

Pharmacokinetics and efficacy of ectolysin P128 in a mouse model of systemic Methicillin Resistant *Staphylococcus aureus* (MRSA) infection

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Abstract

P128 is a recombinant chimeric bacteriophage-derived ectolysin with bactericidal activity against a wide range of Staphylococci including MRSA. P128 is efficacious in animal models of *S. aureus* infection and is under development for Staphylococcal bacteremia. We studied the Pharmacokinetics (PK) of parenteral P128 in BALB/c mice and correlated the PK characteristics with efficacy in systemic MRSA infection. The PK profile was characterized following single dose intravenous (IV) administrations of P128 at 10, 30 and 60 mg/kg. Blood samples were collected at different time points and concentration of P128 in plasma was determined by ELISA.

PK parameters were estimated using non-compartmental analysis in Phoenix WinNonlin software. P128 showed multi-exponential decline with low systemic clearance and low volume of distribution at steady state, and a long terminal half-life ranging between 5.2 - 5.6 h. The C_{max} was 167, 459 and 2213 $\mu\text{g/ml}$ and AUC_{inf} was 12, 48 and 225 $\mu\text{g}\cdot\text{h/ml}$ at 10, 30 and 60 mg/kg, respectively. For testing efficacy the standard neutropenic mouse model of bacteremia was used. BALB/c mice rendered neutropenic with cyclophosphamide and challenged *via* the intraperitoneal route with MRSA COL (10^8 CFU/mouse) were treated 2 h post-infection with P128 (10, 30 and 60 mg/kg) by IV route. Bacterial load in blood was determined at different time points over 24 h by plating and enumerating CFU.

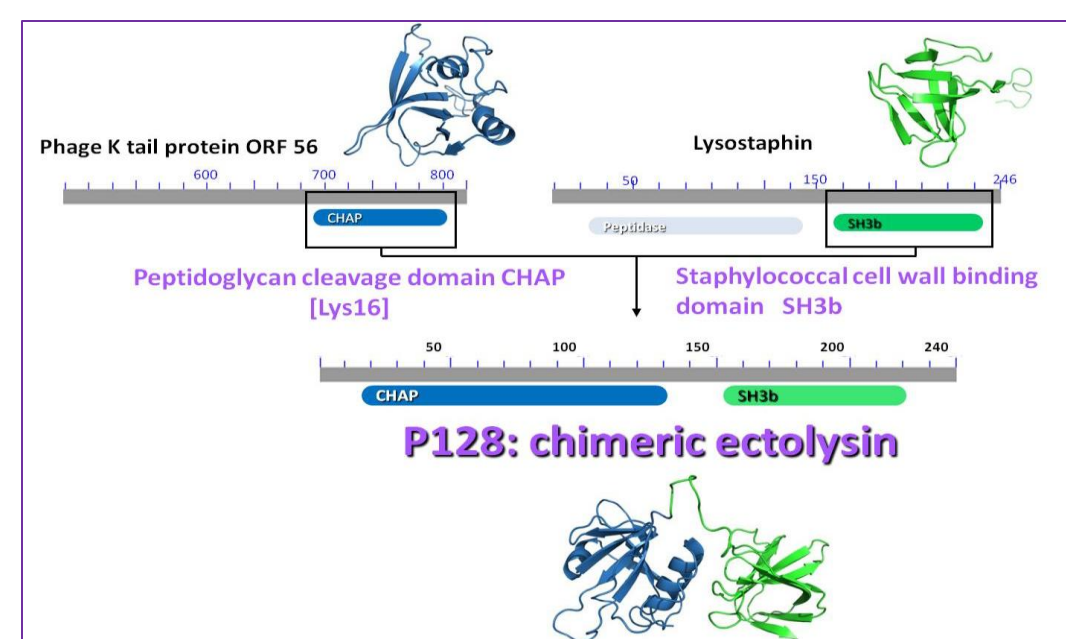
P128 showed dose dependent antibacterial activity following single IV bolus doses. Maximum bactericidal effect was seen within 30 minutes of administration. The $t > MIC$ was 15, 30 and 60 minutes for 10, 30 and 60mg/kg, respectively. CFU counts in blood of untreated animals were in the range of $10^7 - 10^8/\text{ml}$, over 24 hours. In animals treated with 30 or 60mg/kg, CFU counts in blood were at least 2 orders of magnitude lower ($10^4 - 10^6$ CFU/ml) than controls. These doses resulted in high circulating levels of P128 at the initial time points which accounted for the rapid killing of cells. CFU counts remained in the same low range upto 24 h although the concentration of P128 in circulation was well below the MIC during this phase.

