Poster # C-2061
American Society for Microbiology 110th General Meeting
San Diego, CA
May 23-27, 2010

E-101 Solution Demonstrates Antiviral Properties Against Herpes Simplex Virus, Human Immunodeficiency Virus, and Human Influenza A/H1N1 Virus

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ABSTRACT

Background: E-101 Solution is a novel myeloperoxidase-based topical antimicrobial that generates the local production of singlet oxygen and hypochlorous acid. It is composed of 1) porcine myeloperoxidase (pMPO) and glucose oxidase (GO); 2) glucose, which is the substrate for GO; and 3) specific amino acids that stabilize the enzymes once the system has been activated after mixing all of the components. The purpose of this study is to determine the in vitro antiviral efficacy of E-101 Solution against prototype viral strains that cause major unmet public health problems.

Methods: The virucidal properties of E-101 Solution at a concentration of 100 GU/mL pMPO: 16.7 U/mL GO was studied using a virucidal suspension test (in vitro time-kill). Test organisms included herpes simplex virus (HSV), type 1 (ATCC #VR-742); human immunodeficiency virus (HIV-1), strain Mn (ZeptoMetrix Part #0810027CF); and human influenza A (HIA) virus, A/WS/33 H1N1 (ATCC #VR-1520). The reduction in terms of percent and $\log_{10}$ reductions of the original viral challenge was determined at 15-, 30-, and 45-minute exposures. All testing was performed at Bioscience Laboratories, Inc., Bozeman, MT.

Results: Compared with controls, the time-kill suspension test performed against HSV-1 and HIV-1 demonstrated significant reduction in the infectivity of virus by 5.50 $\log_{10}$ (>99.99% reduction) and 4.25 $\log_{10}$ (>99.99% reduction), respectively, following 15-, 30-, and 45-minute exposures. Also, the time-kill suspension test performed against HIA demonstrated reduced infectivity of virus by 2.00 $\log_{10}$ (99.00% reduction) following 15-minute exposure and 4.25 $\log_{10}$ (>99.99% reduction) following 30- and 45-minute exposures.

Conclusions: E-101 Solution is a novel topical antimicrobial agent that appears to be very effective against HSV-1, HIV-1, and HIA in rapidly reducing viral infectivity in vitro. The therapeutic implication is that E-101 Solution, which is known to be effective in killing bacteria and yeast/fungi under both in vitro and in vivo conditions, might be applied to oral, respiratory, genitourinary, and rectal mucosal surfaces to help prevent or treat a broad array of epithelial-related infections and diseases.

INTRODUCTION

E-101 Solution is a novel antimicrobial agent being developed by Exoxemis, Inc. for the prevention of surgical-site infections. E-101 Solution exhibits broad antimicrobial effects due to its mimicry of the host's phagolysosome. The active ingredients of E-101 Solution are glucose oxidase (GO) and porcine myeloperoxidase (pMPO). When these ingredients are mixed with glucose at the time of preparation, the GO produces low levels of hydrogen peroxide. The amount of hydrogen peroxide produced by GO is too low to cause host cellular or tissue injury but is the critical substrate for MPO, which, in turn, produces hypochlorous acid. Hypochlorous acid then reacts with additional hydrogen peroxide to produce singlet oxygen (Figure 1).

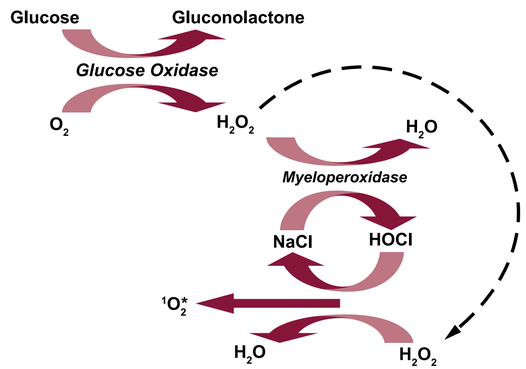
These end products of E-101 Solution are identical to the host's most potent and broadly effective innate antimicrobial factors found in the phagolysosome. Normally, these MPO end products are contained within the phagolysosome of neutrophils and macrophages that have phagocytosized apoptotic intact azurophilic granules of neutrophils. However, E-101 Solution has been formulated for topical application to kill microbes in the topical target area. E-101 Solution is not intended to be administered systemically because MPO is rapidly degraded in normal physiological pH and in the presence of hemoglobulin, serum catalases, and ceruloplasmin. For this reason, it is not surprising that the primary clinical development focus of E-101 Solution is the prevention of surgical-site infection by direct topical application into the surgical wound just after incision and prior to closure. The rationale for this strategy is further supported by extensive in vitro and in vivo preclinical data that

INTRODUCTION (CONT)

E-101 Solution has inherent microbicidal activity against aerobic and anaerobic bacteria and yeast at extremely low concentrations, irrespective of the microbe's antimicrobial susceptibility profile.

