

Preclinical studies of anti-staphylococcal ectolysin P128 for potential hypersensitivity and evaluation of efficacy in *Staphylococcus aureus* bacteremia with renal abscesses in rats

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Abstract

P128 is a novel recombinant chimeric bacteriophage derived ectolysin with potent antistaphylococcal activity and is efficacious in animal models of infection with *S. aureus* including methicillin resistant *Staphylococcus aureus* (MRSA). This study in rats sought to (1) evaluate its potential for causing Type I hypersensitivity; (2) determine circulating levels of P128 following a single intravenous bolus dose of 2.5 mg/kg; and (3) evaluate its efficacy in MRSA-infected rats with bacteremia and renal abscesses. To evaluate P128 for any propensity to elicit Type I hypersensitivity (anaphylaxis), Wistar albino rats were exposed to intravenous P128 at 3 dose levels (0.6, 6.0 and 12.0 mg/kg) and re-dosed after a 15-day resting period. Animals were scored for symptoms of systemic anaphylaxis upon re-exposure to P128. No typical symptoms of systemic anaphylaxis were seen in P128-dosed animals, while the expected reactions were seen in Ovalbumin-dosed positive controls. Intravenous administration of as high as 12 mg/kg of P128 did not result in any toxicity as evidenced by necropsy and histopathology. Following a bolus dose of 2.5mg/kg in rats, P128 concentration in circulation remained above MIC (4 µg/mL) for 15 minutes. The efficacy of P128 was tested in rats challenged intravenously with MRSA USA300 (10⁹ CFU/rat). This bacterial dose resulted in 80 to 100% mortality in control animals by day 15. Bacterial load in organs evaluated at 96 hours post-infection yielded CFU (mean±SE/gram of tissue) counts of 1.9x10⁷±9.8x10⁶ in kidney, 2.4x10⁵±2.2x10⁵ in liver, 2.2x10⁴±1.3x10⁴ in spleen and 2.0x10⁴±1.9x10⁴ in lung. High bacterial counts in the kidney correlated with the presence of numerous diffuse abscesses. Histopathological examination of kidneys revealed massive infiltration of inflammatory cells, indicating *S. aureus* colonization. P128 administered as a bolus dose (0.25, 0.5 or 2.5mg/kg) to challenged animals 2 hours after infection resulted in dose-dependent survival of 10% (0.25mg/kg P128), 64% (0.5mg/kg P128) and 100% (2.5mg/kg P128). P128 treated animals had very few or no abscesses in kidney. Based on its ability to prevent renal abscess formation and to rescue animals from fatal *S. aureus* systemic infection, P128 can be considered a promising candidate for the treatment of MRSA bacteremia as stand-alone therapy or in combination with SoC antibiotics.

Methods: Characterization of hypersensitivity reactions and immune response

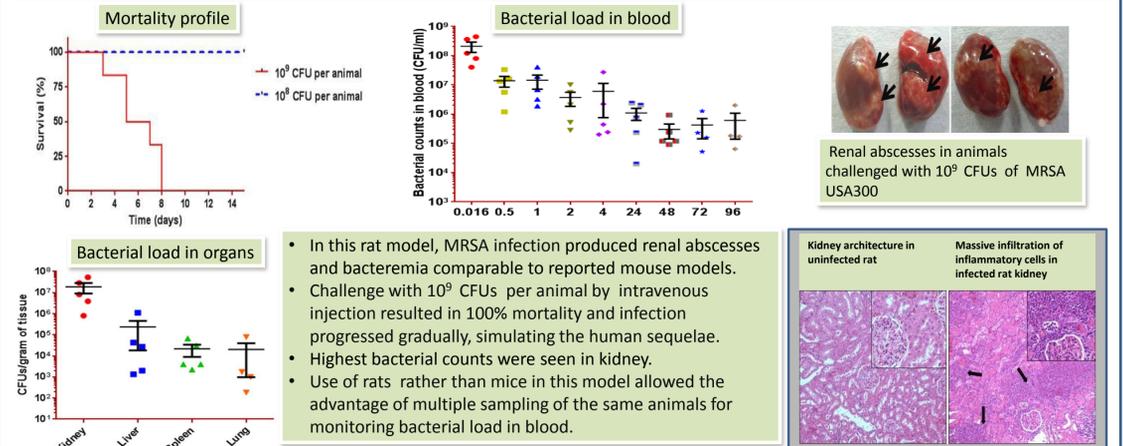
Healthy female Wistar albino rats (8-9 weeks old) were administered a single dose of P128 (0.6, 6.0 or 12.0 mg/kg) or were given the same doses (0.6 or 6.0 mg/kg) daily for 7 days. The animals were re-exposed to the same dosing schedule after a 2-week rest period. Clinical signs, body weight and other attributes were monitored. For clinical signs of systemic anaphylaxis upon re-exposure to P128, animals were observed for 30 minutes after drug administration. Ovalbumin, a known agent that causes anaphylaxis, was used as positive control. To evaluate tissue damage and vasculitis resulting from antigen-antibody interactions, necropsy was performed and organs were examined. Histopathological analysis was performed on several key organs: liver, lymph node, spleen, aorta and lung. Serum was collected 2 weeks after the second single dose or 7-day course of P128 to assess the immune response to P128 and to determine antibody titers.

