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Pharmacokinetic and Pharmacodynamic Studies with CBR-2092, a Novel Rifamycin-Quinolone Hybrid Antibiotic.

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Abstract

Background: CBR-2092 is a novel rifamycin-quinolone hybrid antibiotic in development for the treatment of serious bacterial infections. CBR-2092 exhibits efficacy in various animal infection models including models of acute and biofilm-associated infections. The current studies describe the pharmacokinetics (PK) of CBR-2092 following intravenous (IV) administration to mice, rats, rabbits and dogs as well as the determination of the pharmacodynamic (PD) parameter best correlated with *in vitro* and *in vivo* efficacy.

Methods: In PK studies, CBR-2092 was administered IV in single ascending doses to CD-1 mice, Sprague-Dawley rats, NZW rabbits and beagle dogs and plasma levels determined by LCMS. For *in vivo* PD studies, the mouse neutropenic thigh infection model was used with CBR-2092 doses ranging from 4 to 256 mg/kg administered q2, q8 and q24 hour basis. For *in vitro* PD studies, CBR-2092 dosing regimens (1, 3 and 6 bolus doses with a 2.9h $T_{1/2}$) were simulated in a drip flow reactor system with established *S. epidermidis* biofilms.

Results: PK analysis over the dose ranges tested indicated a $T_{1/2}$ for CBR-2092 of 0.4 to 4.1 h in mice, rats and rabbits and 1.6 to 11.7 h in dogs. The calculated volume of distribution of CBR-2092 ranged from 0.08 to 0.52 and 0.09 to 0.17 L/kg in rodents/rabbits and dogs, respectively. Plasma clearance ranged from 0.07 to 0.36 and 0.01 to 0.08 L/h/kg in rodents/rabbits and dogs, respectively. Calculated AUC and C_{max} values were dose proportional for all species. Data from the neutropenic thigh model and the drip flow biofilm reactor identified the 24h AUC/MIC ratio as the PD index best correlated with efficacy. The static dose of CBR-2092 in the thigh model was 29 mg/kg corresponding to an AUC (total drug) of 53 µg-hr/mL.

Conclusions: CBR-2092 exhibits a linear dose proportional increase in exposure (AUC) when administered IV in mice, rats, rabbits and dogs. The AUC was determined to be the PD parameter best correlated with efficacy both *in vivo* and *in vitro*.

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. CBR-2092 is a new molecular entity that combines rifamycin SV and 4*H*-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.

Pharmacokinetics, the quantitative study of the time course of drug absorption, distribution, metabolism and elimination (ADME) allows for the determination of the therapeutic potential of a given compound. Data collected from pharmacokinetic studies, used in conjunction with efficacy and pharmacodynamic studies, is vital in estimating a target clinical exposure and dose regimens (5,6). In order to determine this relationship, dose escalation studies in several species, both rodent and non-rodent are required. In this study, escalating doses of CBR-2092 were administered intravenously to mice, rats, rabbits and dogs. In addition, pharmacodynamic studies were carried out in immunocompromised mice infected with *Staphylococcus aureus* and in an *in vitro* drip flow model with *S. epidermidis* biofilms in order to determine the pharmacodynamic parameter that best correlates with efficacy (3).

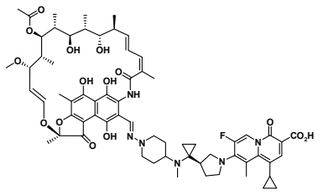
Methods and Materials

Female CD-1 mice, male Sprague-Dawley rats, male New Zealand White rabbits and male/female beagle dogs were administered single intravenous (bolus) escalating doses of CBR-2092 contained in sterile water for injection (WFI). Blood samples were collected over pre-selected time points and plasma collected and analyzed for CBR-2092 by established LCMS methodology. Pharmacokinetic parameters were determined with analysis of plasma levels using WinNonlin Professional Version5.0 (Pharsight Corp). Parameters were; area under the concentration vs time curve to infinity ($AUC_{0\text{-}inf}$), volume of distribution (V_d), plasma clearance (Cl), terminal half-life and maximum observed concentration (C_{first}).

For the murine thigh-infection model, female CD1 mice were rendered neutropenic with Cytoxan. Thigh infections were produced by injection of 10^6 CFU *S. aureus* CB192 (ATCC#6538). Intraperitoneal (IP) dose fractionations consisted of 1, 3, or 12 doses (q24, q8, and q2, respectively) administered over 24 hours and ranging in total dose from 4 mg/kg to 256 mg/kg. After treatment, thighs were removed and bacterial counts were determined and correlated with the PK/PD indices (based on established mouse pharmacokinetic parameters); AUC, C_{max} and T>MIC (1).

