Review Article
Volume 7:3, 2021

Journal of Antimicrobial Agents

ISSN: 2472-1212

Open Access

Antiseptic Action of E-101 Solution, a Myeloperoxidase-Mediated Formulation, in the Presence of Whole Blood Compared to Conventional Wound Antiseptics and Biocides

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Abstract

E-101 solution is a first-in-class myeloperoxidase containing antimicrobial solution developed for topical application. The active ingredients in E-101 solution are two enzymes, porcine myeloperoxidase (pMPO) and glucose oxidase (GO) in an aqueous solution and activated by the addition of a glucose solution. Once activated, the reactive species hydrogen peroxide, hypochlorous acid/hypochlorite (HOCl/OCl$^-$), and singlet oxygen ($^1O_2$) are generated. We evaluated the effect of whole human blood on the performance of E-101 solution compared to commercially available wound antiseptics and commonly used biocides. The wound cleansers NeutroPhase, Microcyn, and Vashe with the active HOCl/OCl$^-$ component were tested according to the USP-51 effectiveness test in the presence of 0, 1, 2 and 5% blood. Comparative time-kill studies against chlorhexidine, povidone-iodine, sodium oxychlorosene were tested in the presence of 0, 2, 5 10, and 20% blood. In the USP-51 test, E-101 solution demonstrated >2 log10 reduction against bacterial and fungal isolates in the presence of 5% blood at days 14 and 28. With the exception of NeutroPhase activity against *S. aureus*, all comparable wound antiseptics demonstrated <2 log10 reduction in the presence of 5% blood at days 14 and 28. Time-kill microbicidal data observed in the presence of blood demonstrated that E-101 solution was the most active biocide, followed by chlorhexidine and povidone-iodine. The presence of 2% blood completely inhibited the activity of sodium oxychlorosene. In summary, E-101 solution remained active in the presence of blood containing catalase and other substances that competitively react with $^1O_2$ and HOCl/OCl$^-$ as a safe and effective wound antiseptic.

Keywords: Antiseptics • Biocide • Wound cleaners • Blood • E-101 solution • Myeloperoxidase • Hypochlorous acid • Chlorhexidine • Povidone-iodine • Sodium oxychlorosene

Introduction and Discussion

Antiseptics are used extensively in hospitals as topical agents to prevent health-care associated infections. Many topical products are currently on the market that contains a variety of chemical agents or biocides [1]. These biocides have a broad-spectrum of activity and are directed to multiple targets. However, the antimicrobial activities of these biocides are influenced by a number of factors including formulation, dilution, synergy, temperature and presence of organic material [2]. In addition, reduced susceptibility to antiseptics as well as intrinsic or acquired resistance and cross-resistance to antibiotics have been reported [1].

E-101 solution is a first-in-class topical myeloperoxidase-mediated formulation developed as an antimicrobial open wound wash solution. It is composed of two enzymes, glucose oxidase (GO) and porcine myeloperoxidase (pMPO) in an aqueous vehicle. Upon topical application of E-101 solution containing glucose, the hydrogen peroxide (H_2O_2) produced in situ by GO drives pMPO-dependent oxidation of chloride to hypochlorous acid (HOCl). Once generated, HOCl (or its conjugate base OCl$^-$, pKa=7.5) participates in a diffusion controlled reaction with a second H_2O_2 molecule to yield singlet molecular oxygen ($^1O_2$), a metastable electronically excited reactant with a microsecond lifetime. Singlet oxygen is a potent electrophilic oxygenating agent capable of reacting with a broad-spectrum of electron rich compounds. E-101 solution demonstrates a broad-spectrum in vitro and in vivo microbicidal activity even in the presence of antimicrobial-resistant pathogens [3-6]. The MPO component selectively binds and kills specific gram-positive bacteria and all gram-negative bacteria tested [7-9] and inhibits endotoxin activity of lipopolysaccharide and lipid A [10]. Preliminary studies have shown that E-101 maintains its antimicrobial activity in the presence of serum and blood [11].

The goals of this study were to determine the effect of whole human blood on the antimicrobial activity of E-101 Solution and three predicate antimicrobial skins and wound solutions in accordance with the USP-51 antimicrobial effectiveness test. Second, to compare the rate of killing of E-101 solution to that of chlorhexidine gluconate, povidone-iodine, and sodium oxychlorosene in the presence of whole human blood.

(This work was presented in part at American Society for Microbiology and Interscience Conference on Antimicrobial Agents and Chemotherapy. San Diego, CA, 2015 [12] and 30th European Congress of Clinical

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Received date: 22 February 2020; **Accepted date:** 08 March, 2021; **Published date:** 15 March, 2021

Microbiology and Infectious Diseases, Paris France, 2020, [13]).