The potent, rapid bactericidal activity and prolonged maintenance of low CFU counts in blood from a single dose of P128, afford significant advantages. With these properties, P128 has potential as a stand-alone therapy or be used in combination with SoC antibiotics for the treatment of MRSA bacteremia.

Introduction

- Methicillin resistant *Staphylococcus aureus* (MRSA) is a leading cause of bacteremia and systemic infections which are difficult to treat, such as those with endocarditis.
- With increasing resistance to antibiotics, there is a dire need for novel agents to control MRSA.
- P128 is a chimeric recombinant ectolysin (phage lysin involved in cleaving the peptidoglycan from outside the bacterium during DNA injection) derived from the tail of *Staphylococcus* phage K.
- P128 specifically kills *Staphylococcus* spp. and shows no inhibition of other bacterial species or of eukaryotic cells even at concentrations as high as 1 mg/ml.
- P128 has demonstrated potent antibiofilm activity on MRSA and coagulase-negative Staph (CoNS) strains in various *in vitro* models including those involving clinically relevant mixed bacterial species.
- P128 is highly synergistic with standard-of-care (SoC) drugs in inhibiting *S. aureus* and CoNS cells, especially those embedded in biofilms.
- These properties make it an attractive candidate for antibacterial drug development.

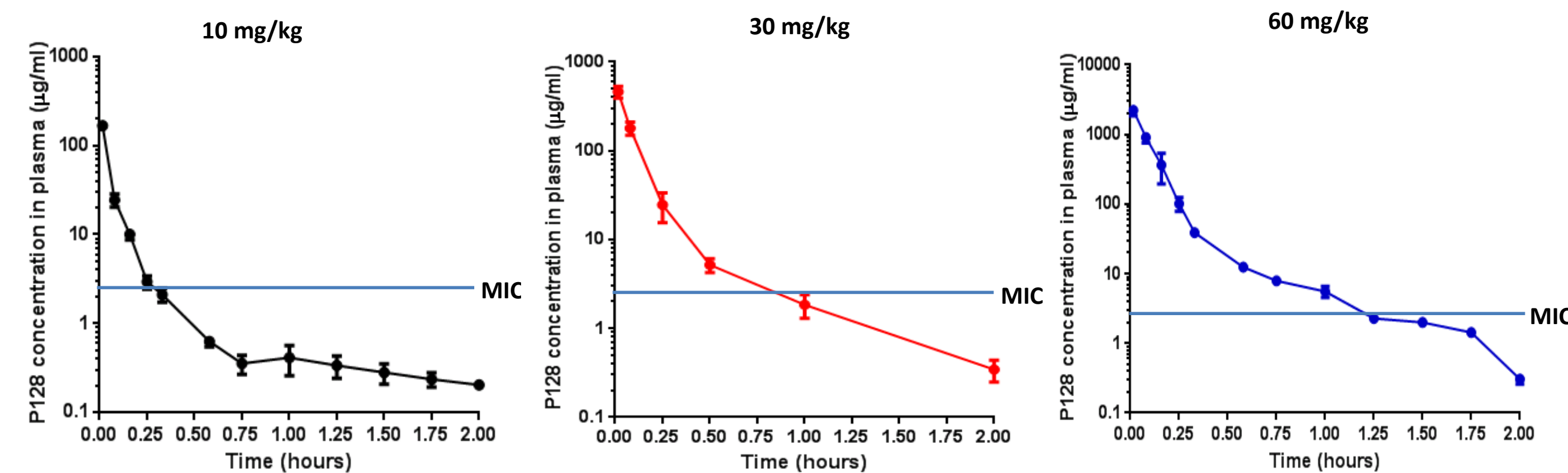
- In this study, we have investigated the Pharmacokinetics (PK) of parenteral P128 in BALB/c mice and explored the relationship between PK and efficacy in mouse model of systemic MRSA infection.



