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In Vitro Studies of the Efficacy of CBR-2092, a Novel Rifamycin-Quinolone Hybrid Antibiotic, in Killing Staphylococcal Cells in Biofilms.

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Abstract

Background: CBR-2092 is a novel rifamycin-quinolone hybrid antibiotic in development for the treatment of serious bacterial infections, including biofilm infections of indwelling medical devices. The *in vitro* activity of CBR-2092 in killing staphylococcal cells in biofilms is described herein in studies employing alternate assay formats.

Methods: Studies of biofilm efficacy were undertaken using the MBEC device (Innovotech Inc.), colony biofilms, a 96-well plate-based format and a drip-flow reactor system. In all cases, efficacy and resistance development were evaluated in parallel by CFU determination.

Results: CBR-2092 was found to exhibit potent anti-staphylococcal biofilm activity with measured Minimum Biofilm Eradication Concentrations (MBECs) of 0.5-4 µg/mL at 24 h. CBR-2092 also proved efficacious against plate-adherent *S. epidermidis* biofilms, reducing viable counts to the limit of detection by 48 h (≥ 7-Log₁₀ CFU reduction). In colony biofilm assays, CBR-2092 exhibits dose-dependent bactericidal properties against mature *S. aureus* biofilms demonstrating up to ~2-Log₁₀ CFU/mL reduction by 24 h and eradication of viable *S. aureus* cells (≤ 125 CFU/mL) with longer dosing. CBR-2092 also demonstrates excellent bactericidal properties (Log₁₀ CFU/cm² reductions at 24 h of 3.3 and 4.8 at 1 and 4 µg/mL, respectively) versus *S. epidermidis* biofilms grown on silicone catheter slices in a drip-flow reactor. In this system, CBR-2092-treated biofilms showed little to no recovery following termination of dosing and recovery in drug-free media.

Conclusions: Data from multiple assay formats indicate that CBR-2092 retains potent bactericidal activity against staphylococcal cells in the biofilm state. In contrast to existing rifamycin class agents, resistance development in biofilm populations is not observed with CBR-2092. These studies hold promise for the potential of CBR-2092 in the treatment of biofilm-associated infections.

Introduction

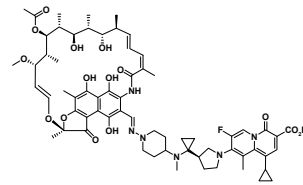
Antibiotics of the rifamycin class have proven efficacy in the treatment of persistent bacterial infections including tuberculosis and biofilm-associated infections of indwelling medical devices (2, 5, 7). However, the relative ease with which bacteria develop resistance to the rifamycins restricts their clinical use to antibiotic combination regimens (1). In a program directed toward the synthesis and evaluation of rifamycin-based multi-functional antibiotics, a series of compounds were prepared that covalently combine rifamycin and quinolone pharmacophores to form stable hybrid antibiotic agents.

CBR-2092 combines the rifamycin SV and 4H-4-oxoquinolizone (3) pharmacophores via a chiral linking group. Herein we report the *in vitro* activity of CBR-2092 in killing staphylococcal cells in biofilms in studies that employ a number of alternate assay formats. Overall, the combined data suggest that CBR-2092 exhibits activity in killing staphylococcal cells in established biofilms that are superior to both parent antibiotics, rifampin+fluoroquinolone cocktails and comparator agents. These data hold promise for the potential utility of CBR-2092 in the treatment of biofilm-associated infections including those of indwelling medical devices.

Methods and Materials

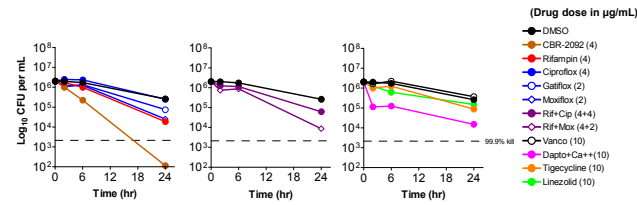
Time-kill studies involving non-growing *S. aureus* were performed using *S. aureus* ATCC #29213 after 1-day pre-incubation in 1x-phosphate buffered saline with 0.1 % (w/v) glucose and 0.002 % (v/v) polysorbate-80 to serve as a surfactant. Studies of biofilm efficacy employed either *S. aureus* ATCC# 6538 or *S. epidermidis* ATCC# 35984 in an adapted colony biofilm assay (6), a 96-well plate assay (6), an enhanced Drip-Flow Reactor system (4) and assays employing the MBEC device (Innovotech Inc.) or the CDC biofilm reactor (Cytergy LLC, Biosurface technologies). In all cases, efficacy and resistance development were evaluated in parallel by determination of remaining viable cell counts on media with or without supplementation with test agents at the indicated concentrations. Rifampin and quinolones were dosed at current CLSI breakpoints.

