

Anti-virulence Effect of TR-700 on MSSA and MRSA Strains Causing Complicated Skin Soft Tissue Infections (cSSTIs)

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Abstract

Background. The phenol-soluble modulins (PSM) peptides (A1-4) play key role in the pathogenesis of CA-MRSA mouse abscess and bacteremia models. PSM3 has the most pronounced pro-inflammatory and cytolytic activity against neutrophils *in vitro*. We determined PSM production in clinical isolates of *Staphylococcus aureus* and the inhibitory potential of TR-700 (TR) on PSM production. **Methods.** MRSA (n=35) and MSSA (n=15) causing cSSTIs were obtained from TR-700 Phase II clinical trials. Study strains and controls (USA300, USA400) were grown in tryptic soy broth for 16 h at 37°C; supernatants were harvested to measure baseline PSM production using MRM mass spectrometry (LC-MS-MS). MICs for TR and clindamycin (CL) were determined for selected strains (n=10) according to CLSI guidelines. Supernatants harvested from 1/2, 1/4, and 1/8 MIC cultures (TR and CL) were measured for PSM A3 with levels normalized to CFU/mL determined by plate counting or a verified standard curve of OD vs CFU. **Results.** PSM (A1-4) levels at baseline were similar for USA300 (22, 12, 13, and 28 mcg/ml) and for 50 clinical isolates (mean values: 26, 14, 13, and 32 mcg/ml) although up to 3-fold higher levels were observed in some strains. For USA 300, TR inhibited PSM A3 production by 100%, 63% and 27% at 1/2, 1/4, and 1/8 MIC respectively. TR exerted variable effects on PSM A3 production in the clinical strains, with complete inhibition at 1/2 MIC for 70% of isolates while the level of PSM production ranged from 46% to 206% of baseline at 1/4 MIC and 68% to 117% at 1/8 MIC. Similar trends were observed for CL. **Conclusion.** Clinical strains causing SSTIs secrete PSM peptides at least equal and up to 3-fold higher than those of USA300 reference CA-MRSA. TR and CL exert significant inhibition of PSM production at 1/2 MIC but more variable and paradoxical effects were observed at lower concentrations. The precise effect of TR and other protein synthesis inhibitors on genes that regulate PSM production merits future investigation.

Introduction (1,2,3)

- Alpha (A) type Phenol-Soluble Modulins (PSMs A1-4) are cytolytic peptides that primarily target neutrophils and elicit inflammatory responses, with PSM A3 being the most potent when tested against human neutrophils. Animal models of infection have demonstrated their role as a virulence factor in SSTI and bacteremia.¹
- Nearly all *Staphylococcus aureus* strains produce PSMs. It has been previously shown that CA-MRSA strains produce higher amounts of PSMs compared to HA-MRSA strains.^{1,2}
- Clindamycin and other protein synthesis inhibiting antibiotics have been shown to inhibit various exotoxins produced by *Staphylococcus aureus* such as alpha-hemolysin, Panton-Valentine leukocidin (PVL), and TSST-1.^{3,4,5}

Study Objectives

- To investigate the level of PSM A1-4 production by *Staphylococcus aureus* isolates causing cSSTIs.
- To determine the inhibitory effect of TR-700 on the production PSM A-3 and compare the effects to that of clindamycin.

Methods

BACTERIAL STRAINS

- Isolates:** 50 unique PVL (+) MRSA (n=35) and MSSA (n=15) isolates causing infections were collected from adults patients with cSSTIs who were enrolled in Phase II clinical trial of TR-700.
- Control strains** from NARSA: NRS 384 (USA 300), NRS 123 (MW2), NRS 483 (USA 1000), and NRS 484 (USA 1100).