Methods: Pharmacokinetics in mice

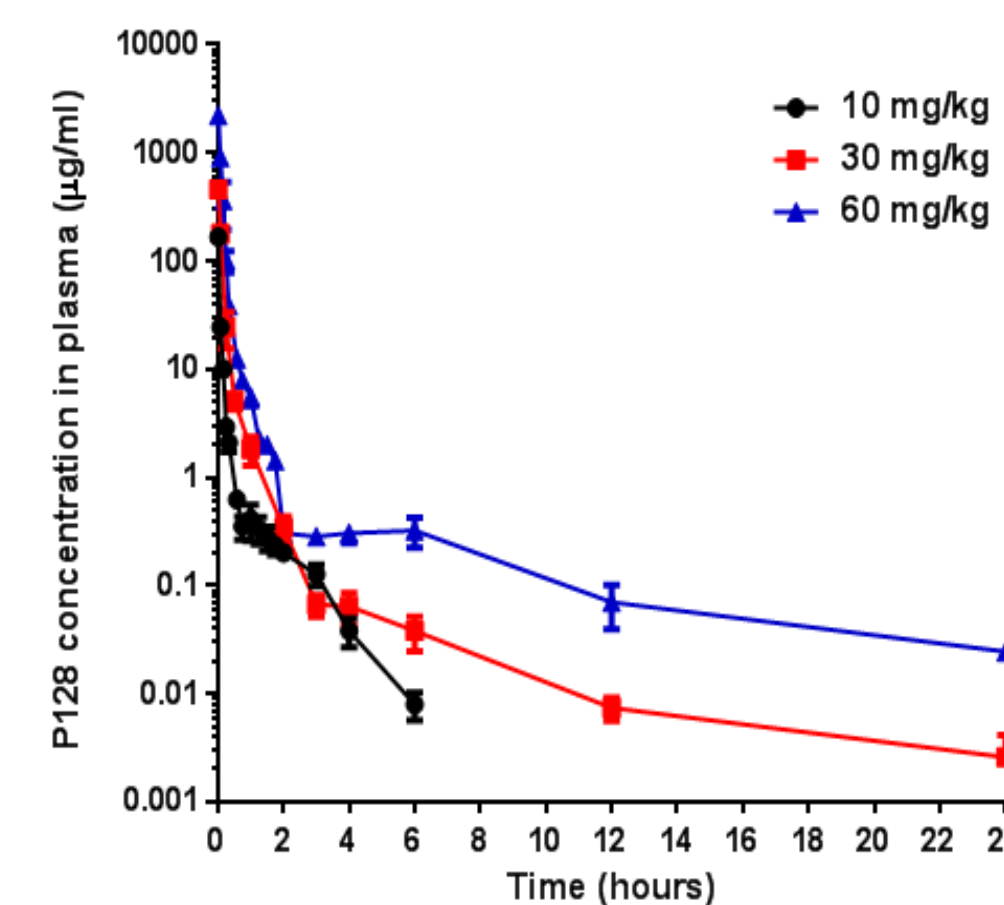
Healthy female BALB/c mice were administered single intravenous bolus doses of 10, 30 or 60 mg/kg. Blood samples were collected at different time points and concentration of P128 in plasma was determined by ELISA which was in a microplate format and utilized affinity-purified rabbit anti-P128 polyclonal antibodies. Pooled mouse plasma used to dilute samples was screened to ensure absence of interference in the assay. The mean concentration of P128 over time was plotted and PK parameters were estimated using non-compartmental analysis using Phoenix WinNonlin software.

Exploratory Data Analysis



- P128 displayed multi-exponential disposition and a slow distribution phase was seen up to 4 h post dose at 30 and 60 mg/kg.
- First order kinetics was observed at 10 and 30 mg/kg; at 60 mg/kg, clearance was non-linear.

Pharmacokinetic characteristics of P128 (Non-compartmental analysis)



PK parameters of P128 following single IV doses of 10, 30 and 60 mg/kg in naïve BALB/c mice

PK Parameter	10 mg/kg	30 mg/kg	60 mg/kg
$t_{1/2}$ (h)	0.83	5.5	5.2
C_{max} ($\mu\text{g/mL}$)	167	459	2212
$AUC_{0-\infty}$ (h. $\mu\text{g/mL}$)	11.6	47.6	225
AUC (%Extrap)	0.082	0.074	0.071
CL (mL/h/kg)	859 [#]	629 [#]	266
V_{ss} (mL/kg)	157 ^s	146 ^s	59.7
AUC/Dose	1.16	1.59	3.75
C_{max}/Dose	16.7	15.3	36.9

