F1-2013

Efficacy of PMX30063 in Experimental Staphylococcal Skin and Skin Structure Infection Models

49th ICAAC Meeting
San Francisco, CA
September 12-15, 2009

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Abstract

Background: PMX30063 (PMX) is a synthetic small molecule mimetic of host defense proteins and is currently in clinical development. PMX possesses a novel mechanism of action, disrupting bacterial cell membranes and is highly active against both Gram+ and Gram- organisms. The current study was performed to evaluate the efficacy of PMX in the mouse subcutaneous abscess (MSCA) and rat granuloma pouch (RGP) infection models.
Methods: For the MSCA model, CD-1 mice were rendered partially neutropenic by IP administration of cyclophosphamide (150 mg/kg day -4). An *S. aureus* (Sa) culture was mixed w/ Cytodex beads to 105 CFU and 0.2 mL injected SC into the flanks of each mouse. Treatment was initiated +2hrs post-infection and continued either once (qd) or twice (bid) a day for 3 days. Bacterial abscess counts were determined at 18 hrs after the last administered dose. For the RGP, sterile pouches were formed on the backs of Sprague-Dawley rats by the injection of sterile air + sesame oil w/ 1% croton oil. Pouches were allowed to form for 5 days after air removal and infected w/ 106 cfu of Sa. Treatment was initiated 2 hrs later and both pouch and blood samples collected at selected time points over 24 hrs for CFU determinations. **Results:** In the MSCA model, PMX demonstrated log CFU reductions of 1.3 – 2.3 for IV dosing regimens (both qd and bid) between 10 - 20 mg/kg as compared to untreated controls. Vancomycin exhibited a 3.3 log reduction at 25 mg/kg bid IP. In the RGP model, PMX effected log CFU reductions in pouch fluid of 1.1 – 2.5 following single IV doses of 4 – 20 mg/kg and up to a 5 log reduction at 10 mg/kg bid (within 6 hrs of administration). Vancomycin at 50 mg/kg exhibited a 5.2 log reduction in the pouch fluid.
Conclusions: PMX30063 demonstrated *in vivo* efficacy in two different rodent models of *S. aureus* skin structure infections and warrants further investigation for its utility in these and other clinically relevant infections.

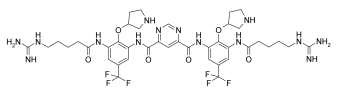
Introduction

PolyMedix uses a proprietary computational *de novo* drug design platform to design biomimetics: small molecules that mimic the activity of certain proteins. The first products developed using this computational platform were novel small molecule antibiotic drugs which mimic the activity of host defense proteins. These are the first and only small molecule defensin mimetics being developed intended for use in systemic infections. By mimicking the activity of the host defense proteins, PolyMedix's compounds have a highly unique mechanism of action: directly lysing bacterial cell membranes, resulting in the destruction of the genetic machinery often responsible for bacterial resistance. Thus, it is unlikely that bacterial resistance can easily develop to these compounds. PMX-30063 has broad and potent antimicrobial activity against Gram-positive and Gram-negative bacteria, including antibiotic-resistant strains. Here we describe studies that evaluate PMX-30063 efficacy in mouse subcutaneous abscess and rat granuloma pouch models infected with *Staphylococcus aureus* ATCC13709.

Methods and Materials

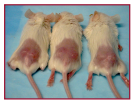
Bacteria: *Staphylococcus aureus* (ATCC13709)- methicillin-susceptiple.
Subcutaneous Abscess: Female, 5 - 6 week old CD-1 mice were used in abscess studies and were rendered neutropenic by a single intraperitoneal (IP) administration of cyclophosphamide (Cytoxan) given at 150 mg/kg day -4 pre-infection. *S. aureus* was grown on appropriate agar media and suspended in tryptic soy broth (TSB) to an absorbance of 1.37 (600_{nm}). This suspension was diluted to the target CFU range in TSB (approx. 5 x 10^6 CFU/mL), and then 4-fold diluted (v/v) into 20 mg/mL sterile dextran beads to generate infecting inocula. Plate counts of inocula determined there to be a mean input of 1.25 x 10^6 CFU/abscess for each study. Prior to infection, mice were anesthetized, shaved, and sterilized. While under anesthesia, mice were shaved, decontaminated (skin), and infected by subcutaneously (SC) injecting 0.2 mL of the inocula into 2 separate sites on the flanks of each animal. All treatments began 2 hours after infection, and compounds were either administered as a 0.1 mL intravenous (IV) injection (PMX-30063) or a 0.2 mL IP injection (Vancomycin). All treatments were continued for either twice-a-day (PMX-30063) or once-a-day (Vancomycin) for a period of three days as indicated for each group. Abscesses were aseptically removed 18 - 24 hours following the final treatment, homogenized, and plated onto charcoal supplemented agar media for final CFU counts.
Rat Granuloma Pouch: Anesthetized male, Sprague-Dawley rats were shaved and decontaminated on the dorsal side from shoulder to mid-back. Pouches were formed by SC injecting 30 mL of sterile air through a 25G needle, and then by injecting 0.5 mL of a 1% croton oil:sesame oil (v:v) mixture through the same needle. The air was removed within 72 hours, and pouches were infected 5 days following air removal with 0.1 mL of a 10^7 CFU/mL suspension of *S. aureus* ATCC13709. All compounds were IV administered beginning 2 hours post-infection, and pouch fluid was sampled for each rat 0.5 – 24 hours post-treatment to determine CFU counts and compound concentration. Additionally, blood samples were collected from the same rats 1 minute to 8 hours post-treatment to determine comparable concentrations in plasma.