Attributes evaluated	Scoring scheme for anaphylaxis
Daily observations for vital signs	0= no symptoms
Body weights	1= Nose licking or rubbing/scratching around the nose
Clinical manifestations of systemic anaphylaxis (Type I hypersensitivity) upon re-administration	2= puffiness around the eyes and mouth, redness of paws, piloerection, defecation, urination
Detection of anti-P128 antibodies	3= sneezing or coughing, retching, gasping respiration, respiratory rales and cyanosis
Gross pathology	4 = convulsion, prostration
Histopathology (Liver, lymph node, spleen, aorta, lung)	5= death

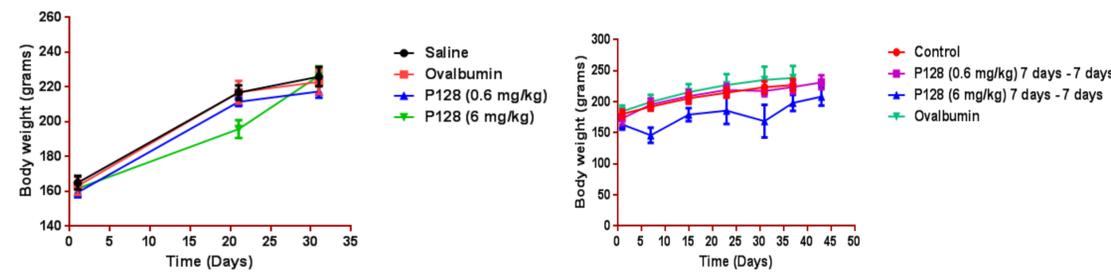
Methods: Evaluation of efficacy in rat-model of bacteremia with renal abscesses

The bacteremia model was established by challenging rats with MRSA strain USA300 intravenously. Animals were monitored for 14 days for mortality. Subsets of animals were euthanized at 96 hours and kidneys were examined for presence of abscesses. Bacterial load in organs was determined in kidney, liver, spleen and lung. Animals challenged at LD₈₀₋₁₀₀ level of bacterial inoculum were administered a single bolus dose of P128 (0.25, 0.5 or 2.5 mg/kg) two hours after infection. Rescue from mortality and change in the nature and number of renal abscesses signified efficacy in this model. To monitor bioavailability of P128, a subset of rats were administered P128 at 2.5mg/kg and plasma levels were determined by ELISA.

