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

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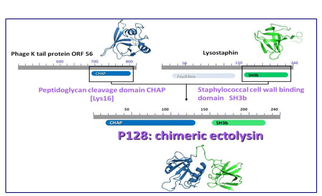
Abstract

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 (10^9 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 $1.9x10^7±9.8x10^6$ in kidney, $2.4x10^5±2.2x10^5$ in liver, $2.2x10^4±1.3x10^4$ in spleen and $2.0x10^4±1.9x10^4$ 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.

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 treat MRSA infections.
- Cell-wall degrading enzymes are promising as antibacterial agents.
- P128 ectolysin has demonstrated potent antistaphylococcal activity alone and in combination with SoC antibiotics in mouse models of bacteremia.
- Because P128 is a non-mammalian protein, we evaluated P128's potential to elicit symptoms typical of hypersensitivity or immunotoxicity in rats.
- We also report efficacy of P128 in a clinically relevant model of bacteremia associated with renal abscesses in rats.
  - P128 is a chimeric recombinant ectolysin with rapid bactericidal activity on sensitive and resistant strains of *S. aureus* and coagulase-negative Staphylococci (CoNS).
  - P128 has demonstrated potent antibiofilm activity on MRSA and CoNS strains and is able to kill antibiotic-tolerant persister cells.
  - Combination regimens are now favoured for treatment of bacterial infections due to rapid development of resistance to single-drug therapy.
  - P128 is highly synergistic with standard-of-care (SoC) antibiotics in inhibiting *S. aureus* and CoNS *in vitro*.
  - P128 is efficacious against MRSA in mouse models of bacteremia and synergizes with SoC antibiotics *in vivo.*
  - These properties make it an attractive candidate for antibacterial drug development
- The aims of this study are (i) Characterization of P128's potential to elicit systemic anaphylaxis ; (ii) Evaluation of efficacy in MRSA-bacteremia with renal abscesses, modeled in rats; and (iii) Determination of bioavailability of P128

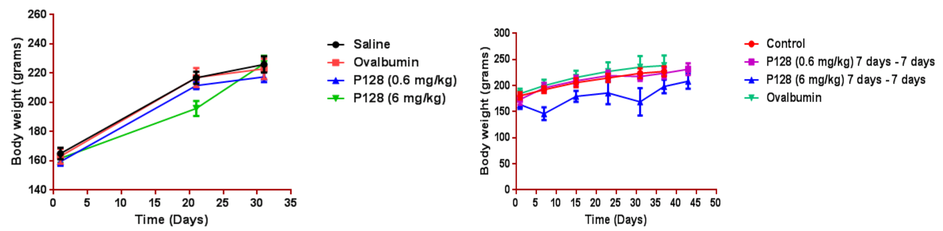


Methods: Characterization of hypersensitivity reactions and immune response

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

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

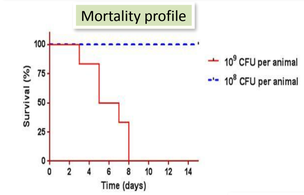
Group (n=8)	First dose / course (Day 1 / Days 1-7)		Re-administration (Day 17 / Days 23-29)	Mean Anaphylaxis Score	Rats positive for ADA (Day 31)	Highest ADA titer
P128, 0.6 mg/kg	Single IV bolus	Animals rested for 2 weeks	Single IV bolus	1.3	6/8	1:320
	7-day course		7-day course	1.0	6/8	1:4096
P128, 6 mg/kg	Single IV bolus		Single IV bolus	2.3	5/8	1:64
	7-day course		7-day course	2.0	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	-

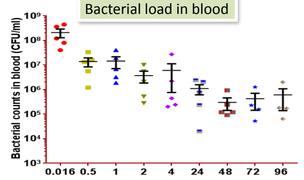
P128 does not elicit clinical signs and symptoms of systemic anaphylaxis and animals positive for ADA show low titers of antibodies

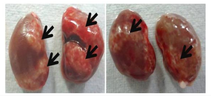
Methods: Evaluation of efficacy in rat-model of bacteremia with renal abscesses

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 LD_{80-100} 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.

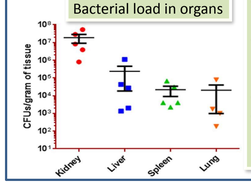
Model Development and Validation



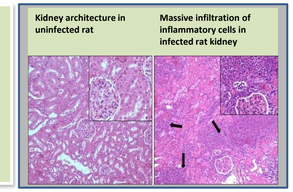




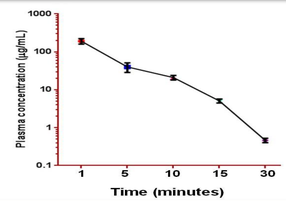
Renal abscesses in animals challenged with 10^9 CFUs of MRSA USA300



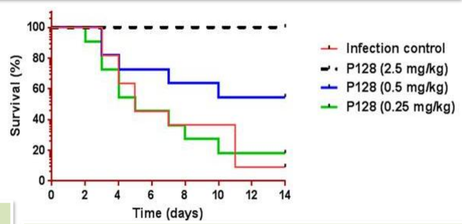
- In this rat model, MRSA infection produced renal abscesses and bacteremia comparable to reported mouse models.
- Challenge with 10^9 CFUs per animal by intravenous injection resulted in 100% mortality and infection progressed gradually, simulating the human sequelae.
- Highest bacterial counts were seen in kidney.
- Use of rats rather than mice in this model allowed the advantage of multiple sampling of the same animals for monitoring bacterial load in blood.



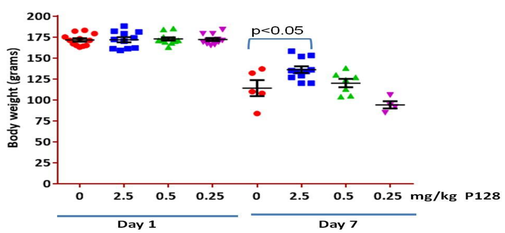
Efficacy and bioavailability of P128 in rats



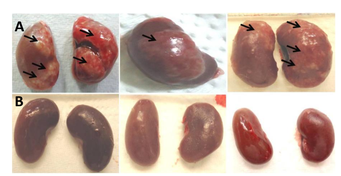
Plasma bioavailability of P128 in rats following IV bolus injection of 2.5 mg/kg.



P128 rescues animals from mortality in a dose-dependent manner



Body weights of P128 (2.5 mg/kg) treated animals are significantly different from control on day 7



Diffuse renal abscesses in untreated group (A) compared to P128-treated animals (B).
P128 prevents formation of renal abscesses

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

1. Nair S, *et al.,* (2016). Antimicrob Agents Chemother. 60(12):7280-7289.
2. Vipra AA *et al*., (2012). BMC Microbiol. 2012; 12: 41.
3. Paul, VD *et al*., (2011). BMC Microbiol. 11: 226.
4. Shishehbor F et al., (2010). Iran J Allergy Asthma Immunol. 9(1): 27-34

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