Materials and Methods

Antiseptics and reagents

Comparative antiseptics used in the USP-51 antimicrobial effectiveness test included E-101 solution (Exoxemis, Inc., Little Rock, AR), NeutroPhase skin and wound cleanser (NovaBay Pharmaceuticals, Inc. Bowling Green, OH), Microcyn antimicrobial skin wound cleanser (Oculus Innovative Sciences, Petaluna, CA), and Vashe wound therapy+solution (SteadMed, Fort Worth, TX,). Stock solutions of E-101 solution were prepared at Exoxemis, Inc. (Omaha, NE). E-101 enzyme solution contained two enzymes, porcine myeloperoxidase (pMPO), glucose oxidase (GO), derived from Aspergillus niger, and proprietary amino acids in an aqueous formulation vehicle consisting of 150 mM sodium chloride and 0.02% (wt/vol) polysorbate 80, pH 6.5, 20 mM solution, and phosphate buffer. The stock concentration of pMPO and GO were 2.5 mg/ml and 0.5 mg/ml, respectively. The E-101 substrate solution contained 300 mM glucose in the same aqueous formulation as the enzyme solution. The enzyme and substrate solutions were packaged in two separate vials and mixed together by swirling for approximately 10 seconds. The activated test product was held at room temperature for 20 to 30 minutes before inoculation of challenge microorganisms. The final concentration of activated E-101 was 0.83 mg pMPO/ml. NeutroPhase, Microcyn, and Vashe all contain a stabilized organic derivative of hypochlorous acid (HOCl) at concentrations of HOCl tested of 0.03%, 0.003%, and 0.033%, respectively. Solutions were prepared according to the manufacturer's directions.

Comparative antiseptics used in the time kill assays included E-101 solution, chlorhexidine-digluconate (Sigma-Aldrich, St. Louis, MO), povidone-iodine (Purdue Products, LLP, Stamford, CT), and sodium oxychlorosene (Clorpactin wcs-90, Guardian Laboratories, Hauppauge, NY). All antiseptics were prepared according to manufacturer's direction at skin application concentrations. Solutions were prepared at non-toxic use concentrations [14,15],

The final concentrations of E-101 solution, chlorhexidine, povidone-iodine and sodium oxychlorosene were 0.83 mg pMPO/ml, 0.2 mg/ml, 3.5 mg/ml, and 4 mg/ml respectively.

The neutralization solution used for E-101 solution in the USP-51 assay was Dey/Engley (D/E) broth supplemented with 1% bovine catalase (Sigma-Aldrich, St. Louis, MO). All other antiseptics tested in the USP-51 assay were neutralized in D/E broth without the addition of catalase. The neutralization solution used in the comparative time-kill assay was composed of 3 g/liter lecithin, 30 ml/liter polysorbate 80, 1 g/liter L-histidine, and 30 g/liter saponin prepared in sterile distilled water [16]. The components were purchased from Sigma Aldrich. For E-101 solution, the neutralizer was supplemented with 1% bovine catalase. Volunteer donor whole blood (irradiated, leukocytes reduced and washed red blood cells) was obtained from the Blood Bank Department at Indiana University Health Pathology Laboratory (Indianapolis, IN).

Microbes

The USP-51 antimicrobial effectiveness test used the following five microorganisms; Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027, Staphylococcus aureus ATCC 6538, Candida albicans ATCC 10231, and Aspergillus braziliensis ATCC 16404. The bacteria were plated to Trypticase soy agar with 5% Sheep Blood (TSA) and plates were incubated at 35±2°C for 18-24 hours. Overnight bacterial cultures were re-suspended in sterile phosphate buffered saline (PBS) to approximate a 0.5 McFarland Standard (1.5 x 10^8 CFU/ml). Each suspension was diluted so that the appropriate volume (0.1 – 1% of the volume of test solutions) is added to each test solutions to achieve an approximate concentration of 1 x 10^6 CFU/ml. Bacterial suspensions were used within 30 minutes of preparation. Candida albicans was plated to Sabourauds dextrose agar (SDA) and plates were incubated at 22.5 + ±2°C for 44-52 hours. Individual colonies of *C. albicans* were suspended in PBS to approximate a 1.0 McFarland Standard (3.0 x 10^8 CFU/ml). The suspension was diluted so that the appropriate volume (0.1 – 1% of the volume of test solutions) is added to each test solutions to achieve a concentration of 1 x 10^6 CFU/ml. This suspension was used within 30 minutes of preparation. Aspergillus brasiliensis was plated to SDA and plates were incubated at 22.5+ ±2°C for 6-10 days. A suspension of *A. brasiliensis* was prepared by washing surface growth from a 6-10 day culture using sterile PBS supplemented with 0.1 Triton X-100 (v/v). The concentration was adjusted to approximate a 2.0 McFarland Standard (6.0 x 10^8 CFU/ml). The suspension was diluted so that the appropriate volume (0.1 – 1% of the volume of test solutions) is added to each test solution to achieve a concentration of 1 x 10^5 and 1 x 10^6 CFU/ml. The final *A. brasiliensis* suspension was refrigerated for up to 10 days of preparation.