Chemical Structure of PMX30063



N^4,N^6-bis(2-((R)-pyrrolidin-3-yloxy)-3-((4-carbamoylbutyl)guanidine)-5-(trifluoromethyl) phenyl)pyrimidine-4,6-dicarboxamide

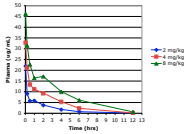
Mouse Subcutaneous Abscess and Rat Granuloma Pouch Models



Subcutaneous abscesses on the dorsal surface of CD-1 mice following subcutaneous injection of ATCC13709 with dextran (Cytodex) beads.

Granuloma pouch on Sprague-Dawley rat after injection of croton/sesame oil followed by pouch injection of ATCC13709 in gastric mucin.

Pharmacokinetics of PMX30063 in CD-1 Mice Following Intravenous Administration[a]



PK Parameter	Units	2 mg/kg	4 mg/kg	8 mg/kg
AUC_{0-t}	ug-hr/mL	23.96	57.03	106.30
C_{max}	ug/mL	21.90	32.90	46.30
$T_{1/2}$	hr	1.90	1.50	2.00
V_{ss}	L/kg	0.20	0.18	0.24
Cl	L/hr/kg	0.08	0.07	0.07
MRT_{last}	hr	2.40	2.60	3.20

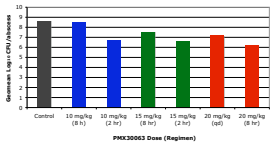
- PMX30063 exhibits a dose dependant increase in plasma exposure (AUC) following intravenous administration in mice.
- The elimination half-life, volume of distribution and clearance were consistent across the dose range from 2 - 8 mg/kg.

[a] from Pharmacokinetic-Pharmacodynamic Relationships for PMX30063 in the Mouse Thigh Burden Model. 2009. 49th ICAAC, Poster F1-2014

Efficacy of PMX30063 in the Mouse Subcutaneous Abscess Model following Intravenous Administration

Compound	Dose (mg/kg)	Regimen	Geometric Mean Log_{10} CFU	SD	Mean Log reduction[a]
PMX30063	4	2x/day - 8 hr interval	8.94	0.19	-0.33
	10	2x/day - 8 hr interval	8.48	0.76	0.13
	10	2x/day - 2 hr interval	6.73	0.7	1.88
	15	2x/day - 8 hr interval	7.51	0.88	1.1
	15	2x/day - 2 hr interval	6.63	1.16	1.98
	20	1x/day	7.2	0.57	1.41
	20	2x/day - 8 hr interval	6.18	0.56	2.43
Vancomycin	25	2x/day - 8 hr interval	5.49	0.75	3.12
Infection control	NA	NA	8.61	0.29	NA

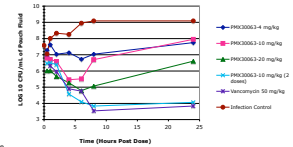
[a] relative to infection (untreated) control at end of study



- The infection control group exhibited a bacterial density of 8.61 log_{10} CFU.
- PMX30063 (8 hr dosing interval) demonstrated a dose response with mean log reductions of -0.33 to 2.43 over a dose range from 4 - 20 mg/kg.
- PMX30063 administered at 10 and 15 mg/kg at a 2 hr dosing interval achieved greater efficacy (CFU reduction) than the same doses over an 8 hr interval.

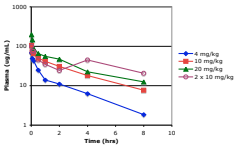
Efficacy of PMX30063 in the Rat Granuloma Pouch Model Following Intravenous Administration

Time (hrs post-dose)	PMX30063 4 mg/kg	PMX30063 10 mg/kg	PMX30063 20 mg/kg	PMX30063 2x10 mg/kg	Vancomycin 50 mg/kg	Infection Control
	Mean LOG_{10} CFU/mL of Pouch Fluid					
0.5	7.28	6.8	6.01	6.46	7.01	7.05
1	7.62	6.71	6.02	6.51	6.31	8.01
2	7.03	6.59	5.66	6.39	5.95	8.33
4	7.13	5.46	5.27	4.57	4.9	8.26
6	6.73	5.51	4.78	4.09	4.75	8.95
8	7.03	6.7	5.06	3.83	3.53	9.09
24	7.75	7.95	6.6	4.06	3.83	9.09

