need to develop new agents against *S. aureus* infections.

CBR-2092 is under development for the treatment of serious or life-threatening bacterial infections including those caused by pathogens that have developed or acquired resistance to commonly used antibiotics. CBR-2092 is a new molecular entity that combines rifamycin SV and 4H-4-oxoquinolizone pharmacophores into a single, stable molecule. The resulting drug is not a pro-drug, but a single molecule that acts as a multifunctional antibiotic exhibiting many of the properties of the parent pharmacophores.

PURPOSE OF THIS STUDY

To compare efficacy of CBR-2092 vs. vancomycin in the treatment of MRSA experimental endocarditis in rabbits.

METHODS AND MATERIALS

***S. aureus* strain:** A clinical isolate of methicillin-resistant *S. aureus* (MRSA) 67-0 has been used in the current investigation. This strain has been studied in a number of prior experimental MRSA endocarditis studies in rabbits (1,2). It produces β-lactamase and is oxacillin-resistant, but vancomycin-susceptible.

MICs/MBCs: MICs and MBCs were determined according to Clinical Laboratory Standards Institute (CLSI). The MIC was taken as the lowest drug concentration at which observable growth was inhibited. The MBC was taken as the lowest concentration of each drug that resulted in more than 99.9% reduction of the initial inoculum.

Killing curve: The killing assays were determined in CAMHB inoculated with the test organism at 10⁸ CFU/mL initial inoculum, with either i) no drug exposure (control); ii) CBR-2092 at 1 µg/mL; iii) CBR-2092 at 4 µg/mL; or iv) vancomycin at 8 µg/mL. CFU's were determined at time points through 50 h of incubation at 37°C by serial dilution and plating on charcoal.

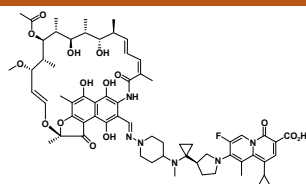
Rabbit model of endocarditis: Endocarditis model was established in New Zealand White rabbits by positioning a catheter across the aortic valve as previously described (3). Animals were infected 48 h after catheterization by iv. injection of 5 × 10⁸ CFU *S. aureus* 67-0. At 24 h after infection, rabbits were randomly assigned to one of the following groups: untreated controls; CBR-2092 at either 10, 25 or 40 mg/kg (iv, bid); or vancomycin at 15 mg/kg (iv, bid). All treatments were for 3 days. At 24 hrs after the last antibiotic dose, all animals were sacrificed with a lethal dose of sodium pentobarbital. For relapse studies, catheterized and infected animals received CBR-2092 or vancomycin for 3 days and then were allowed to reside drug-free for an additional 3 days. At sacrifice, vegetations, kidneys and spleen were removed and quantitatively cultured. To monitor *in vivo* resistance development, homogenate tissues were cultured in parallel on TSB agar plates containing 1 µg/mL CBR-2092. Culture results will be expressed as mean log₁₀CFU per gram of tissue (± SD).

Pharmacokinetics (PK) study: Serum concentrations of CBR-2092 were determined from blood samples obtained at selected time points after dosing. Serum was collected and frozen at -70°C. CBR-2092 serum levels were determined by an established LCMS assay.

Statistical analyses: The Kruskal-Wallis ANOVA was utilized with the Tukey post-hoc correction for multiple group comparisons to compare target tissue bacterial densities among the various genetic constructs, in the presence or absence of antibiotic therapy. A p < 0.05 were used to define a statistically significant.

RESULTS

Panel 1: CBR-2092 Structure



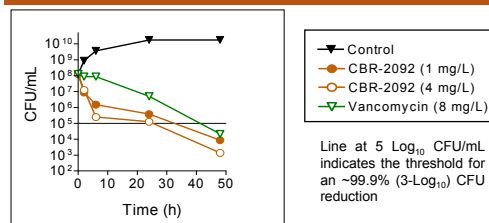
R-3-[4-[1-[1-(3-Carboxy-1-cyclopropyl-7-fluoro-9-methyl-4-oxo-4H-quinolizine-9-yl)-pyrrolidin-3-yl]-cyclopropyl]-methylamino]-piperidin-1-ylimino]-methylenyl]-rifamycin SV

Panel 2: MICs/MBCs of CBR-2092 and Vancomycin for *S. aureus* 67-0

Compound	MIC ^a (µg/mL)	MBC ^b (µg/mL)
CBR-2092	0.008	0.5
Vancomycin	1.0	1.0

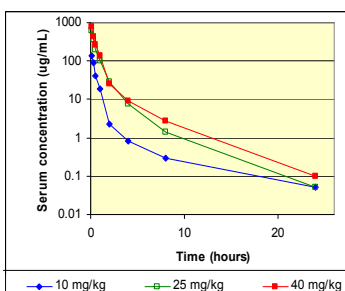
^a MIC as determined in cation adjusted MHB with 0.002 % (v/v) polysorbate-80 as wetting agent; ^b MBC is the drug concentration required to kill 99.9% (3-Log₁₀) input CFU.

Panel 3: *In vitro* killing curve for *S. aureus* 67-0



Line at 5 Log₁₀ CFU/mL indicates the threshold for an ~99.9% (3-Log₁₀) CFU reduction

Panel 4: PK of CBR-2092 in rabbits following a single iv dose



PK parameters	10 mg/kg	25 mg/kg	40 mg/kg
AUC _{0-∞} (µg·h/mL)	72.1	371.2	452.2
V _d (L/kg)	0.24	0.08	0.12
Cl (L/h/kg)	0.14	0.07	0.09
MRT (h)	1.68	1.15	1.35
Half-life (h)	3.87	3.52	3.41
C _{max} (µg/mL)	140.7	632.9	762.9

AUC: area under the concentration curve; V_d: calculated volume of distribution; Cl: clearance; MRT: mean residence time; Half-life: elimination half-life; C_{max}: maximum observed concentration

Panel 5: Efficacy of CBR-2092 in the rabbit endocarditis model

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*P < 0.05 vs. Controls; ** P < 0.05 vs. Vanco and Controls.

INTERPRETATIONS

The MICs / MBCs of CBR-2092 and vancomycin on the test *S. aureus* strain were 0.008/0.5 and 1.0/1.0 µg/mL, respectively (panel 2).

CBR-2092 at both 1 or 4 µg/mL had faster and greater bactericidal effects as compared to vancomycin at 8 µg/mL at a starting inoculum of 10⁸ CFU/mL over 48 hrs incubation (panel 3).

The peak concentrations of CBR-2092 (iv) in serum were at 5 mins post dose and increased in a dose-dependent manner. Of note, the half-life of CBR-2092 was about 3.4-3.8 hrs in the rabbit (panel 4).

All regimens significantly decreased *S. aureus* densities as compared to untreated controls in all target tissues (see panel 5; P < 0.05 vs. controls). Of note, CBR-2092 at 40 mg/kg treatment had maximal efficacy in decreasing bacterial densities in all three target tissues.

In contrast to vancomycin, sterile tissue cultures were observed in vegetations, kidneys and spleens for all CBR-2092 treatment groups following relapse (panel 5).

No CBR-2092 resistance development was observed after the *in vivo* passage and treatment experiments.

CONCLUSIONS

These results suggest that higher doses of CBR-2092 exhibited better efficacy as compared to vancomycin both for treatment and in the prevention of relapse, in this severe MRSA infective endocarditis model.

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