Poster #A-024

Antimicrobial Activity of E-101 Solution, A New Myeloperoxidase-Based Antimicrobial Agent Versus Mupirocin against Staphylococcus aureus Phenotypes

G.A. Denys[1], P. Grover[2], C. Pillar[2], J.T. Stephens, Jr.[3]
[1]Clarian Pathology Laboratory, Indianapolis, IN, [2]Eurofins Medinet, Anti-Infective Services, Chantilly, VA, [3]Exoxemis, Inc., Little Rock, AR

Contract Information:
medinfo@exoxemis.com

ABSTRACT

Background: E-101 Solution (E-101) is a new broad-spectrum topical agent containing myeloperoxidase (MPO) developed for the potential prevention and control of infections due to resistant and problem pathogens. MPO exerts potent oxidative microbicidal action against both Gram positive and Gram-negative organisms. Mupirocin (MUP) is a common topical antibiotic used to eradicate *Staphylococcus aureus* (SA) from the anterior nares and skin and control methicillin-resistant *S. aureus* (MRSA) outbreaks. Because of the concern of developing MUP-resistance, we compared the in vitro activity of E-101 and MUP against different phenotypes of SA.
Methods: MICs were determined using a modification of the CLSI broth microdilution guidelines against 109 non-duplicate clinically-significant strains of SA. Phenotypes of SA tested included; methicillin susceptible and resistant (MSSA/MRSA), vancomycin intermediate and resistant (VISA/VRSA), and Panton-Valentine Leukocidin (PVL) positive.
Results: As shown in the table, E-101 was more potent than MUP against all phenotypes. Only 4 strains showed elevated MICs to MUP; 2-MRSA (4 and >32 µg/ml), 1-MSSA (>32 µg/ml), 1-VISA/VRSA (4 µg/ml). However, comparative MICs for these strains to E-101 remained low (0.015-0.03 µg MPO/ml).

			MIC (µg MPO/ml)		
Agent	Phenotype	Total N	Range	MIC_{50}	MIC_{90}
E-101	All	109	0.008-0.06	0.015	0.03
	MSSA	55	0.008-0.03	0.015	0.03
	MRSA	45	0.008-0.015	0.015	0.03
	PVL-positive	4	0.03-0.06	-	-
	VISA/VRSA	5	0.015-0.03	-	-
			MIC µg/ml		
Agent	Phenotype	Total N	Range	MIC_{50}	MIC_{90}
Mupirocin	All	109	≤0.03->32	0.06	0.12
	MSSA	55	≤0.03->32	0.06	0.12
	MRSA	45	≤0.03->32	0.06	0.12
	PVL-positive	4	≤0.03- 0.06	-	-
	VISA/VRSA	5	≤0.03- 4	-	-

Conclusion: E-101 was highly active in vitro against all SA tested, regardless of the phenotype and resistance to MUP. Further investigation of E-101 for clinical application is warranted.

INTRODUCTION

Staphylococcus aureus is a leading cause of infections in hospitals and other health care settings in the United States and has developed resistance to a broad-spectrum of antibiotics commonly used to treat it. Wound site infections including surgical site infections caused by *S. aureus* remain a major source of morbidity and mortality (2).
Patients usually become rapidly colonized with *S. aureus* from the exogeneous environment or their own skin flora as the source of infection.
E-101 is a novel drug product developed for topical/local application and contains 2 therapeutic enzymes; porcine myeloperoxidase (MPO) and glucose oxidase (GO) from *Aspergillus Niger*. It is prepared from 2 different aqueous solutions, an enzyme solution, and a substrate solution, which are packaged in separate vials. The enzyme solution contains the enzymes MPO and GO, with selected amino acids formulated in a phosphate buffer. The substrate solution contains glucose (dextrose, USP) in the same phosphate buffer. The enzyme solution and substrate solution are combined in appropriate proportions prior to use. The mechanism of action of E-101 Solution involves the selective binding of MPO to the surface of target microorganisms (1), the in situ generation of hydrogen peroxide by GO and glucose, the MPO catalyzed oxidation of chloride ion by hydrogen peroxide to generate hypochlorous acid, and the nonenzymatic oxidation of additional hydrogen peroxide by hypochlorous acid to yield singlet oxygen (3,7). The half life of singlet oxygen, a potent oxygenating agent (4,6), is approximately 1 microsecond and restricts its sphere of reactivity to about 0.1-0.2 µm, approximately the width of a bacterial cell wall (5), thus avoiding bystander damage. Moreover, the hydrogen peroxide and hypochlorous acid produced is sufficient for singlet oxygen generation, but not substantial enough to cause host tissue toxicity.
The purpose of this study was to determine the in vitro activity of two topical agents E-101 compared to mupirocin against different phenotypes of *S. aureus* from diverse geographical locations. Because there are no interpretive breakpoints for E-101, mupirocin was tested for comparative purposes only.