MIC DETERMINATION AND COLLECTION OF SUPERNATANT

- A modified version of CLSI macrobroth dilution was used:
 - TSB was used in place of Mueller-Hinton broth.
 - Bacteria was added to each test tube containing 2-fold drug dilutions to make a final inoculum of $\sim 1 \times 10^8$ CFU/ml in 4ml of TSB, then cultures were incubated overnight at 37°C with shaking at 200 rpm.
 - MIC was read as the first tube containing no visible growth. Supernatants were then collected from cultures tubes containing 1/2 MIC, 1/4 MIC, and 1/8 MIC plus control tubes with no antibiotic.
 - Supernatants were stored in -80°C with addition of 2 mg/ml ascorbic acid until assayed by UPLC/MS/MS.
 - Plate counting was performed for each tube at the time of harvest of supernatants or OD_{600nm} was measured and compared to standard curve to estimate CFUs.

UPLC/MS/MS

- Concentration of PSM A1, 2, 3, and 4 in TSB culture media were determined using an UPLC-tandem mass spectrometric assay.
- Solvents and assay reagents were purchased from Fisher Scientific (Madison, WI). PSM A1, 2, 3, 4, A1 D5 (internal standard), and PSM A3 D5 (internal standard) were synthesized by The City of Hope Medical Center.
- Instrumentation consisted of a Waters Acquity UPLC system in line with a Waters Quattro Premier XE Triple Quadrupole Mass Spectrometer (Waters, Milford, MA)
- The detector settings were as follows: capillary voltage, 4.8 kV; cone voltage for PSM alpha 1, 2, 3, 4, and PSM A3 D5 were 75 V, 78V, 80V, 75V, and 85V, respectively; collision cell voltage for PSM alpha 1, 2, 3, 4, and PSM A3 D5 were 90 eV, 88 eV, 105 eV, 88 eV, and 95 eV, respectively; source temperature, 125°C; desolvation temperature, 450°C; cone gas flow, 80 liter/h; and desolvation gas flow, 700 liter/h.
- The mass transitions monitored for PSM A1, 2, 3, 4, A1 D5 and PSM A3 D5 were 1144.5→86.3, 1153.56→86.3, 1318.22→120.2, 1100.46→86.3, 1147.85→86.3 and 1320.15→124.94, respectively.
- Chromatography consisted of gradient separation across a Jupiter 4 μ Proteo 90A 150 x2.0 mm analytical column (Phenomenex, Torrance, CA) using mobile phase A: 0.1% TFA in water, and B: 0.1% TFA in acetonitrile.
- The column temperature was 30 °C. The following gradient program was used: 60% B (0-2 min), 85% B (2.1 min), 94% B (6.5 min), 60% B (6.6 min), 60% (9 min). The flow rate was 0.3 ml/min. The total run time was 9 minutes, and retention times for PSM A1, 2, 3, 4, and PSM A3 D5 were 4.07, 3.97, 3.48, 5.07, and 3.48 minutes, respectively. The standard curve of quantitation was from 0.1 μ g/ml to 10 μ g/ml.

Results

Figure 1. Baseline PSM production by clinical isolates (n=50)

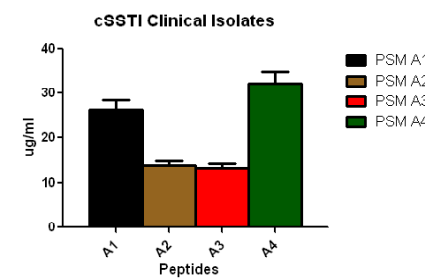


Figure 2. Baseline PSM production by control strains

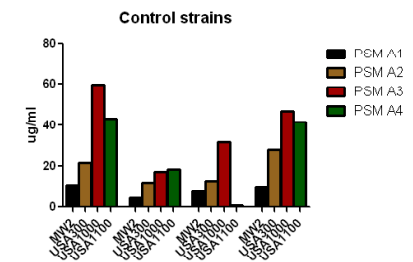


Figure 3. Growth kinetics of MRSA and MSSA clinical isolates with TR-700 at sub-MICs