The comparative time-kill assay used the following four microorganisms; Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 43300, and Candida auris CDC B11903. Logarithmic-phase growth suspensions were prepared in PBS to achieve a concentration of approximately 1 x 10^6 CFU/ml.

USP-51 antimicrobial effectiveness test

The initial USP-51 preservative challenge test with neutralization verification for E-101 solution was performed at Microchem Laboratories (Round Rock, TX) as previously described [17]. The neutralization control consisted of Dey/Engley broth supplemented with 1% catalase. In this study, the USP-51 antimicrobial effectiveness test was performed with the modification of added blood. The antiseptic solutions were tested against five American Type Culture Collection microorganisms in the presence of 0, 1, 2, and 5% whole human blood. The initial concentration of each microorganism was determined by inoculating control PBS using standard 10-fold serial dilutions and plating to the appropriate culture media. Four tubes containing 10 ml of each antiseptic solution was prepared and 0, 0.1, 0.2 and 0.5 ml of blood were added to tube 1, 2, 3, and 4, respectively. Within 20±5 minutes of E-101 solution preparation, 0.1 ml of challenge microorganisms was added and mixed by swirling for 10 seconds. Similarly, comparator antiseptics were inoculated with 0.1 ml of challenge microorganisms. The inoculated tubes were incubated at room temperature for up to 28 days. A 0.1 ml volume of inoculated test solutions and control tubes were sampled at 1, 7, 14, and 28 days for quantitative culture. Bacterial plates were incubated at 35±2°C for 48±4 hrs. Fungal plates were incubated at 22.5+±2°C for 48±4 hrs. After 48 hours, surviving microorganisms were counted and the log10 reduction from the initial population was determined. The effectiveness of each antiseptic was based on the USP-51 criteria. For comparison purposes, a 2.0 log10 or greater reduction from the initial inoculum in the presence of blood at day 14 and 28 was used for antimicrobial effectiveness. A neutralization control was also performed for each test microorganism and test antiseptic supplemented with blood, wherein 1.0 ml of test antiseptic was added to 9.0 ml of appropriate neutralizer broth. A neutralizing control was comprised of 1.0 ml PBS added to 9.0 ml neutralizing broth. For each antiseptic and PBS control, a set of 4 tubes containing neutralizing broth and antiseptic (10 ml) was supplemented with 0, 1% (0.01 ml), 2% (0.02 ml), and 5% (0.05 ml) blood. Each tube was then inoculated with 0.01 ml for microorganism suspension. Test and control tubes were mixed and held at room temperature for 10 minutes and a 0.01 ml aliquot plated in duplicate to the appropriate growth media. Quantitative colony counts were performed on days 1, 7, 14, and 28. The neutralization test counts (CFU) were compared to neutralization control counts after 24-48 hour of incubation. Neutralization was validated if the recovery percentages were ≥50% when compared to the control counts.

Comparative time-kill assay in the presence of whole blood

The time-kill studies were conducted as previously described with modifications [11,16]. Reaction tubes were prepared to contain the appropriate logarithmic growth (10^6 CFU), antiseptic, and 0%, 2%, 5%,

10%, and 20% blood. Antimicrobial activity was evaluated by mixing 800 µl of antiseptic with 100 µl blood +PBS dilution (example final 5% blood=50 µl blood +50 µl saline) and 100 µl microbial suspension. At the desired contact times (5, 15, 30, and 60 min), 100 µl of the test mixture was added to 800 µl neutralizer solution and 100 µl PBS. After a further 5-min incubation, the neutralized mixture was 10-fold serially diluted in PBS and 100 µl of test mixture or 100 µl of each dilution and homogeneously spread onto a TSA or SAB plate and incubated at 35°C for 24 (bacteria) or 48 (yeast) hrs. The log10 CFU at each time point was determined and compared to the growth control. Due to in-test dilution, antiseptics were tested at 80% of final concentrations.

