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Efficacy of CBR-2092, a Novel Rifamycin-Quinolone Hybrid Antibiotic, in Rodent Models of Bacterial Infection.

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Abstract

Background: CBR-2092 is a novel rifamycin-quinolone hybrid antibiotic in development for the treatment of serious bacterial infections. These studies assess CBR-2092 efficacy in various rodent models of infection including acute systemic infections and chronic biofilm-associated infections of indwelling medical devices.

Methods: The murine septicemia model employed *S. aureus* (Sau), *S. epidermidis* (Sepi), *S. pneumoniae* (Spn) and *S. pyogenes* (Spy). Skin abscess and granuloma pouch models of chronic localized infections employed Sau. A central venous catheter (CVC) model and a subcutaneous catheter implant model were used to assess efficacy in biofilm-associated Sau infections.

Results: In the septicemia model, ED₅₀ values for CBR-2092 ranged from 1.4 to 3.8 mg/kg for MSSA, MRSA and FQ-resistant Sau and 4.8 to 10.7 mg/kg for Sepi and Spy infections. In the skin abscess model, CBR-2092 exhibited efficacy comparable to rifampin or cSSSI comparators with log₁₀ CFU reductions of > 3.1 to 5.3 observed for MRSA, Sepi or Spy. In the rat CVC infection model, CBR-2092 exhibited equivalent efficacy to rifampin, but significantly improved over cSSSI comparator agents with log₁₀ CFU reductions of 2.8 to 3.4 observed for MSSA and FQ-resistant Sau. CBR-2092 showed effective penetration into granuloma pouch fluid and reduced Sau counts by 1 to 3.4 log₁₀ CFU after single IV doses of 5 to 25 mg/kg. In the mouse subcutaneous catheter implant model, CBR-2092 was more efficacious than rifampin and cSSSI comparators achieving log₁₀ CFU reductions of 5.03, 3.98 and 2.51 against susceptible, FQ-resistant and rifampin-resistant Sau isolates, respectively. In addition, CBR-2092 exhibited continued suppression of the catheter-localized biofilm infections after therapy was withdrawn.

Conclusions: CBR-2092 exhibits efficacy in murine models of acute, chronic and biofilm-associated infections and therein is a promising development candidate for the treatment of serious bacterial infections in humans.

Introduction

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. Key target pathogens for CBR-2092 include staphylococci, streptococci, and pneumococci and potential usage in the hospital setting includes treatment of complicated skin and skin structure infections and other acute infectious disease syndromes.

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, and effectively addresses the resistance development liability commonly associated with rifamycin use.

These studies assess the efficacy of CBR-2092 in a variety of rodent models of infection including a systemic murine septicemia model (5), subcutaneous abscess (3,4), penetration and efficacy in the rat granuloma pouch (2), central venous catheter (CVC) infection in rats (1) and a chronic biofilm-associated infection model for indwelling medical devices (6,7).

Methods and Materials

Murine Acute Lethal Septicemia - Female CD-1 mice were infected with a pre-determined bacterial inoculum resulting in the death of untreated controls within 24-48 hr. A single IV treatment was initiated 30 min post-infection and survival ratios monitored for 7 days for ED₅₀ determination by Probit analysis (8).

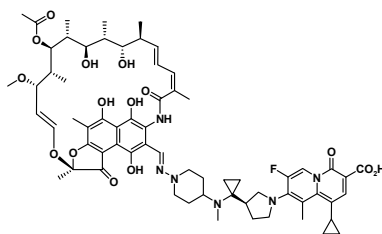
Subcutaneous abscess - Abscesses were formed on the dorsal surface of female CD-1 mice by SC injection of bacteria + dextran (Cytodex) beads. Treatment (IP) was initiated 2 hrs later and continued for 3 days bid. Abscesses were removed and plated for CFU determination + 18 hrs after the last treatment.