From an infectious disease perspective, the fact that E-101 Solution does not cause resistance under rigorous in vitro conditions provides impetus for its development to reduce overall antibiotic usage in the general surgical population and reduce selection for more resistant pathogens that constitute a major public health dilemma, such as methicillin-resistant (MRSA) and vancomycin-resistant (VRSA) *Staphylococcus aureus*, and multidrug-resistant (MDR) gram-negative bacteria. Additional clinical indications for E-101 Solution application beyond surgical-site infection prevention are being considered, if it can be applied topically to specific mucosal surfaces. The purpose of this exploratory study was to assess whether E-101 Solution might have virucidal effects against prototype DNA double-stranded (HSV-1), RNA single-stranded (HIA), and RNA retrovirus (HIV-1) viruses.

Figure 1. Schematic of the mechanism of action of E-101 Solution. The enzymatic activity of glucose oxidase produces a steady state of hydrogen peroxide that is critical for porcine myeloperoxidase to generate its end products, hypochlorous acid and singlet oxygen.



METHODS

Compound: E-101 Solution (Exoxemis, Inc., Little Rock, AR) was evaluated at a 90% (v/v) concentration to achieve a 100 GU/mL pMPO and 16.7 U/mL GO final test concentration/virus mixtures. A GU (guaiacol unit) is the amount of MPO enzyme that catalyzes the conversion of 1 micromole of hydrogen peroxide per minute at 25ºC. A U (unit) is the amount of GO enzyme that catalyzes the oxidation of 1 micromole of glucose per minute at 25ºC. The concentration of pMPO:GO in E-101 Solution is fixed at 5.625:1, based on their measures of activity.

Cell Lines and Virus Isolates: Vero (African green monkey [ATCC #CCL-81]), and Madin Darby Canis Kidney (MDCK [ATCC #CCL-34]) cell lines were used for the virucidal suspension tests of HSV-1 (ATCC #VR-260) and HIA/WS/33 H1N1 (ATCC #VR-1520), respectively. Cell line C8166 (human T-cell leukemia), obtained from ECACC (ECACC #88051601), was used as the host system for (HIV-1, strain Mn (ZeptoMetrix Part #0810027CF) propagation and titration.

METHODS (CONT)

Neutralization Test. A neutralization test was performed versus HSV-1 prior to the virucidal suspension test. Catalase (300 U/mL) in Dey-Engley Broth was determined to be effective in neutralizing the virucidal activity of E-101 Solution while nontoxic to both virus and cell culture used.

Virucidal Suspension Test. The study design summary for the virucidal suspension test and controls is shown in Table 1. Each challenge virus was added to a sample of E-101 Solution and mixed thoroughly to achieve a 100 GU/mL pMPO final E-101 Solution concentration/virus mixture. The mixtures were held for 15, 30, and 45 minutes. Immediately after each exposure time, the mixture was neutralized to stop virucidal activity and diluted in maintenance medium. Each dilution was plated in 4 replicates. The plates were incubated in CO_2 for 5 to 14 days and monitored for cytopathic/cytotoxic effect.

Table 1. Virucidal suspension test and study design

Parameter	Summary	Replicates
Virucidal suspension test	Virus+E-101 Solution → Exposure → Neutralization → Dilution→ Plating	4 per group
Virus control	Virus+Diluent → Neutralization → Dilution → Plating	4 per group
Cytotoxicity control	E-101 Solution+Diluent → Neutralization → Dilution → Plating	4 per group
Neutralization control	E-101 Solution+Diluent → Neutralization → Virus Inoculation → Dilution → Plating	4 per group
Negative control	Maintenance medium	4 per group

Calculations. Viral titers were calculated as 50% tissue culture infectious dose ($TCID_{50}$) by the Spearman-Karber method. The $\log_{10}$ reduction [($\log_{10}$ $TCID_{50}$ *virus control*) – ($\log_{10}$ $TCID_{50}$ *Virucidal Suspension Test*)] and percent reduction [(1-$TCID_{50}$ *test*/$TCID_{50}$ *virus control*) x 100] in viral infectivity resulting from treatment were calculated.