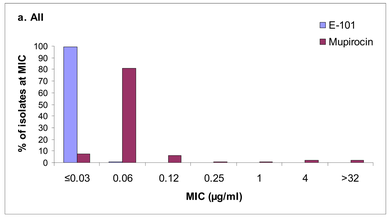

Figure 1. MIC distribution (%) for E-101 and mupirocin against all *S. aureus* isolates (n=109)

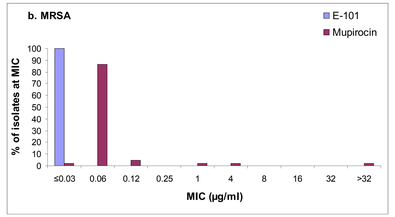

Figure 2. MIC distribution (%) for E-101 and mupirocin against MRSA isolates (n=45)

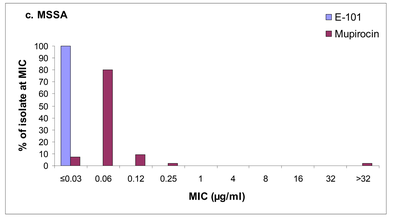

Figure 3. MIC distribution (%) for E-101 and mupirocin against MSSA isolates (n=55)

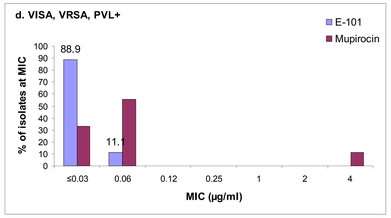

Figure 4. MIC distribution (%) for E-101 and mupirocin against VISA/VRSA and PVL+ isolates (n=9)

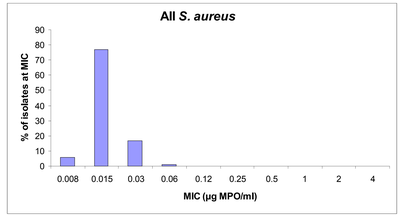

Figure 5. MIC distribution (%) for E-101 against all *S. aureus* phenotypes (n=109)

Table 1. In vitro activity of E-101 against *S. aureus* phenotypes

		MIC (µg MPO/ml)		
Phenotype	Total N	Range	MIC_{50}	MIC_{90}
All	109	0.008-0.06	0.015	0.03
MSSA	55	0.008-0.03	0.015	0.03
MRSA	45	0.008-0.015	0.015	0.03
PVL-positive	4	0.03-0.06	-	-
VISA/VRSA	5	0.015-0.03	-	-

Table 2. In vitro activity of mupirocin against *S. aureus* phenotypes

		MIC (µg MPO/ml)		
Phenotype	Total N	Range	MIC_{50}	MIC_{90}
All	109	≤0.03->32	0.06	0.12
MSSA	55	≤0.03->32	0.06	0.12
MRSA	45	≤0.03->32	0.06	0.12
PVL-positive	4	≤0.03-0.06	-	-
VISA/VRSA	5	≤0.03-4	-	-

METHODS

Bacterial Strains:
A total of 109 clinical isolates of *Staphylococcus aureus* were obtained from the culture collection of Eurofins Medinet Anti-Infective Services (Chantilly, VA), and represent diverse geographical regions. These isolates included methicillin-resistant *S. aureus* (MRSA, n=45), methicillin-susceptible *S. aureus* (MSSA, n=55), vancomycin-intermediate/-resistant *S. aureus* (VISA/VRSA, n=5), and Panton-Valentine Leukocidin (PVL) positive S. aureus (PVL+, n=4). All PVL and VISA/VRSA isolates were provided by NARSA (Network on Antimicrobial Resistance in *Staphylococcus aureus*).
Antimicrobial Agents:
E-101 Solution is comprised of 2 aqueous solutions designated as enzyme solution and substrate solution. These 2 solutions were prepared by AAI International (Charleston, SC). Mupirocin lithium was obtained from GlaxoSmithKline, Inc. (Philadelphia, PA). Antimicrobial agents were tested over doubling dilutions ranging between 0.004-8 µg MPO/ml for E-101 and ≤ 0.03-32 µg/ml for mupirocin.
Susceptibility Testing:
MICs were determined by broth microdilution method according to CLSI-defined methodology (8). Test modifications were made to accommodate the rapid in vitro activity of E-101. Both mupirocin and enzyme solution were diluted in double strength cation