[#]16-22% of Mouse Kidney Blood Flow [3.9 L/h/kg]

^s> Mouse Blood volume [0.085 L/kg] and < Mouse total Body water [0.725 L/kg]

- Systemic clearance was low and similar at 10 and 30 mg/kg [22% MKBF] but decreased at 60 mg/kg.
- Volume of distribution at steady state was low [more than mouse blood volume and less than mouse total body water].
- A long terminal half-life of 5.2-5.6 h was observed at 30 and 60 mg/kg

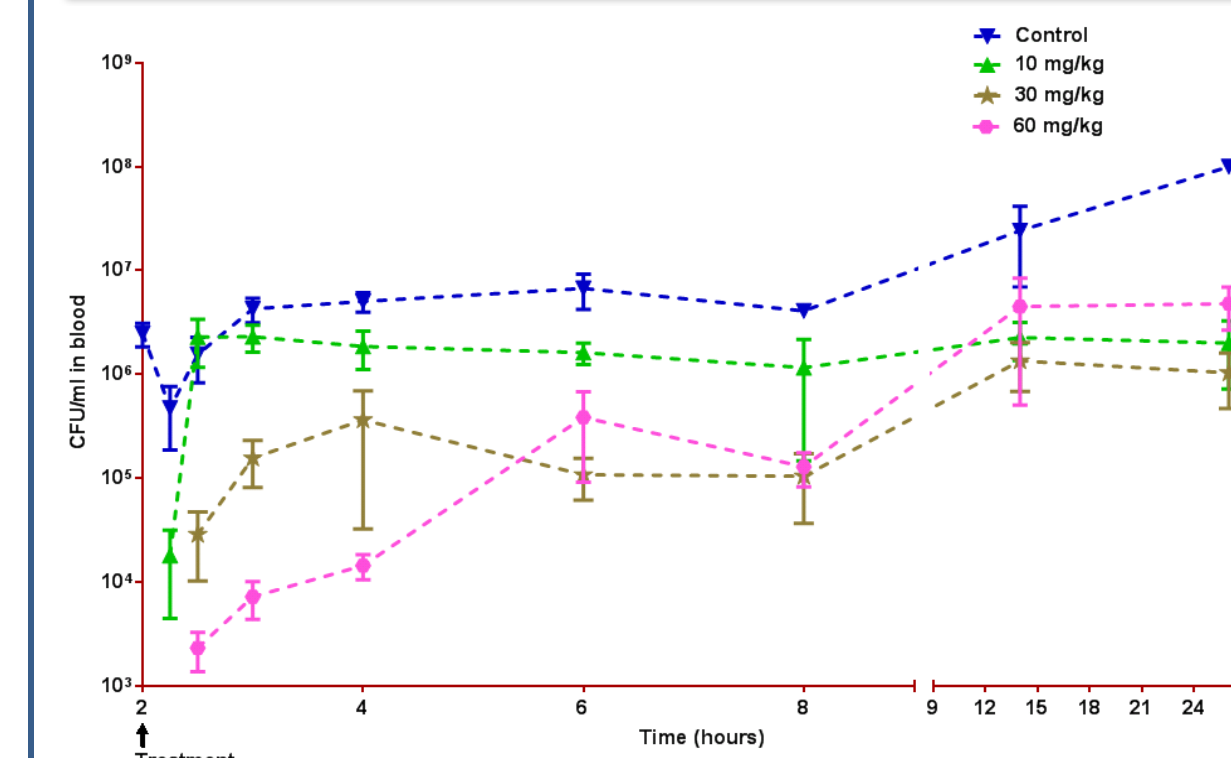
Conclusions

- PK of P128 was characterized by low systemic clearance and low V_{ss} with long $t_{1/2}$.
- P128 showed rapid antibacterial effect.
- Comparison of PK and PD time courses showed that rapid bactericidal effect was related to dose-dependent increase plasma exposure of P128, and that this anti-bactericidal effect persisted through later time points when the circulating P128 concentrations were lower.
- P128 is a potential candidate for treatment of MRSA bacteremia as a stand-alone therapy or in combination with SoC antibiotics because of its rapid bactericidal effect and prolonged maintenance of low CFU counts in blood following administration of a single dose.