Results

USP-51 antimicrobial effectiveness test

E-101 solution met the antimicrobial effectiveness criteria in the USP-51 preservative challenge test. Based on recovery percentages achieved of >50%, the neutralization validation results demonstrated the D/E broth supplemented with 1% catalase was an adequate neutralizer for use. A summary of the modified USP-51 test results appears in Table 1. In the absence of blood added, E-101 solution and comparable antiseptics all met the passing criteria of ≥2 log10 reduction from the initial inoculum count against all challenge organisms at days 1, 7, and 14 with no increase in growth at day 28 of viability sampling. The addition of increasing concentrations of blood interfered with the antimicrobial activity of the antiseptics to various degrees. At days 14 and 28, E-101 solution demonstrated the greatest effectiveness against all challenge microorganisms. The reactive oxidants of E-101 solution demonstrated a ≥2 log10 reduction CFU even in presence of 5% blood containing catalase and other competitive substrates. NeutroPhase was the next antiseptic demonstrating activity in the presence of 1 and 2% blood with the exception of *S. aureus*, <2 log10¬ CFU kill was observed in the presence of 5% blood against the other challenge microorganisms. Vashe demonstrated moderate activity in the presence of 2% blood against the bacterial strains tested and was active against the fungi in the presence of 1% blood. The presence of as low as 1% blood inhibited the antimicrobial activity of Microcyn (<2 log10 reduction CFU). Based on recovery percentages of >50%, the neutralization validation was achieved for all antiseptics in the absence of blood at days 1, 14 and 28. Some exceptions in recovery of test microorganisms was observed in the presence of blood. At day 1, neutralization in the presence of blood was validated for all antiseptics. At day 14, neutralization recovery counts was validated with the exception of *S. aureus* against NeuroPhase and E-101 solution in the presence of 1% and 2% blood, respectively. At day 28, recovery counts of <50% in the presence of blood was observed for *S. aureus* and *E. coli* against E-101 solution. The reduction in counts for NeutroPhase and E-101 solution against *S. aureus* and *E. coli* may have resulted in a lower pH in the growth media at the longer recovery periods.

Table1. USP-51 antimicrobial effectiveness of E-101 solution and comparator wound antiseptics in the presence of whole human blood. Active ingredients: E-101 solution (0.83 mg pMPO/ml and 0.167 mg GO/ml; 0.083%); NeutroPhase (0.3 mg/ml HOCl, 0.03%); Vashe (0.33 mg/ml, 0.033%); Microcyn (0.03 mg/ml HOCl, 0.003%).

Log10 reduction of CFU/ml versus time (days)																	
Organism	**% blood**	**E-101 solution**				**NeutroPhase**				**Vashe**				**Microcyn**			
		1	**7**	**14**	**28**	**1**	**7**	**14**	**28**	**1**	**7**	**14**	**28**	**1**	**7**	**14**	**28**
C. albicans	0	-	6.447	6.447	6.447	-	6.447	6.447	6.447	-	6.447	6.447	6.447	-	6.447	6.447	6.447
	1	-	2.566	3.368	6.447	-	6.447	6.447	6.447	-	6.447	6.447	6.447	-	3.602	2.97	1.125
	2	-	1.271	2.368	6.447	-	6.447	6.447	6.447	-	0.333	0.168	1.049	-	0.301	1.125	1.333
	5	-	1.105	2.301	6.447	-	-0.316	-0.456	0.368	-	-0.301	-0.434	1.067	-	-0.186	0.049	1.049
S. aureus	0	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301
	1	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	0.87	1	1.125	1.372
	2	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	6.301	-0.29	-0.079	1.155	1.87
	5	2.362	6.301	6.301	6.301	2.382	4.456	6.301	4.301	0.046	0.071	1.561	1.393	0.155	-0.312	1	-0.591
E. coli	0	6.362	6.362	6.362	6.362	6.362	6.362	6.362	6.362	6.362	6.362	6.362	6.362	6.362	6.362	6.362	6.362
	1	6.362	6.362	6.362	6.362	6.362	6.362	6.362	6.362	3.061	6.362	6.362	6.362	2.699	2.427	0.964	2
	2	6.362	6.362	6.362	6.362	6.362	6.362	6.362	6.362	0.76	0.83	6.362	6.362	0.805	0.186	-0.386	0.68
	5	6.362	6.362	6.362	6.362	-1.07	0.709	0.248	-0.036	-0.195	0.061	-0.053	-0.752	-0.229	-0.96	-0.115	-0.036
P. aeruginosa	0	6.462	6.462	6.462	6.462	6.462	6.462	6.462	6.462	6.462	6.462	6.462	6.462	6.462	6.462	6.462	6.462
	1	6.462	6.462	6.462	6.462	6.462	6.462	6.462	6.462	6.462	6.462	6.462	6.462	1.611	0.533	-0.191	-0.395
	2	6.462	6.462	6.462	6.462	0.971	4.462	6.462	6.462	1.047	1.684	3.582	6.462	1.383	-0.219	-0.684	-1.21

	5	6.462	6.462	6.462	6.462	1.064	1.485	1.582	0.348	0.781	0.316	-1.191	-1.918	-1.88	-1.094	-1.617	-2.117
A. brasiliensis	0	-	6.477	6.477	6.477	-	6.477	6.477	6.477	-	6.477	6.477	6.477	-	6.477	6.477	6.477
	1	-	6.477	6.477	6.477	-	6.477	6.477	6.477	-	3.574	2.436	3.198	-	1.273	1.436	1.273
	2	-	6.477	6.477	6.477	-	1.778	1.62	1.398	-	1.398	1.273	0.761	-	1.363	1.363	1.273
	5	-	1.714	3.331	3.745	-	1.301	0.854	1.155	-	1.301	1.477	1.247	-	1.398	1.398	1.398