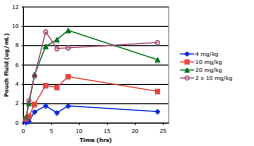


- Pouch titers of the *S. aureus* ATCC13709 increased from 7.05 log_{10} cfu/mL to 9.09 log_{10} cfu/mL over 24 hours.
- PMX30063 at 4 - 20 mg/kg reduced Log_{10} CFU/mL counts by as much as 2.2 - 4.9 at 6 hrs post-dose when compared to the corresponding infection control.
- Regrowth in the pouch was observed for PMX30063 dosing regimens of 4, 10 and 20 mg/kg, but not 2 x 10 mg/kg, by 24 hours.
- As compared to 24-hr controls, vancomycin (50 mg/kg) and PMX30063 (2 x 10 mg/kg) reduced the bacterial pouch burden by 5.3 and 5.0 Log_{10} CFU/mL compared, respectively. .

Pharmacokinetics of PMX30063 in Rat Plasma Following IV Administration



Pharmacokinetics of PMX30063 in Granuloma Pouch Fluid Following IV Administration



Parameter	4 mg/kg		10 mg/kg		20 mg/kg		2 x 10 mg/kg	
	Plasma	Pouch	Plasma	Pouch	Plasma	Pouch	Plasma	Pouch
AUC_{0-t} (ug/hr/mL)	75.8	33	191.8	88.2	271.5	181.1	283.7	180.1
C_{max} (ug/mL)	68.1	1.8	104.2	4.8	198	9.6	100.5	9.4
$T_{1/2}$ (hr)	2.3	27.9	2.8	29.3	2.9	29	4.8	ND

- PMX30063 appears to penetrate into the granuloma pouch fluid in a dose dependent manner.
- The percent penetration (AUC_{0-t} pouch / AUC_{0-t} plasma) was calculated to be 44 - 66% over the dose range of 4 - 20 mg/kg.

Summary and Conclusions

- PMX30063's unique mechanism of action, directly lysing bacterial cell membranes, results in the destruction of the genetic machinery often responsible for bacterial resistance.
- In the subcutaneous abscess model, PMX30063 (8 hr dosing interval) demonstrated a dose response with mean log reductions of -0.33 to 2.43 over the dose range of 4 to 20 mg/kg.
- PMX30063 administered at 10 or 15 mg/kg over a 2 hr dosing interval achieved greater efficacy (CFU reduction) than the same doses over an 8 hr interval.
- In the rat granuloma pouch model, PMX30063 at 4 - 20 mg/kg reduced Log_{10} CFU counts by as much as 2.2 - 4.9 at 6 hrs post-dose when compared to the corresponding infection control.
- Regrowth(increase in Log_{10} CFU from 4-6 hrs) in the pouch was observed for PMX30063 when given as a single dose at 4, 10 and 20 mg/kg, but not when 10 mg/kg was given in two separate doses
- Vancomycin (50 mg/kg) and PMX30063 (2 x 10 mg/kg) reduced the bacterial pouch burden 5.3 and 5.0 Log_{10} CFU/mL compared to control at 24 hours, respectively.
- Pharmacokinetic analysis of the pouch fluid showed that PMX30063 achieves sustained levels in excess of 6 ug/mL from 4 through 24 hrs at doses of 20 mg/kg per day.
- Based on total measured exposure (AUC_{0-t}), penetration of PMX30063 into the granuloma pouch fluid was 44 - 66%.
- PMX30063 demonstrated *in vivo* efficacy in two different rodent models of *S. aureus* skin structure infections and warrants further investigation for its utility in these and other clinically relevant infections.

References

1) Bunce, C., L. Wheeler, G. Reed, J. Musser, and N. Barg. 1992. Murine model of cutaneous infection with gram-positive cocci. Infect. Immun. 60:2636-2640.
2) Ford, C. W., D. Hamel, D. Stapert, and R. J. Yancey. 1989. Establishment of an experimental model of a *Staphylococcus aureus* abscess in mice by the use of dextran and gelatin microcarriers. J. Med. Microbiol. 28:259-266.
3) Ford, C. W., J. C. Hamel, D. Stapert, and J. Yancey, R.J. 1987. Antibiotic Therapy of an Experimental *Staphylococcus epidermidis* Subcutaneous Abscess in Mice. Zbl. Bakt. Suppl. 16:247-257.
4) National Research Council. 1996. Guide for the Care and Use of Laboratory Animals. National Research Council.
5) Taubes, G. 2008. The bacteria fight back. Science. 312(18 July 2008): 356-361.
6) Scott, R.,et.al. *In vivo* efficacy and safety of a novel host defense protein mimetic, PMX30063; 48th ICAAC, Washington, DC.
7) Scott, R., et.al. *In vitro* antimicrobial activities of a novel host defense protein mimetic, PMX30063. 48th ICAAC, Washington, DC.

Acknowledgments

This study was funded and supported by Polymedix Inc., Radnor, PA. The authors would like to acknowledge Yongjiang Xu (Polymedix) for the synthesis of PMX30063 and Yuri Khmelnitsky (AMRI) for bioanalysis of the plasma and pouch samples.