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ABSTRACT

Background: This study compares the efficacy of CBR-2092, a novel rifamycin-quinolone hybrid antibiotic, and rifampin+quinolone combinations against a Quinolone-Resistant MRSA (QRMRSA) strain in a rabbit model of infective endocarditis (IE).

Methods: IE was induced with a QRMRSA (*norAup*, *parCS80F*) after transarotid-transaortic valve indwelling catheterization. At 24h post-infection, animals received either: no therapy (Control); CBR-2092 40 mg/kg, iv, bid; Ciprofloxacin (Cipro) 20 mg/kg, iv, tid + Rifampin (Rif) 10 mg/kg, im, bid; or Levofloxacin (Levo) 20 mg/kg, iv, bid + Rifampin 10 mg/kg, im, bid, all for 3 days. At 24h post-dose, target tissues were cultured. Relapse was also investigated 3 days after treatment was discontinued.

Results: MICs/MBCs of CBR-2092, Cipro, Levo and Rif were 0.008/0.5, 16/16, 2/2 and 0.008/2 µg/mL, respectively. CBR-2092 significantly reduced QMRSA densities in all target tissues at the end of therapy and relapse. Levo+Rif significantly reduced densities but to a lower extent than CBR-2092. Cipro+Rif exhibited the lowest overall efficacy.

Group (# animals)	Log ₁₀ CFU/g. tissue ± SD		
	Vegetation	Kidney	Spleen
Control (11)	8.8 ± 0.5	7.5 ± 0.7	6.1 ± 0.9
CBR-2092 (5)	3.2 ± 1.8*	3.3 ± 2.1*	3.7 ± 1.9*
CBR-2092 relapse (6)	3.6 ± 1.2*	4.2 ± 1.1*	3.9 ± 0.7*
Cipro+Rif (6)	7.0 ± 2.2	5.3 ± 0.9*	5.5 ± 1.2
Cipro+Rif relapse (6)	5.9 ± 1.9	5.4 ± 1.6*	4.9 ± 1.5
Levo+Rif (7)	5.1 ± 0.6*	4.6 ± 1.0*	4.4 ± 0.6*
Levo+Rif relapse (6)	5.4 ± 0.3*	5.1 ± 0.6*	5.3 ± 0.6

*P < 0.05 vs. Controls.

Conclusion: CBR-2092 exhibits excellent efficacy against a QRMRSA strain in the IE model.

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. These observations raise 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-oxoquinolizine 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 the efficacy of CBR-2092 vs. clinically available quinolone + rifampin combinations in the treatment of quinolone resistant MRSA experimental endocarditis in rabbits.

METHODS AND MATERIALS

***S. aureus* strain:** The *S. aureus* strain (CB1834) used in this investigation was a laboratory engineered derivative of a clinical isolate of methicillin-resistant *S. aureus* (MRSA) 67-0. This strain exhibits elevated FQ efflux (*norA^{up}*) and possesses a prevalent Cip resistance mutation (*parC^{S80F}*). CB1834 has the same MIC for CBR-2092 and rifampin as MRSA 67-0, but is resistant to ciprofloxacin with intermediate susceptibility to levofloxacin.

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.

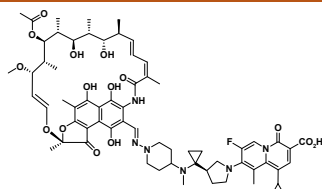
Killing curve: The killing assays were performed in CAMHB inoculated with the test organism at 10⁸ CFU/mL initial inoculum, with either i) no drug exposure (control); ii) CBR-2092 at 1 µg/mL; iii) CBR-2092 at 4 µg/mL; or iv) rifampin at 1 µg/mL and v) levofloxacin at 4 µg/mL. CFU's were determined at 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 (1,2). Animals were infected 48 h after catheterization by iv. injection of 10⁸ CFU *S. aureus* CB1834. At 24 hr after infection, rabbits were randomly assigned to one of the following groups: no therapy (Control); CBR-2092 40 mg/kg, iv, bid; Ciprofloxacin (Cipro) 20 mg/kg, iv, tid + Rifampin (Rif) 10 mg/kg, im, bid; or Levofloxacin (Levo) 20 mg/kg, iv, bid + Rifampin 10 mg/kg, im, bid, all for 3 days. All treatments were for 3 days. At 24h post-dose, target tissues were cultured. Relapse was also investigated 3 days after treatment was discontinued. To monitor *in vivo* CBR-2092 resistance development, homogenate tissues were cultured in parallel on TSB agar plates containing 1 mg/mL CBR-2092. Culture results will be expressed as mean log₁₀CFU per gram of tissue (± SD).