Comparative time-kill assay

Time-kill data demonstrated the rapid bactericidal activity of all antiseptics without the presence of blood (Table 2). The most active antiseptic tested in the presence of blood was of E-101 solution. A fungicidal reduction (≥5 log10) in viability of C. auris was obtained within 60 min in the presence of 2% blood. A bactericidal reduction in viability of *S. aureus* was obtained within 60 min in the presence of 5% blood. E-101 solution remained highly active in the presence of 10% and 20% blood against *E. coli* and *P. aeruginosa*. A bactericidal reduction in viability of E coli in the presence of 20% blood was obtained within 15 min. A bactericidal reduction of *P. aeruginosa* in the presence of 10% blood and >2 log10 reduction in viability in the presence of 20% blood was obtained within 30 min.

Table 2. Comparative microbicidal activities of E-101 solution, chlorhexidine, povidone iodine and sodium oxychlorosene in the presence of human whole blood. Concentrations: E-101 solution 0.83 mg pMPO/ml (0.083%); chlorhexidine 0.2 mg/ml (0.02%); povidone-iodine 3.5 mg/ml (0.35%); sodium oxychlorosene 4.0 mg/ml (0.4%).

Log10 reduction ofCFU/ml versus time (min)																	
Organism	**% blood**	**E-101 solution**				**Chlorhexidine**				**Povidone-iodine**				**Sodium oxychlorosene**			
		5	**15**	**30**	**60**	**5**	**15**	**30**	**60**	**5**	**15**	**30**	**60**	**5**	**15**	**30**	**60**
C. aureus	0	1.8	6.158	6.158	6.158	2	4	4	6.301	6.297	6.297	6.297	6.297	6.544	6.544	6.544	6.544
	2	0.012	0.816	2.079	6.158	1.393	2.921	3.699	6.301	6.297	6.297	6.297	6.297	0.053	0.082	0.097	-0.024
	5	0.035	0.434	1.094	1.903	1.488	2.058	2.561	3.125	-0.085	-0.146	0.053	0.053	-0.047	0	-0.036	-0.069
	10	-0.325	-0.118	0.085	0.68	0.359	0.66	0.925	1.183	-0.178	-0.185	-0.199	-0.167	0.182	0.202	0.164	0.129
	20	-0.179	-0.204	-0.028	0.148	-0.026	0.058	0.204	0.125	-0.196	-0.229	-0.189	-0.216	0.182	0.053	0.129	0.053
S. aureus	0	1.523	6.415	6.415	6.415	3.449	6.352	6.352	6.352	6.568	6.568	6.568	6.568	6.398	6.398	6.398	6.398
	2	0.446	6.415	6.415	6.415	1.724	1.946	2.219	2.351	6.568	6.568	6.568	6.568	-0.064	-0.121	0	0.097
	5	0.088	0.569	1.21	5.716	1.423	1.303	1.449	1.526	0.392	0.153	0.091	0.226	-0.053	-0.1	-0.053	-0.014
	10	0.374	0.813	1.318	2.602	1.101	1.168	1.193	1.363	0.226	0.454	0.527	0.364	0.056	0.076	0.056	0.056
	20	0.257	0.281	0.351	0.451	0.122	0.76	0.855	1.062	0.106	0.063	0.137	0.063	0.495	0.585	0.578	0.495
E. coli	0	4.146	6.748	6.478	6.478	6.74	6.74	6.74	6.74	6.724	6.724	6.724	6.724	6.914	6.914	6.914	6.914
	2	4.146	6.748	6.748	6.748	6.74	6.74	6.74	6.74	6.724	6.724	6.724	6.724	0.027	0.101	0.005	-0.095
	5	3.748	6.748	6.748	6.748	6.74	6.74	6.74	6.74	0.247	0.293	0.402	0.219	0.088	0.094	-0.036	0
	10	4.049	6.748	6.748	6.748	4.74	6.74	6.74	6.74	0.293	0.344	0.133	0.168	-0.04	-0.095	-0.059	0.081
	20	4.748	6.748	6.748	6.748	2.51	3.895	4.439	6.74	0.133	0.168	0.071	0.091	0.056	0.011	0.136	-0.016
P. aeruginosa	0	3.038	6.618	6.618	6.618	6.623	6.623	6.623	6.623	6.813	6.813	6.813	6.813	6.602	6.602	6.602	6.602
	2	1.087	3.84	6.618	6.618	6.623	6.623	6.623	6.623	6.813	6.813	6.813	6.813	0.011	0.034	-0.031	0.222
	5	0.481	2.087	6.618	6.618	6.623	6.623	6.623	6.623	-0.062	0.097	0.269	0.336	0.084	0.222	0.111	0.187
	10	0.539	3.773	6.618	6.618	6.623	6.623	6.623	6.623	0.073	0.123	0.021	0.065	0.204	0.022	0.24	0.24
	20	0.995	1.276	2.975	3.919	1.079	2.916	4.021	6.623	0.123	0.222	0.065	0.179	0.125	0.24	0.187	0.125