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-ylimino]-methylene]-rifamycin SV
- Chemical Formula:** C₆₅ H₈₁ F N₆ O₁₅
- Molecular Weight:** 1205.38 Daltons

Panel 2: CBR-2092 Time-kill studies – non-growing *S. aureus*



CBR-2092 profiled in time kill assay vs non-growing *S. aureus* in 1xPBS, 0.1% glucose.

- CBR-2092 retains excellent time-dependent cidal activity versus non-growing *S. aureus* cells
- CBR-2092 exhibits better cidal activity compared to benchmark agents or Rif + Fq cocktails
- Only CBR-2092 achieves 99.9% CFU reduction in < 24 h

Panel 3: CBR-2092 dose response in plate adherence biofilm model

Antibiotic dose tested (µg/mL)	Remaining adherent CFU/mL following 48 h treatment						
	Control	CBR-2092	Rifampin	Ciproflox	Gatiflox	Vancomycin	Linezolid
1	8.8 ± 0.1	1.5 ± 0.1	8.6 ± 0.1	8.6 ± 0.1	7.6 ± 0.0	—	—
4	8.8 ± 0.1	< 1.4	8.0 ± 0.8	4.9 ± 0.1	3.5 ± 0.5	8.7 ± 0.2	8.7 ± 0.1
16	8.8 ± 0.1	< 1.4	8.5 ± 0.2	5.9 ± 0.2	2.9 ± 0.1	8.7 ± 0.2	8.0 ± 0.7

CBR-2092 activity profiled against *S. epidermidis* in 96-well plate adherence biofilm model.

- CBR-2092 exhibits excellent anti-biofilm activity across a range of concentrations
- Rifampin shows limited efficacy - due to resistance development (not shown)
- Quinolones exhibit dose dependent efficacy that is inferior to CBR-2092
- Vancomycin and linezolid exhibit limited efficacy in this model

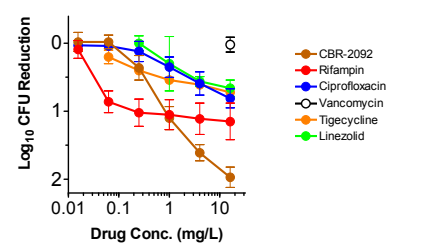
Panel 4: CBR-2092 MBEC - Calgary Biofilm Device

Antimicrobial agent	MIC (µg/mL)		MBC _{99.9} (µg/mL)		MBEC (µg/mL)
	CLSI assay	Assay with CBD	CLSI assay	Assay with CBD	0 CFU/peg - recovery method
CBR-2092	≤ 0.015	0.03	2	2 - 8	0.5 - 4
Rifampin	≤ 0.015	0.06	2	4	> 32
Ciprofloxacin	0.24	1	0.5	32	> 32
Moxifloxacin	0.06	0.12 - 0.24	0.12	2	> 32
Rif+Cip (1:1)	≤ 0.03	0.24	16	16	> 32
Vancomycin	1	8 - 16	2	16 - > 32	> 32
Linezolid	2	8	> 32	> 32	> 32

CBR-2092 activity assessed through MIC, MBC, or MBEC endpoints using the Calgary Biofilm Device (CBD).