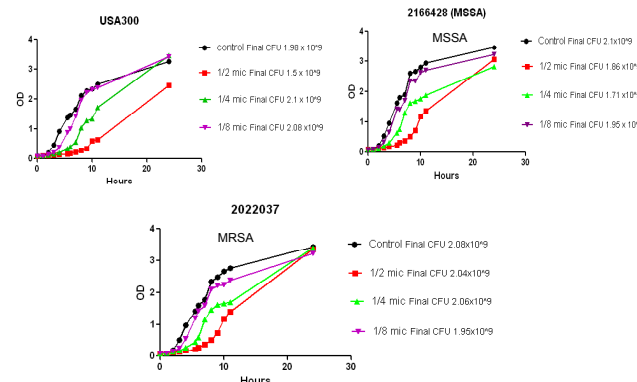


Figure 4. Effect of clindamycin on PSM A3 at sub-MICs

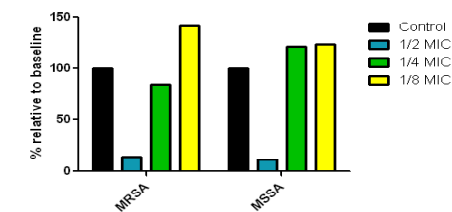


Figure 5. Effect of TR-700 on PSM A3 at sub-MICs

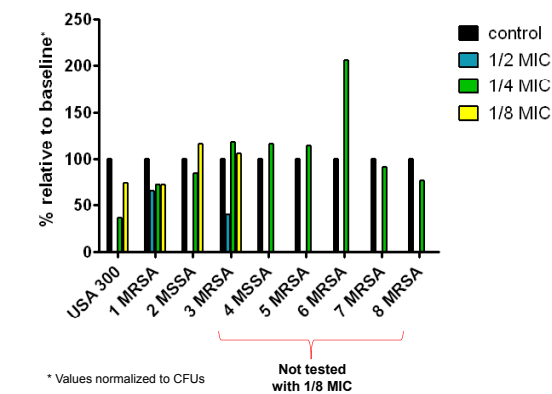


Table 1. Effect of TR-700 and clindamycin on PSM A3 production at sub-MICs (% relative to baseline)

	TR at 1/2 MIC	CL at 1/2 MIC	TR at 1/4 MIC	CL at 1/4 MIC	TR at 1/8 MIC	CL at 1/8 MIC
Mean % (\pm std)	15% (27)	12.5% (2.2)	106% (55.5)	102.5% (26)	87% (22)	132% (13)

Summary

- The MRSA/MSSA clinical isolates mean PSM production most closely fit that of USA300. Although some strains produced up to 3 times the amount of PSMs compared to USA300.
- TR-700 had some effect on growth even at sub-inhibitory concentrations, with the most pronounced effect at 1/2 MIC.
- Clindamycin at 1/2 MIC inhibited PSM A3 production a mean decrease of 87.5% from baseline. At 1/4 MIC, PSM A3 production slightly increased in the MSSA strain and slightly decreased in the MRSA strain. At 1/8 MIC both strains had an increase in PSM A3 production.
- TR-700 at 1/2 MIC concentrations inhibited PSM A3 production in all 9 isolates tested; PSM A3 levels were below the assay limit of the detection (100 ng/ml) in 78% of isolates. At 1/4 MIC a slight decrease was seen in 5/9 isolates, and a slight increase in the remaining four isolates. At 1/8 MIC, a slight decrease in 2/4 isolates tested and slight increase in the remaining two isolates.

Conclusions

- PVL (+) clinical isolates of *Staphylococcus aureus* (MSSA and MRSA) vary in their level of PSMs production.
- TR-700 and clindamycin greatly inhibited PSM A3 production at 1/2 MIC of each drug, with 100% inhibition in most isolates for TR-700.
- TR-700 and clindamycin at 1/4 and 1/8 MICs had varying effects on PSM A3 production, causing a slight decrease in some isolates while a slight increase in others.
- At appropriate concentrations ($\geq 1/2$ MIC) TR-700 and clindamycin may provide beneficial effects by attenuating virulence attributed to PSMs production.
- Antibiotic dosing with protein synthesis inhibitors should be guided by MIC values to avoid potential induction in PSM A3 production at low antibiotic concentrations.

References

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