METHODS (CONT)

adjusted Mueller Hinton Broth (CAMHB) and dispensed in microdilution trays. Isolates were prepared by suspending several colonies (4-6) from an overnight culture on Trypticase Soy Agar (TSA) with 5% sheep blood into sterile saline and the density adjusted to a 0.5 McFarland standard (~10^8 CFU/ml). Standardized bacterial suspensions were further diluted in double strength substrate solution so that approximately 5×10^5 CFU/ml was mixed with serial drug or enzyme dilutions. The addition of substrate solution to the enzyme solution activates the enzyme system, which in turn exerts its rapid mode of action. The microdilution trays were incubated in ambient air at 35°C for 18-24 hours. The MIC was determined by observing the lowest concentration of antimicrobial agent that completely inhibited the growth of the organism.
Quality Control:
CLSI-recommended QC strain *Staphylococcus aureus* ATCC 29213 and cefazolin (GlaxoSmithKline) was tested to monitor the accuracy of MICs by the modified CLSI method.
Data analysis:
The MIC_{50} and MIC_{90} values, the concentrations at which 50% and 90% of the isolates were inhibited, and MIC distribution histograms were calculated for comparative purposes.

RESULTS

- Overall, E-101 was highly active against all phenotypes of *S. aureus* with a MIC_{90} of 0.03 µg MPO/ml (Table 1). MIC_{90}s for E-101 were 4 two-fold dilutions more potent than mupirocin, 0.12 µg/ml (Table 2).
- The MIC distribution (%) for all phenotypes shows that E-101 was highly active and was ≥ 2 two-fold dilutions more potent than mupirocin (Figures 1).
- Elevated MICs to mupirocin (Figures 2-4) were observed in 1 MSSA strain (>32 µg/ml), 2 MRSA strains (4 and >32 µg/ml), and 1 VISA/VRSA strain (4 µg/ml). Comparable MIC for these strains to E-101 were 0.015 to 0.03 µg MPO/ml.
- The MIC distribution (%) for E-101 against all phenotypes is shown in Figure 5. The mean MIC was 0.015 µg MPO/ml.

CONCLUSION

- In this study, we confirmed the potent anti-staphylococcal activity of E-101 to several resistant phenotypes of *S. aureus*.
- MIC distribution histograms showed that the MIC range for E-101 is very narrow for all *S. aureus* phenotypes compared to mupirocin.
- E-101 shows promise as a topical agent for managing complicated and uncomplicated skin and soft tissue infections.

REFERENCES

1. Allen, R., and J. Stephens Jr. 2008. Myeloperoxidase shows selective bacterial binding, p. A1-3497, 48th ICAAC/IDSA 46th Annual Meeting Washington, DC.

2. Engemann, J. J., Y. Carmeli, S. E. Cosgrove, V. G. Fowler, M. Z. Bronstein, S. L. Trivette, J. P. Briggs, D. J. Sexton, and K. S. Kaye. 2003. Adverse clinical and economic outcomes attributable to methicillin resistance among patients with *Staphylococcus aureus* surgical site infection. Clin Infect Dis 36:592-8.

3. Kiryu, C., M. Makiuchi, J. Miyazaki, T. Fujinaga, and K. Kakinuma. 1999. Physiological production of singlet molecular oxygen in the myeloperoxidase-H2O2-chloride system. FEBS Lett 443:154-8.

4. Parker, J. G., and W. D. Stanbro. 1984. Dependence of photosensitized singlet oxygen production on porphyrin structure and solvent. Prog Clin Biol Res 170:259-84.

5. Rogers M.A.J. 1983. Time resolved studies of 1.27 µm luminescence from singlet oxygen generated in homogeneous and microheterogeneous fluids. Photochem Photobiol. 37(1):99-103.

6. Straight, R., and J. Spikes. 1985. Photosensitized Oxidation of Biomolecules, p. 91-143. In A. A. Frimer (ed.), Singlet O_2. CRC Press, Boca Raton, Fla.

7. Tatsuzawa, H., T. Maruyama, K. Hori, Y. Sano, and M. Nakano. 1999. Singlet oxygen ((1)Delta(g)O(2)) as the principal oxidant in myeloperoxidase-mediated bacterial killing in neutrophil phagosome. Biochem Biophys Res Commun 262:647-50.

8. Wikler, M. A., and Clinical and Laboratory Standards Institute. 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically : approved standard, 7th ed. Clinical and Laboratory Standards Institute, Wayne, PA.