Chlorhexidine demonstrated a bactericidal reduction in *E. coli* and P. aeruginosa in the presence of 10% and 20% within 30 and 60 min, respectively. A fungicidal reduction of C. auris in the presence of 2% blood and >2 log10 reduction in viability in the presence of 5% blood was obtained within 60 min. Only a >2 log10 reduction of *S. aureus* was observed in the presence of 2% blood. Povidone-iodine demonstrated rapid within 5 min antimicrobial activity in the presence of 2% blood. No significant antimicrobial activity was observed at the higher blood concentrations tested. Sodium oxychlorosene was inhibited by the presence of 2% or greater blood.

Discussion

Topical antiseptics exhibit broad-spectrum antimicrobial activity. Unlike antibiotics that tend to have specific targets, biocides have multiple targets. Antiseptics are less selective in terms of specific targeted microbicidal action and are used to kill or reduce the overall bioburden in open wounds [18]. E-101 solution exerts a potent and broad-spectrum antimicrobial action against gram-positive and gram-negative bacteria including multidrug-resistant pathogens and fungi [3-6,19]. The MPO component of this antimicrobial solution has been shown to selectively bind to many target pathogens sparing normal flora, but at high concentrations, MPO demonstrates microbial action against essentially all microbes [7,8]. Even at higher concentrations selective MPO binding results in selective microbicidal activity with minimal bystander damage to host cells, including erythrocyte. In addition to microbicidal activity, MPO has been reported to inactivate exotoxins [20,21] and inhibit endotoxin activity of lipopolysaccharide and lipid A [10].

The purpose of the USP-51 preservative effectiveness test was to evaluate the effect of aqueous product formulations containing antimicrobial ingredients by inhibiting growth and reducing microbial contamination over extended storage times. We modified the test to challenge the efficacy of E-101 solution and competitor products in the presence of whole human blood. The hypochlorous-based antiseptics evaluated by the USP-51 antimicrobial effectiveness test, were prepared at dilutions recommended for safety by manufacturers for topical application. Their antimicrobial activity was markedly diminished or eliminated in the presence of blood when tested at recommended use solution concentrations. The reactive oxidants of E-101 solution are focally generated and less susceptible to the inhibitory effect of blood containing catalase and other competitive substrates that competitively react with available $^1O_2$ and HOCl. As demonstrated elsewhere and herein, E-101 solution is superior to HOCl/OCl^- in the presence of erythrocytes [7]. Because of the concerns over skin cells and tissue damage and wound healing, topical solutions of skin and wound cleansers are formulated to minimize cytotoxicity with varying degree of antimicrobial activity [22]. In addition, conventional topical antiseptics with relatively low molecular weights (<800 daltons) and high water and lipid solubility have the greatest potential for absorption into the systemic circulation through intact or broken skin and mucus surfaces. Concerns of systemic absorption of antiseptics include potential systemic toxicity and selection of cross-antibiotic resistance. The most commonly used biocides used in wound cleanser products in clinical practice today are chlorhexidine and povidone-iodine. The likelihood of antiseptic systemic absorption after topical application based on molecular weight is likely for povidone-iodine (364.9 daltons) and unlikely for chlorhexidine-gluconate (897.8 daltons). The potential for systemic adsorption of E-101 solution is highly unlikely since the molecular weights of pMPO and GO are 150,000 and 160,000 daltons, respectively.

Antiseptics can also be separated according to the molecular size of biocide. Small molecules such as free iodine from povidone-iodine can penetrate bacterial membranes through porins and cause oxidation of key proteins, nucleotides, and fatty acids within the bacterial cytoplasm. Larger molecules such as chlorhexidine cannot pass through porins and inflict damage to the outer and inner membrane causing leakage of cell contents [23]. Similarly, E-101 solution has been proposed to exert microbicidal activity at the membrane level [11]. Time-kill studies showed chlorhexidine, povidone-iodine, sodium oxychlorosene, and E-101 solution to be rapidly microbicidal at non-toxic concentrations when tested in the absence of blood. E-101 solution and chlorhexidine were the most active in the presence of blood. Chlorhexidine is the most widely used biocide in antiseptic products. However, its activity is pH dependent and previously demonstrated to be highly reduced in the presence of organic matter [24]. Likewise, the activity of povidone-iodine is susceptible to neutralization in the presence of organic matter [22,25]. Sodium oxychlorosene is chlorine releasing topical antiseptic used for treating localized infections [26]. The antimicrobial activity of the sodium oxychlorosene was greatly diminished in the presence of low concentrations of blood.