Rat Granuloma Pouch - Pouches were formed by SC injection of 30 mL sterile air + sesame oil w/ 1% croton oil. Air was bled out 5 days later and pouches allowed to form over 7 days. Pouches were then infected w/ approx. 10⁸ CFU of *S. aureus* CB192 (ATCC[®] 6538) and a single IV treatment administered + 2 hrs. Blood and pouch samples were collected and analyzed for CBR-2092 levels and bacterial CFU counts.

Rat CVC - Male Sprague-Dawley rats w/ pre-existing JVC were infected with 1 mL of bacterial inoculum directly through the catheter and then flushed. Treatment was initiated + 3 days post-infection, bid x 4 days, via the lateral tail vein. Catheters were excised + 24 hrs post-dose for CFU determination.

Mouse Biofilm Implant - Teflon catheter segments (1 cm) were exposed for 2 hrs to 10⁶ CFU *S. aureus* cultures in TSB+G to establish biofilms. Two colonized catheters were implanted SC on the backs of female *Balb/c* mice. Treatments were initiated 7 days post surgery by bid IP injections for up to 14 days. Catheters were excised and processed for CFU enumeration.

Panel 1: CBR-2092 Structure



- Chemical Name:** R-3-[[4-[1-[[3-Carboxy-1-cyclopropyl-7-fluoro-9-methyl-4-oxo-4H-quinolizine-8-yl)-pyrrolidin-3-yl-cyclopropyl]-methylamino]-piperidin-1-yl-imino]-methylene]-rifamycin SV
- Chemical Formula:** C₄₈₅ H₈₁ F N₆ O₁₅
- Molecular Weight:** 1205.38 Daltons

Panel 2: CBR-2092 *in vitro* antimicrobial activity against test strains

Compound	Minimum Inhibitory Concentration (µg/mL)									
	<i>S. aureus</i> CB192	<i>S. aureus</i> CB899	<i>S. aureus</i> CB969	<i>S. aureus</i> CB785	<i>S. aureus</i> CB901	<i>S. epiderm.</i> CB1260	<i>S. pneumo.</i> CB1352	<i>S. pyog.</i> CB1208	MSSA	Smith
CBR-2092	0.008	0.03	0.008	0.5	0.015	0.008	0.12	0.12	0.008	0.008
Rifampin	0.008	0.008	0.015	> 4	0.008	0.008	0.06	0.06	0.008	0.008
Garfloxacin	0.12	0.06	2	0.12	4	0.24	0.5	0.5	0.12	0.06
Ciprofloxacin	0.24	0.12	16	0.24	16	0.5	1	0.5	0.24	0.12
Vancomycin	1	1	2	1	1	2	0.5	0.5	1	1
Daptomycin	0.24	0.5	0.5	0.5	0.5	0.5	0.24	0.06	0.24	0.5
Linezolid	2	4	2	2	4	2	2	2	2	4
Azithromycin	0.5	1	0.5	0.5	> 16	> 16	0.24	16	0.5	1
Tigecycline	0.12	0.24	0.12	0.24	0.24	0.5	0.06	0.03	0.12	0.24

FQ-R: fluoroquinolone resistant; Rif-R: rifampin-resistant; ML-R: macrolide-resistant

- Microtiter broth MICs performed in accordance with CLSI guidelines.
- MICs were determined for CBR-2092 in the presence of 0.002% polysorbate 80.
- CBR-2092 exhibits potent activity against MSSA, MRSA, FQ-R and Rif-R *S. aureus* isolates, as well as *S. epidermidis*, *Strep. pneumoniae* and *Strep. pyogenes*.