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-8-yl)-pyrrolidin-3-yl]-cyclopropyl)-methylamino]-piperidin-1-ylimino]-methyl-1-yl]-rifamycin SV

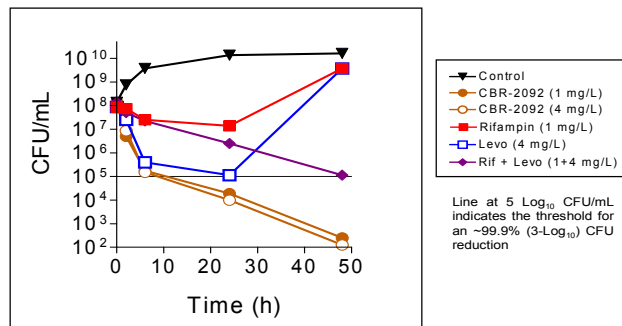
Panel 2: *S. aureus* strains used for this study

Strains	Description	Genotype
67-0	MRSA, virulent in rabbit endocarditis, Rifampin and Quinolone-sensitive	<i>norA^{WT}</i> ; <i>parC^{WT}</i>
CB1834	QRMRSA, 67-0 selected for <i>NorA</i> gain of function with Hoesch H33342 and Norfloxacin, and for <i>ParC^{S80F}</i> with Tosufloxacin	<i>norA^{up}</i> ; <i>parC^{S80F}</i>

Panel 3: MICs/MBCs of CBR-2092, Cipro, Levo, and Rif on *S. aureus* 67-0 and CB1834

Strains	MICs / MBCs (µg/mL)			
	CBR-2092	Cipro	Levo	Rifampin
67-0	0.008 / 0.5	0.5 / 0.5	0.24 / 0.24	0.008 / 2.0
CB1834	0.008 / 0.5	16 / 16	2.0 / 2.0	0.008 / 2.0

Panel 4: *In vitro* killing curve for QRMRSA CB1834



Panel 5: Efficacy of CBR-2092 in the rabbit endocarditis model for QRMRSA CB1834

Group (# animals)	Log ₁₀ CFU/g. tissue ± SD		
	Vegetation	Kidney	Spleen
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*P < 0.05 vs. Controls

INTERPRETATIONS

- The MICs / MBCs of CBR-2092 on the test *S. aureus* strain are 0.008/0.5 µg/mL, while the MICs / MBCs of cipro, levo and rif are 16/16, 2/2 and 0.008/2.0 µg/mL, respectively (panel 3).
- CBR-2092 at both 1 or 4 µg/mL had faster and greater bactericidal effects as compared to levo at 4 µg/mL and levo + rif (4+1 µg/mL) at an initial inoculum of 10⁸ CFU/mL over 48 hrs incubation (panel 4). In addition, no re-growth was observed in CBR-2092 groups (panel 4).
- Therapy with CBR-2092 significantly reduced *S. aureus* densities in all three target tissues at both end-of-treatment and after three days relapse as compared with untreated controls (panel 5). In contrast, cipro+rif did not significantly lower bacterial counts in vegetations and spleen either at the end-of-therapy or after three days therapy; but significantly decreased *S. aureus* densities in kidneys were observed in this treatment group. For the levo+rif group, the combination significantly reduced *S. aureus* densities at both end-of-therapy and three days post-therapy in all three target tissues.
- CBR-2092 treatment showed significantly greater efficacy in reduction of vegetation bacterial counts as directly compared with the two Rif + FQ combinations at both end-of-therapy and 3 days post-therapy.
- No CBR-2092 resistance development was observed after the *in vivo* passage and treatment experiments.
- Similar *S. aureus* counts were seen in the both combination groups cultured on the plates with/without either cipro or levo in the respective groups, suggesting retention of their quinolone-resistant phenotypes during the treatment and following treatment.

CONCLUSIONS

These results suggest that CBR-2092 had the best efficacy in decreasing *S. aureus* densities in all three target tissues as compared with all other treatment groups and when compared to untreated controls. CBR-2092 also had a significantly better impact in reduction of *S. aureus* densities within vegetations as compared with the two combination regimens in this QRMRSA endocarditis model when compared head-to-head.

REFERENCES

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