Antiseptic formulations use a variety of biocides that act at various rates and persistence intervals, show various levels of toxicity and are capable of promoting resistance. Concerns of widespread use may lead to selection of resistance. Because antiseptics have multiple targets and are broad spectrum in nature the likelihood of resistance selection is low. However, bacterial resistance to chlorhexidine has been well documented [1]. Bacterial resistance to antiseptics have been due to reduced susceptibility, intrinsic or acquired resistance to the active ingredient or biocide [1]. Several factors such as intrinsic antimicrobial activity, resistant pathogen, over dilution of antiseptic or use of contaminated antiseptics have resulted in hospital-acquired outbreaks [27]. Another concern is the recent increase in cross-resistance between conventional antiseptics and broad-spectrum antibiotics [28-31]. A targeted approach to combating antiseptic resistance in healthcare facilities and has been proposed under the authority of the antimicrobial stewardship program [32,33].

Conclusion

E-101 solution exhibits many desirable attributes of an effective antiseptic for open wounds. It demonstrates rapid and broad-spectrum microbicidal activity against gram-positive and gram-negative bacteria, including multidrug-resistant pathogens, yeast and fungi. The bactericidal activity of E-101 solution is enhanced by the selective binding of MPO to the surfaces of target pathogens sparing normal flora. Unlike other antiseptics, MPO can bind and neutralizes endotoxins and exotoxins. E-101 solution does not select for antiseptic resistance or cross-resistance to antibiotics in vitro. The likelihood of resistance to occur is remote because of the combustive oxygenation mechanism of action. As shown in this study, the microbial activity of E-101 solution remains effective in the presence of blood. The active ingredients of E-101 solution are not systemically absorbed thereby eliminating systemic toxicity and do not cause cellular or local damage. These results provide support for future clinical testing of E-101 solution in the treatment of open wound infections.

Conflict of Interest Statement

Gerald A. Denys and Robert C. Allen serves as consultants for Exoxemis, Inc. Jackson T Stephens, Jr. is president and CEO of Exoxemis, Inc. Jessica L. Carpenter declares no conflict of interest regarding the publication of this paper.

Acknowledgement

We express appreciation to Anagha Tulpule for providing technical support during the USP-51 antimicrobial effectiveness test studies. This study was supported by Exoxemis, Inc.

References

1. McDonnell, Gerald and Denver Russell. “Antiseptics and disinfectants:

activity, action and resistance".Clin Micro Rev (1999) 12:147-179.