Panel 3: CBR-2092 efficacy - Murine acute lethal septicemia model

Compound	ED ₅₀ mg/kg (95% confidence limits) ^a							
	<i>S. aureus</i> CB192	<i>S. aureus</i> Smith	<i>S. aureus</i> CB901 ^b	<i>S. aureus</i> CB969 ^b	<i>S. epiderm.</i> CB1260	<i>S. pneumo.</i> CB1352	<i>S. pyog.</i> CB1208	LD ₅₀ (no./test)
CBR-2092	1.5	3.8	2.5	1.4	4.3	28.7	5.1	(0.86 - 2.6)
Vancomycin	2.2	0.3	7.1	1.4	6.1	0.6	0.13	(2.3 - 5.6)
Rifampin	(1.7 - 4.1)	(0.26 - 0.39)	(5.1 - 10.8)	(NC)	(4.6 - 10.4)	(0.5 - 0.7)	(0.09 - 0.17)	(0.09 - 0.14)
Garfloxacin	2.7	0.26	> 60	> 60	3.9	13.1	4.3	(0.06 - 0.14)
LD ₅₀	3.7 x 10 ³	3.1 x 10 ³	2.2 x 10 ³	7.1 x 10 ³	5.5 x 10 ³	1 x 10 ³	9.7 x 10 ³	(20)

^a seven day survival ratio from three separate tests, pooled for estimation of median effective dose and 95% upper and lower confidence limits by a computerized program for joint probit analysis; ^b gyrA⁵⁸⁴ + parC¹⁰⁷; NC not calculated

- Single intravenous treatment initiated 30 minutes post-infection.
- Five mice at each of five dose levels per compound were utilized for ED₅₀ determinations.
- CBR-2092 was efficacious against infections with susceptible and resistant *S. aureus* exhibiting ED₅₀ values ranging from 1.4 - 3.8 mg/kg.

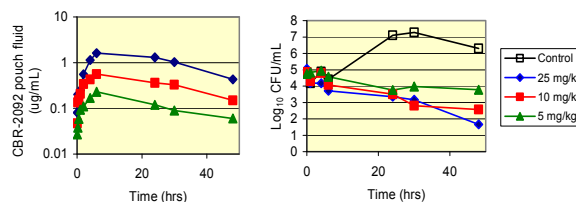
Panel 4: CBR-2092 efficacy - Mouse subcutaneous abscess model

Compound	Log ₁₀ CFU reduction		
	<i>S. aureus</i> ^a	<i>S. pyogenes</i> ^b	<i>S. epidermidis</i> ^b
CBR-2092	> 3.1	5.3	3.4
Rifampin	> 3.1	> 5.4	3.1
Daptomycin	3.0	> 5.4	2.2
Vancomycin	3.0	5.3	2.0
Linezolid	2.3	5.3	1.2
Tigecycline	3.1	> 5.4	NT

^a methicillin and fluoroquinolone resistant; ^b macrolide - resistant
NT = Not tested

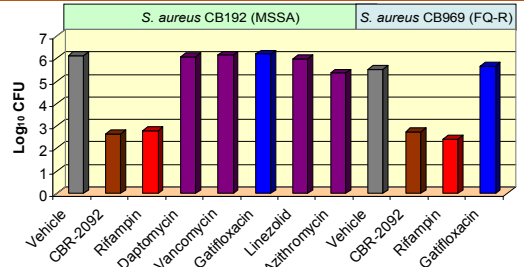
- Infection established by subcutaneous injection of bacteria + Cytodex beads.
- Treatment (IP) 25 mg/kg bid for three days, abscesses were excised + 24 hrs post-dose.
- CBR-2092 exhibits comparable efficacy to commonly used cSSSI antimicrobial agents for *S. aureus* and *S. pyogenes* and was the most efficacious against *S. epidermidis*.

Panel 5: Efficacy of CBR-2092 in the rat granuloma pouch model



- Single intravenous treatment initiated 2 hrs post-infection with *S. aureus* CB192.
- Pouch fluid samples removed at pre-selected time points and analyzed.
- CBR-2092 penetrated into the granuloma pouch and effectively reduced bacterial counts by 1.0, 2.3 and 3.4 log₁₀ CFU, from the start of therapy, at single doses of 5, 10 and 25 mg/kg, respectively.

Panel 6: CBR-2092 efficacy in the rat CVC model



- Stable, mature biofilm infection introduced on tip of jugular vein catheter.
- All treatments administered at 10 mg/kg bid x 4 days via the tail vein.
- CBR-2092 demonstrates superior efficacy against both susceptible and FQ-R *S. aureus* strains as compared to Daptomycin, Vancomycin, Garfloxacin, Linezolid and Azithromycin.