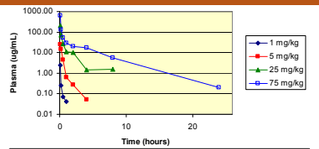
In the drip flow reactor system silicone catheters were inoculated with 10^5 *S. epidermidis* CB191 (ATCC# 35984) cells in TSB and grown for 48 hr by dripping 1/10 strength TSB supplemented with 0.04% P-80 and 4% BSA at 4 mL/hr over inoculated samples to produce mature biofilms. Biofilms were treated for 24 hr with multiple CBR-2092 regimens that simulated 1, 3, or 6 bolus doses and a half life of 2.9 hr. Surviving cells were recovered and plated for viable counts to determine treatment efficacy (2,4).

Panel 1: CBR-2092 Structure



- **Chemical Name:** *R*-3-[(4-{1-[l-(3-Carboxy-1-cyclopropyl-7-fluoro-9-methyl-4-oxo-4*H*-quinolizine-8-yl)-pyrrolidin-3-yl-cyclopropyl]-methylamino}-piperidin-1-ylimino)-methylenyl]-rifamycin SV
- **Chemical Formula:** $C_{65}H_{81}FN_6O_{15}$
- **Molecular Weight:** 1205.38 Daltons

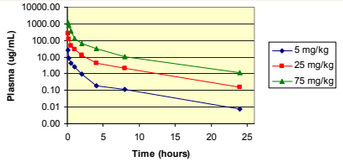
Panel 2: CBR-2092 Pharmacokinetics in Female CD-1 Mice



Parameter	Dosage (mg/kg) 1	5	25	75
$AUC_{0\text{-}inf}$ (ug*h/mL)	0.75	10.1	98.7	346
V_d (L/kg)	0.16	0.18	0.32	0.52
Cl (L/h/kg)	1.3	0.5	0.25	0.22
Half-life (h)	0.4	1.2	3.5	3.5
C_{first} (ug/mL)	2.5	24.3	202	629

- Pharmacokinetics following a single IV bolus dose of CBR-2092 via tail vein injection.
- Plasma analysis performed by LCMS with 3 mice pooled per time point.
- CBR-2092 exhibits predictable increases in exposure (AUC) and C_{first} with dose.

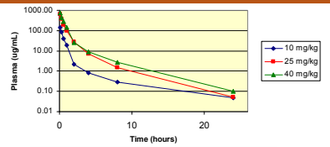
Panel 3: CBR-2092 Pharmacokinetics in Sprague-Dawley Rats



Parameter	Dosage (mg/kg) 5	25	75
$AUC_{0\text{-}inf}$ (ug*h/mL)	14	168	923
V_d (L/kg)	0.51	0.31	0.18
Cl (L/h/kg)	0.36	0.15	0.08
Half-life (h)	1.6	2.5	4.1
C_{first} (ug/mL)	27.4	267.3	1217

- Pharmacokinetics following a single IV bolus dose of CBR-2092 via jugular vein catheter.
- Linear, but non-proportional, increase in exposure parameters with dose were observed.
- Mean observed plasma half-life (T½) = 2.7 hr with a volume of distribution range of 0.2 – 0.5 L/kg.

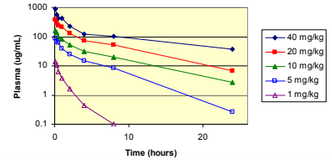
Panel 4: CBR-2092 Pharmacokinetics in New Zealand White Rabbits



Parameter	Dosage (mg/kg) 10	25	40
$AUC_{0\text{-}inf}$ (ug*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
Half-life (h)	3.87	3.52	3.41
C_{first} (ug/mL)	140.7	632.9	762.9

- Pharmacokinetics following a single IV bolus dose of CBR-2092 via jugular vein canula.
- Linear, but non-proportional, increase in exposure parameters with dose were observed.
- Calculated plasma half-life of 3.4 – 3.9 for doses between 10 and 40 mg/kg.

Panel 5: CBR-2092 Pharmacokinetics in Beagle Dogs

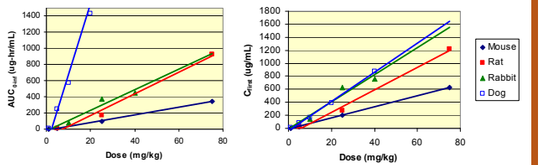


Parameter	Dosage (mg/kg) 1	5	10	20	40
$AUC_{0\text{-}inf}$ (ug*h/mL)	14.1	253	572	1432	3407
V_d (L/kg)	0.09	0.09	0.11	0.09	0.17
Cl (L/h/kg)	0.08	0.02	0.02	0.01	0.012
Half-life (h)	1.6	3.4	5.2	5.7	11.7
C_{first} (ug/mL)	14.2	86	163	390	879

- Pharmacokinetics following a single IV bolus dose of CBR-2092 via femoral vein.
- Proportional and generally linear increase in both AUC and C_{first} with increased dose.
- Observed plasma half-life of 3.4 – 11.7 for doses between 5 to 40 mg/kg.