Methods: Efficacy of P128 in mouse model of systemic methicillin resistant *Staphylococcus aureus* (MRSA) infection

Healthy female BALB/c mice were rendered neutropenic with cyclophosphamide (150 mg/kg, 4 days prior and 100 mg/kg, 1 day prior to infection) and challenged *via* the intraperitoneal route with MRSA COL (10^8 CFU/mouse). At 2 h post-infection, animals were treated with P128 (10, 30 or 60 mg/kg) IV. The bacterial load in blood was determined at different time points over 24 h by plating and enumerating CFU.

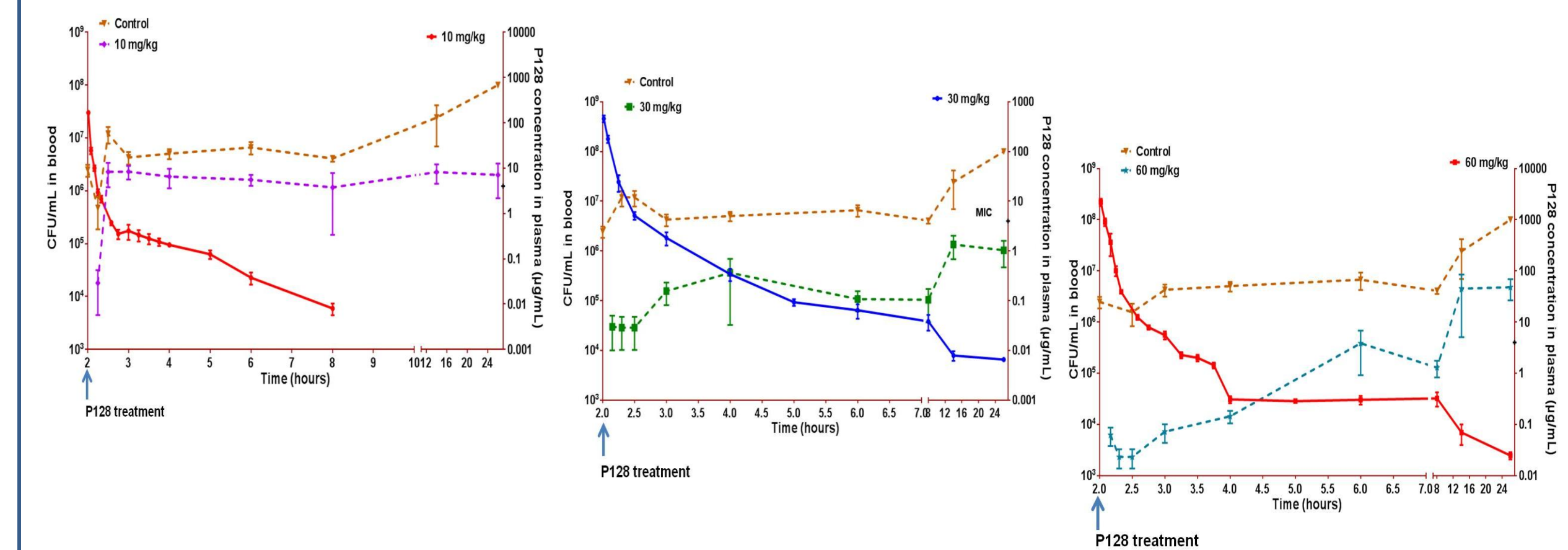
Efficacy of P128 in mouse model of systemic MRSA infection



Dose (mg/kg)	Reduction in CFUs (Log_{10}) with respect to control						
	15 mins	30 mins	1 hr	2 hr	4 hr	6 hr	24 hr
10 mg	0.54	0.72	0.27	0.43	0.61	0.55	1.70
30 mg	0.74	2.62	1.44	1.14	1.79	1.59	1.98
60 mg	1.43	2.83	2.77	2.54	1.24	1.50	1.32

- P128 treatment resulted in rapid and dose-dependant bactericidal effect.
- Maximum bactericidal effect was observed for all the test dose levels, at 30 mins post-treatment.

PK/PD of P128 in mouse model of systemic MRSA infection



- Rapid bactericidal effect of P128 was observed at the initial time points when the P128 concentration in blood was higher.
- Low CFU counts were maintained in blood even at 24 hours following treatment, indicating prolonged antibacterial effect exerted by P128.

References

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All animal experiments were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee, GangaGen Biotechnologies Pvt Ltd, Bangalore, India.

Authors have been listed in alphabetical order.