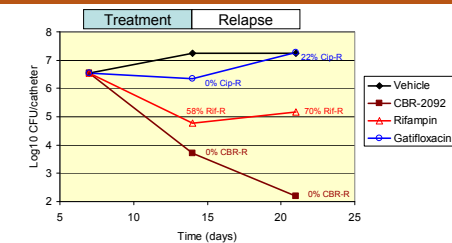
Panel 7: Efficacy of CBR-2092 in mouse catheter biofilm model

Compound	Log ₁₀ CFU Reduction		
	<i>S. aureus</i> CB192	<i>S. aureus</i> CB969	<i>S. aureus</i> CB785
CBR-2092	5.03	3.98	2.51
Rifampin	2.09	2.25	0.03
Garfloxacin	1.67	0.01	2.34
Ciprofloxacin	NT	0.16	1.01
Daptomycin	0.57	2.45	2.64
Vancomycin	0.03	0.01	NT
Linezolid	NT	0.83	NT

FQ-R: fluoroquinolone resistant; Rif-R: rifampin-resistant

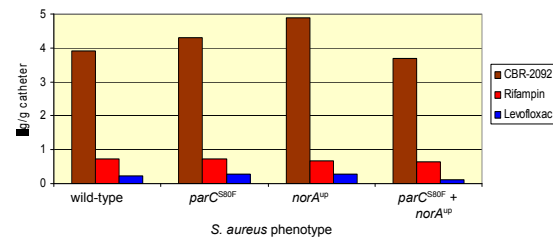
- All compounds administered at 25 mg/kg IP bid on days 7 - 21 post-infection.
- CBR-2092 demonstrated superior efficacy profile against biofilm infections with wild-type, fluoroquinolone-resistant and rifampin-resistant *S. aureus* isolates.
- No resistance to CBR-2092 emerged during treatment.

Panel 8: Effect of relapse on CBR-2092 efficacy in the mouse catheter biofilm infection model



- All compounds administered at 25 mg/kg IP bid on days 7 - 14 post-infection (no treatment was administered on days 15 - 20) with *S. aureus* CB192.
- CBR-2092 exhibits sustained antimicrobial efficacy after treatment and during relapse.
- No CBR-2092 resistance emerged during or after treatment and relapse.

Panel 9: CBR-2092 levels in biofilm infected catheters



- Subcutaneously implanted teflon catheter infected with isogenic strains of *S. aureus*.
- All compounds administered IP bid on days 7 - 21 post-infection.
- CBR-2092 (25 mg/kg) achieves higher (and accumulating) levels in infected catheters compared to either Rifampin or Levofloxacin (administered as a 20 + 25 mg/kg cocktail).

Summary and Conclusions

Summary

- CBR-2092 exhibited efficacy in an acute infection of a mouse lethal septicemia model against susceptible and fluoroquinolone-resistant *S. aureus* as well as infections with MRSA, *S. epidermidis*, *S. pyogenes* and *S. pneumoniae*.
- CBR-2092 efficacy was equivalent to rifampin and cSSSI agents (Vancomycin, Daptomycin and Linezolid) vs FQ-R MRSA, *S. pyogenes* and *S. epidermidis* in a murine subcutaneous abscess infection model.
- CBR-2092 penetrated into, achieved sustained levels, and effectively reduced *S. aureus* CFUs from pouch fluid in the rat granuloma pouch infection model.
- In chronic infection models involving biofilm formation (rat CVC and mouse implant), CBR-2092 demonstrated superior efficacy and resistance profiles against infections with wild-type, fluoroquinolone-resistant and rifampin-resistant *S. aureus* strains.
- No resistance to CBR-2092 emerged during treatment in any of the infection models described herein.

Conclusions

- CBR-2092 demonstrates excellent activity / efficacy in *in vivo* models of biofilm infections while retaining activity in animal models of acute infections.
- Currently in Phase I development, CBR-2092 has the potential for:
 - Treatment of acute infections in patients with foreign body complications.
 - Stand alone agent for treatment of device-associated infections.

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