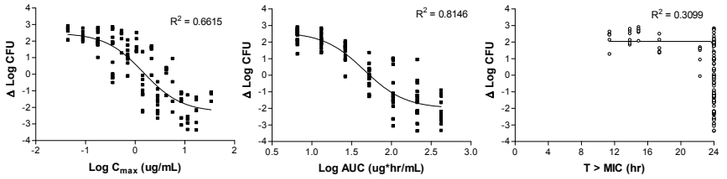
Panel 6: CBR-2092 Pharmacokinetics: Species Comparison

Relationship of administered IV doses of CBR-2092 with calculated $AUC_{0\text{-}inf}$ and C_{first} for mice, rats, rabbits and dogs



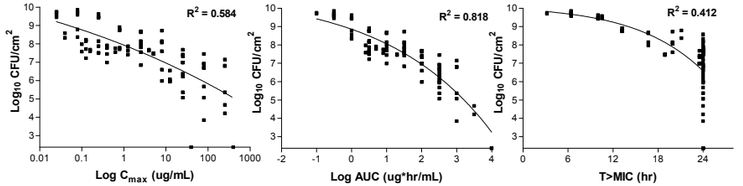
- Association between administered CBR-2092 dose, AUC and C_{first} across species.
- General observed increase in both AUC and C_{first} per dose in higher species.
- Correlation of dose to $AUC_{0\text{-}inf}$ and C_{first} yields a mean correlation $R^2 = 0.96$ for all species.

Panel 7: CBR-2092 Pharmacodynamics in the Murine Neutropenic Thigh Infection Model



- Mouse neutropenic thigh infection with *S. aureus* CB192 (ATCC# 6538).
- Dose fractionations (q24, q8, and q2) administered over 24 hours from 4 to 256 mg/kg.
- The 24 hr AUC is the PK/PD index that best correlates ($R^2 = 0.8146$) with observed antimicrobial efficacy.
- The static dose of CBR-2092 in the neutropenic thigh model was 29 mg/kg.

Panel 8: CBR-2092 Pharmacodynamics : *In vitro* Drip Flow Reactor



- Silicone catheter channels were infected with *S. epidermidis* CB191 (ATCC# 35984) biofilm.
- Pharmacokinetic simulations of 1, 3, or 6 bolus doses with a half life of 2.9 hr were achieved.
- The 24 hr AUC is the PK/PD index that best correlates ($R^2 = 0.818$) with observed anti-biofilm efficacy.

Summary and Conclusions

Summary

Pharmacokinetics of single intravenous doses of CBR-2092

- The plasma elimination half-life for CBR-2092 was 0.4 to 4.1 hr in mice, rats and rabbits and 1.6 to 11.7 hr in dogs.
- The calculated volume of distribution for CBR-2092 ranged from 0.08 to 0.52 in mice, rats and rabbits and 0.09 to 0.17 L/kg in dogs.
- Plasma clearance was slower in the higher species, ranging from 0.07 to 0.36 and 0.01 to 0.08 L/h/kg in mice, rats, rabbits and dogs, respectively.
- AUC and C_{first} values increased with increasing dose for all species and were generally linear.

Pharmacodynamics of CBR-2092 in an *in vivo* neutropenic mouse thigh infection model and an *in vitro* drip flow biofilm reactor

- The PK/PD parameter that best correlates with the *in vitro* efficacy of CBR-2092 for the treatment of *S. epidermidis* biofilms is the 24 hr AUC.
- The 24 hr AUC was also the PK/PD parameter that best correlated with efficacy in the neutropenic thigh infection model with *S. aureus*.
- The static dose of CBR-2092 in the neutropenic thigh model was 29 mg/kg which corresponds to a plasma $AUC_{0\text{-}inf}$ (total drug) of 53 µg-hr/mL.

Conclusions

- CBR-2092 exhibits dose dependant and predictable pharmacokinetics in four species following intravenous administration.
- The 24 hr area under the concentration vs time curve (AUC) is the pharmacodynamic parameter most predictive of efficacy for both *in vivo* acute and *in vitro* biofilm infections.

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