RESULTS

The results of the time-kill suspension test performed for each of the 3 prototype viruses when tested at a final E-101 Solution concentration of 100 GU/mL pMPO is shown in Tables 2 through 4. E-101 Solution reduced the infectivity of HSV-1 and HIV-1 by 5.50 $\log_{10}$ (>99.99%) and 4.25 $\log_{10}$ (>99.99%), respectively, following 15 minutes of exposure. E-101 Solution reduced the infectivity of HIA virus, 2.00 $\log_{10}$ (>99.00%) at 15 minutes and 4.25 $\log_{10}$ (>99.99%) following 30 minutes of exposure.

Table 2. Time-kill suspension test results for HSV-1

	Virus Control	Test Exposure Time (min)		
		15	30	45
$TCID_{50}$	7.00 $\log_{10}$	1.50 $\log_{10}$	1.50 $\log_{10}$	1.50 $\log_{10}$
Log_{10} reduction	NA	5.50 $\log_{10}$	5.50 $\log_{10}$	5.50 $\log_{10}$
Percent reduction	NA	>99.99%	>99.99%	>99.99%

RESULTS (CONT)

Table 3. Time-kill suspension test results for HIV-1

	Virus Control	Test Exposure Time (min)		
		15	30	45
$TCID_{50}$	6.75 $\log_{10}$	2.50 $\log_{10}$	2.50 $\log_{10}$	2.50 $\log_{10}$
Log_{10} reduction	NA	4.25 $\log_{10}$	4.25 $\log_{10}$	4.25 $\log_{10}$
Percent reduction	NA	>99.99%	>99.99%	>99.99%

Table 4. Time-kill suspension results for HIA/WS/33/H1N1 virus

	Virus Control	Test Exposure Time (min)		
		15	30	45
$TCID_{50}$	5.75 $\log_{10}$	3.75 $\log_{10}$	1.50 $\log_{10}$	1.50 $\log_{10}$
Log_{10} reduction	NA	2.00 $\log_{10}$	4.25 $\log_{10}$	4.25 $\log_{10}$
Percent reduction	NA	>99.00%	>99.99%	>99.99%

DISCUSSION

The viruses studied in this report represent major causes of human disease. Each of these viruses utilizes a pathogenic process that involves human mucosa as the infection entry gate followed by different pathways of virus dissemination into susceptible organs. The viral replication cycle promotes further infectivity or dissemination of disease to other individuals. If E-101 Solution could be properly applied to oral, respiratory, genitourinary, and/or rectal sites and lead to sufficient end products of MPO in situ, it might interfere with the infectivity of these viruses. In fact, it would be expected that other locally designated microbes (irrespective of whether they are bacteria, yeast/fungi, or viruses) would be killed because the intrinsic nature of MPO-mediated microbial injury is ubiquitous for all microbes. The combination of an effective topical antiseptic plus a systemic antimicrobial would be expected to provide complementary killing or anti-infectivity effects against target microbes that have a mucosal phase of colonization and/or epithelial attachment prior to systemic dissemination. It would be expected that this would decrease the severity of disease in an individual or reduce the transmission of disease in the population. In this study, E-101 Solution had uniform virucidal effects on HSV-1, HIA virus, and HIV-1. The presumed virucidal mechanism of action of E-101 Solution on these viruses is direct oxidative damage. In an era of increasing viral resistance to drugs, the therapeutic application of E-101 Solution for these viral infections is attractive, because there are no known mechanisms to avoid the killing effects of MPO-mediated microbial injury once it has produced its end products.

CONCLUSION

There are a variety of clinical indications where E-101 Solution might be an effective supplement as a microbicide for a number of viruses when applied to mucosal surfaces. It is envisioned that E-101 Solution could significantly reduce the epithelial viral load of these viruses, thereby providing therapeutic benefit to an infected individual and preventing dissemination of viral and other microbial diseases within the population.