2. Russell, Denver. "Mechanisms of bacterial resistance to antibiotics and biocides" Progr Med Chem" (1998) 35:133-197.
3. Denys, Gerald, Parveen Grover, Peter O'Hanley and Jackson Stephens. "In vitro antibacterial activity of E-101 Solution, a novel myeloperoxidase-mediated antimicrobial, against gram-positive and gram-negative pathogens" J Antimicrob Chemother (2011) 66:335-342.
4. Denys, Gerald, John Davis, Peter O'Hanley and Jackson Stephens Jr. "In vitro and in vivo activities of E-1O1 solution against Acinetobacter baumannii isolates from U.S. military personnel" Antimicrob Agents Chemother (2011) 55:3603-3608.
5. Denys, Gerald, John Davis, Peter O'Hanley and Jackson Stephens Jr. "E-101 solution, a novel myeloperoxidase mediated topical antimicrobial demonstrates in vivo efficacy in whole animal models of surgical infection prevention" J Antimicrob Photon (2014) 129:278-287.
6. Denys, Gerald, Chris Pillar, Daniel Sahm and Peter O'Hanley, et al."Five-year longitudinal assessment (2008 to 2012) of E-101 solution activity against clinical target and antimicrobial-resistant pathogens".Antimicrob Agents Chemother (2014) 58:4911-4914.
7. Allen, Robert and Jackson Stephens, Jr. "Myeloperoxidase selectively binds and selectively kills microbes" Infect Immun (2011) 79:474-485.
8. Allen, Robert and Jackson Stephens, Jr. "Reduced-oxidized difference spectral analysis and chemiluminescence-based Scatchard analysis demonstrate selective binding of myeloperoxidase to microbes". Luminescence (2011) 26: 208-21.
9. Selvaraj, Ratnam, Jan Maciej Zgliczynski, Benoy Paul, and Anthony Sbarra. "Enhanced killing of myeloperoxidase-coated bacteria in the myeloperoxidase-H_2O_2-Cl system" J Infect Dis 137(1978):481-485.
10. Allen, Robert, Mary Henery, John Allen and Roger Hawks, et al. "Myeloperoxidase and eosinophil peroxidase inhibit endotoxin activities and increase mouse survival in lipopolysaccharide lethal dose 90% model" J Immunology Research. 2019.
11. Denys, Gerald, Neil Devoe, Polyxeni Gudis Megan May and Robert Allen, et al. "Mechanism of microbicidal action of E-101 solution, a myeloperoxidase-mediated antimicrobial, and its oxidative products" Infect Immun (2019)87:1-11
12. Denys, Gerald, Kim Koch, Robert Allen and Peter O'Hanley, et al. "Performance of haloperoxidase-containing enzyme products and comparator biocides on Staphylococcus aureus and Pseudomonas aeruginosa in the presence of human whole blood" abstr C-131, Abstr Am Soc Microbiol and Interscience Conference on Antimicrobial Agents and Chemotherapy. San Diego, CA. 2015.
13. Denys, Gerald, Jessica Carpenter, Robert Allen and Jackson Stephens, Jr. "Inhibitory effect of whole blood on the antiseptic action of E-101 solution, a myeloperoxidase mediated formulation, compared to conventional wound cleansers" abstr 2957, Congr Clin Microbiol Infect Dis, 2020.
14. Severyns, Mathieu, Annabelle Lejeune, G Rocoux and Georges Lejeune. "Non-toxic antiseptic irrigation with chlorhexidine in experimental revascularization in the rat" J. Hosp Infect.(1991) 17:197-206.
15. Glick, Philip, B Joseph Guglielmo, Robert Tranbaugh and Kevin Turley. "Iodine toxicity in a patient treated with continuous povidone-iodine mediastinal irrigation" Ann Thorac Surg (1985)39:478-480.
16. Tote, Kim, Tessa Horemans, Dirk Berghe and Louis Maes, et al. "Inhibitory effect of biocides on the masses and matrices of Staphylococcus aureus and Pseudomonas aeruginosa biofilms" Appl Envirn Microbiol (2010)76:3135-3242.
17. The United States Pharmacopeia Convention, 2009 USPC Official 8/1/09 – 11/30/09 General Chapters: <51> Antimicrobial Effectiveness Testing. 23 October 2009.
18. Atiyeh, Bishara, Saad Dibo and Shady Hayek. "Wound cleaning, topical antiseptics and wound healing" Int Wound J (2009)6:420-430.
19. Denys, Gerald, Robert Allen, Peter O'Hanley, and Jackson Stephens Jr. "E-101 solution, a first in class topical anti-infective shows fungicidal activity in vitro against Candida species" poster 23B Abstr 11th ASM Conference on Candida and Candidiasis. San Francisco, CA, 2012.
20. Agner, Kjell. "Detoxicating effect of verdoperoxidase on toxins" Nature (1947) 159:271-27.
21. Ooi, Winnie, Harold Levine, Thomas LaMont, and Robert Clark. "Inactivation of Clostridium difficile cytotoxin by the neutrophil myeloperoxidase system" J Infect Dis 149(1984):215-219.
22. Drosou, Anna, Anna Falabella and Robert Kirsner. "Antiseptics on wounds: An area of controversy" Wounds (2003) 15:149-166.
23. Lachapelle, Jean-Marie, Olivier Castel, Alejandro Fueyo Casado and Bernard Leroy, et al. "Antiseptics in the era of bacterial resistance: a focus on povidone iodine" Clin Pract (2013) 10:579-592.
24. Russel, Denver and Martin Day. "Antimicrobial activity of chlorhexidine" J Hosp Infect (1993) 25:229-238.
25. Zamora, Jose, Martin Price, Pei-Chin Chuang and Layne Gentry. "Inhibition of povidone-iodine's bactericidal activity by common organic substances: an experimental study" Surgery (1985)98:25-29.
26. Wettlaufer, John. "Abacterial cystitis: Treatment with sodium oxychlorosene" J Urol. (1976)116:434-435.
27. Weber, David, William Rutala and Emily Sickbert-Bennett. "Outbreaks associated with contaminated antiseptics and disinfectants" Antimicrob Agents Chemother. (2007)51:4217-4224.
28. Gilbert, Peter and Andrew McBain. "Potential impact of increased use of biocides in consumer products on prevalence of antibiotic resistance?" Clin Micro Rev. (2003)16:189-208.
29. Russell, Denver. "Biocides and pharmacologically active drugs as residues and in the environment: Is there a correlation with antibiotic resistance?" Am J Infect Control. (2002)30:495-498.
30. Fraise, Adam. "Biocide abuse and antibiotic resistance: A cause for concern?" J Antimicrob Chemother. (2002)49:11-12.
31. Htun, Htet Lin, Pei-Yun Hon, Matthew Holden and Brenda Ang, et al. "Chlorhexidine and octenidine use, carriage of qac genes, and reduced antiseptic susceptibility in methicillin-resistant Staphylococcus aureus isolates from a healthcare network" Clin Microbiol Infect (2019)25:1154.
32. Kemp, Gunter. "Acquired resistance to chlorhexidine is it time to establish an 'antiseptic stewardship' initiative?" J Hosp Infect (2016)94:213-227.
33. Wiemken, Timothy. "Skin antiseptics in healthcare facilities: Is a targeted approach necessary?" BMC Public Health (2019)19:1158.