- CBR-2092 exhibits potent rifampin-like *in vitro* potency by MIC or MBC endpoint
- Comparator agents or parental class controls exhibit anticipated *in vitro* activities
- Only CBR-2092 exhibits an MBEC endpoint (0.5 - 4 µg/mL) versus 24 h old biofilms

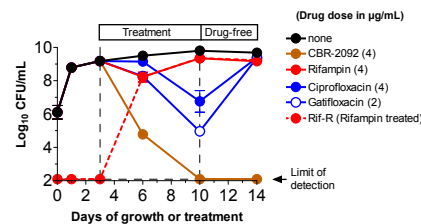
Panel 5: CBR-2092 dose-response - Colony Biofilm



CBR-2092 activity profiled in 24 h colony biofilm model with *S. aureus*.

- Dose dependent bactericidal properties observed for CBR-2092
- At doses of > 1 µg/mL, CBR-2092 exhibits better overall biofilm efficacy than comparators
- Unlike rifampin, no resistance development observed for CBR-2092 (not shown)

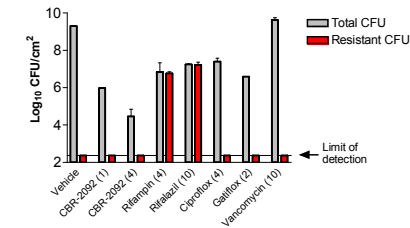
Panel 6: CBR-2092 efficacy and resistance - Colony Biofilm



CBR-2092 activity profiled in long-term colony biofilm model with *S. aureus*.

- CBR-2092 achieves apparent sterilization of the biofilm with 7 days treatment
- Rifampin efficacious initially, but fails due to outgrowth of Rif-R sub-population
- Ciprofloxacin or gatifloxacin are efficacious, but fail to eliminate biofilm 'persisters'

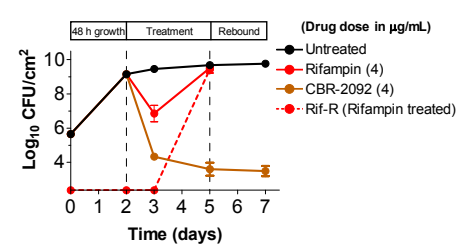
Panel 7: CBR-2092 dose response - Drip Flow Reactor



CBR-2092 profiled in enhanced Drip-flow reactor assay versus *S. epidermidis* with 24 h treatments.

- CBR-2092 exhibits dose-dependent cidal activity that is superior to comparators
- Rifampin & rifalazil exhibit limited efficacy owing to resistance development
- Ciprofloxacin and gatifloxacin suppress resistance development, but exhibit inferior efficacy compared to CBR-2092

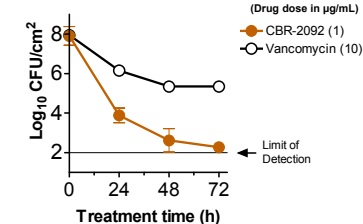
Panel 8: CBR-2092 efficacy - Drip Flow Reactor



CBR-2092 profiled in long-term Drip-flow reactor assay versus *S. epidermidis*.

- Rifampin efficacious early, but treatment fails due to resistance development
- CBR-2092 treatment reduces viable CFU to near the limit of detection without resistance development
- CBR-2092 treated samples show no recovery once treatment is terminated

Panel 9: CBR-2092 efficacy - CDC Biofilm Reactor



CBR-2092 profiled using CDC biofilm reactor model versus *S. aureus*.

- Vancomycin exhibits limited efficacy in the CDC biofilm reactor model
- CBR-2092 treatment reduces viable CFU to near the limit of detection without resistance development

Summary and Conclusion

Summary:

- CBR-2092 is a novel rifamycin-quinolone hybrid antibiotic in development for the treatment of serious bacterial infections, including biofilm infections of indwelling medical devices.
- CBR-2092 exhibits excellent *in vitro* potency, including significant bactericidal activity versus non-growing *S. aureus*.
- CBR-2092 was demonstrated to exhibit superior efficacy in multiple *in vitro* assays of biofilm efficacy:
 - Enhanced killing of staphylococci in established biofilms.
 - No observed resistance development.
- Studies using commercial biofilm devices, including the MBEC Calgary device and CDC reactor model, further substantiate the overall potential of CBR-2092 to treat staphylococci cultivated in the biofilm state.

Conclusion:

- Data from multiple discrete assay formats indicate that CBR-2092 retains potent bactericidal activity against staphylococcal cells in the biofilm state without selecting for antibacterial resistance.

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