Model Development and Validation



Body weights in control and P128-treated animals



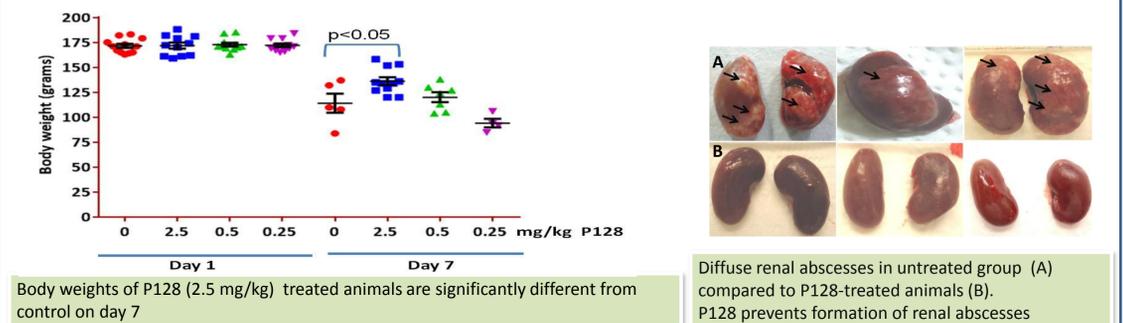
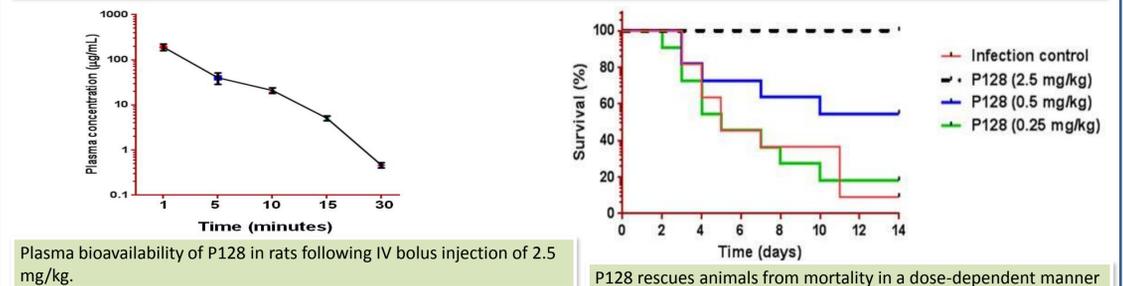
P128 treatment (single IV bolus or 7 day course) did not result in significant loss of body weight in comparison to control group

Anaphylaxis score and Anti-Drug Antibody (ADA) titer

Group (n=8)	First dose / course (Day 1 / Days 1-7)	Re-administration (Day 17 / Days 23-29)	Mean Anaphylaxis Score	Rats positive for ADA (Day 31)	Highest ADA titer
P128, 0.6 mg/kg	Single IV bolus	Single IV bolus	1.3	6/8	1:320
	7-day course	7-day course	1.0	6/8	1:4096
P128, 6 mg/kg	Single IV bolus	Animals rested for 2 weeks	Single IV bolus	5/8	1:64
	7-day course		7-day course	0/8	-
P128, 12 mg/kg	Single IV bolus	Single IV bolus	1.9	4/8	1:20
Saline	7-day course	Single IV bolus	1.0	NA	-
Ovalbumin 10 µg/rat	Single SC bolus	Single IV bolus (25 µg)	3.4	NA	-

P128 does not elicit clinical signs and symptoms of systemic anaphylaxis and animals positive for ADA show low titers of antibodies

Efficacy and bioavailability of P128 in rats



Conclusions

P128 did not elicit systemic hypersensitivity or cause tissue damage through antigen-antibody complex deposition in tissues

- No abnormal clinical signs were observed.
- No clinical signs of systemic anaphylactic reaction indicative of Type I hypersensitivity were observed following readministration of P128.
- Anti-P128 antibodies detected at relatively low titer only.
- No P128-related tissue injury (vasculitis or glomerulonephritis) typical of Type III hypersensitivity was detected by histopathology.

P128 was efficacious in rescuing animals from fatal MRSA USA300 bacteremia

- This rat model of bacteremia with renal abscesses progresses to mortality over 2 weeks, simulating human infection.
- Untreated animals succumb to systemic infection along with formation of abscesses in the kidneys.
- A single dose of 2.5mg/kg of P128 rescues animals from mortality and prevents formation of renal abscesses.

Based on its ability to rescue animals from fatal *S. aureus* systemic infection and absence of systemic anaphylaxis, P128 is a promising candidate for the treatment of MRSA bacteremia.

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

