In Vitro Efficacy of a New Formulation Combining Topical Exosome-Loaded Gel with Antibiotic against Gram Positive and Negative Bacteria

UNIVERSITY OF MIAMI MILLER SCHOOL of MEDICINE

Joel Gil[1], Michael Solis[1], Ryan Strong[1], Roger Cassagnol[1], Melissa Wright[2], Kate Johnson[2] and Stephen C. Davis[1]

[1] Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, Miller School of Medicine, University of Miami, Florida

[2] Luna Labs USA, Charlottesville, Virginia

UNIVERSITY OF MIAMI MILLER SCHOOL of MEDICINE

Abstract

Introduction:

Bacterial resistance to antibiotics is an important problem, especially in non-healing wounds. Two of the most commons pathogens associated with these infections are *Staphylococcus aureus* and *Acinetobacter baumannii*. Exosomes are lipid bilayer-delimited particles that are naturally released from almost all types of cells but, unlike a cell, cannot replicate. They serve as a fundamental intercellular communication system and have shown efficacy in tissue repair. In this *in vitro* study, we examined the use of antibiotic-loaded exosomes formulated with synthetic liposomes to treat and prevent wound infections.

Methods:

Fresh cultures of bacterial pathogenic isolate obtained directly from American Type Culture Collection (ATCC), Rockville, Maryland, were used in these studies, specifically Methicillin Resistant *Staphylococcus aureus* MRSA USA300 (MRSA USA300) and *Acinetobacter baumannii* ATCC 19606 (AB19606). Prior to evaluating zones of inhibition, minimal inhibitory concentrations (MIC) for each pathogen were determined. A modified Kirby Bauer technique was then used to demonstrate the efficacy of topical exosome-based formulations containing the antibiotic Moxifloxacin (MOX). Inhibition zones were measured using ImageJ. Results were tabulated, and statistical analysis was performed to demonstrate the differences between treatments.

Results:

MIC testing resulted in an inhibitory MOX concentration of 32 µg/ml and 256 µg/ml for AB19606 and MRSA USA300, respectively. MOX alone and in combination with topical exosome-loaded gels resulted in significant ($p \leq 0.05$) zones of inhibition compared to vehicles and untreated controls. Inhibition zone areas against MRSA USA300 and AB19606 ranged between 30-43 cm^2.

Discussion:

Our results demonstrated that MOX can be loaded into the topical exosome-loaded gels and has significant antimicrobial activity against two common wound pathogens. These results warrant future preclinical and clinical studies to substantiate their use in preventing or treating wound infections.

Introduction

Delayed wound healing is frequently associated with wound infections.[1] Resolving these infections is made difficult by drug resistant pathogens such as the ESKAPE pathogens *S. aureus* and *A. baumannii*.[2] Methicillin Resistant *S. aureus* produces the greatest burden among notable MDR organisms on the healthcare system, with estimated cost in the United States of $1.2 billion annually. Carbapenem resistant *Acinetobacter* infections produce the greatest cost per community acquired infection.[3] This study investigated the effifacy of a novel encapsulated antibiotic against MRSA and *A. baumannii* clinical isolates through a tube dilution test and a modified Kirby-Bauer assay.

References

1. Rahim, K., Saleha, S., Zhu, X., Huo, L., Basit, A., & Franco, O. L. (2017). Bacterial contribution in chronicity of wounds. Microbial Ecology, 73(3), 710–721. doi:10.1007/s00248-016-0867-9
2. Vázquez-López, R., Solano-Gálvez, S. G., Juárez Vignon-Whaley, J. J., Abello Vaamonde, J. A., Padró Alonzo, L. A., Rivera Reséndiz, A., … Barrientos Fortes, T. (2020). Acinetobacter baumannii Resistance: A Real Challenge for Clinicians. Antibiotics (Basel, Switzerland), 9(4), 205. doi:10.3390/antibiotics9040205
3. Nelson, R. E., Hatfield, K. M., Wolford, H., Samore, M. H., Scott, R. D., Reddy, S. C., … Baggs, J. (2021). National estimates of healthcare costs associated with multidrug-resistant bacterial infections among hospitalized patients in the United States. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America, 72(Suppl 1), S17–S26. doi:10.1093/cid/ciaa1581

Materials and Methods

1. Microorganisms:

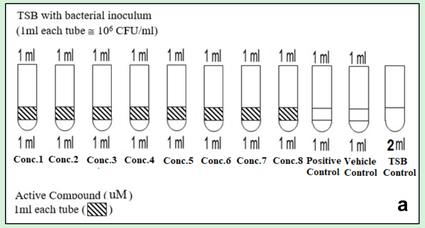
- Fresh cultures of Methicillin Resistant *Staphylococcus aureus* (MRSA) USA300 and *Acinetobacter baumannii* ATCC 19606 (AB19606) were used in this study.

2. In vitro Studies:

- This study was conducted with several phases or tasks. First, minimum inhibitory concentrations of Moxifloxacin against MRSA and AB19606 were determined.
- The second task investigated the zone of inhibition produced after encapsulation of MOX into hybrid exosomes at varying concentrations compared to unencapsulated
- The third task investigated change in efficacy after incorporation of the MOX-loaded hybrid vesicles into gels for potential topical application.
- The fourth task was conducted to evaluate efficacy after varying storage temperatures and times.

3. Minimum Inhibitory Concentration (MIC)

- Overnight growth of bacterial cultures was suspended in sterile tryptic soy broth (TSB).
- 1 mL of challenge inoculum suspension was combined with 1 mL of treatment per tube for a concentration of approximately 10^6 CFU/mL. 3 replicates were performed per treatment (a).
- Tubes were incubated for 24 hours at 37ºC.
- Degree of turbidity was assessed, and bacterial concentration was quantified by plating serial dilutions of each replicate onto tryptic soy agar (TSA) plates.
- The plates were incubated overnight at 37ºC and CFUs were tabulated to determine the MIC.



Contact Information

Stephen C. Davis / Research Professor

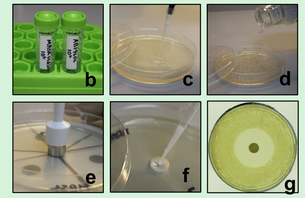
Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery.

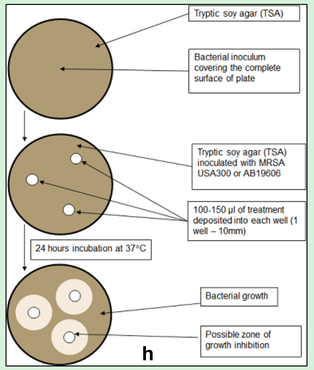
Miller School of Medicine, University of Miami

sdavis@med.miami.edu. Ph: 305.243.4897

4. Zone of Inhibition Assays

- A challenge inoculum with a concentration of 10^6 CFU/mL was prepared by scraping overnight growth of bacterial cultures into TSB (b).
- 100 µL of this inoculum was deposited onto TSA plates and spread evenly with sterile glass beads (c)(d).
- Three punch biopsies (10 mm) were made per plate with the agar within removed to produce wells (e).
- 100-150 µL of each treatment was deposited into these wells (f).
- Treatments was allowed 2-3 hours for diffusion, the plates then were incubated for 24 hours at 37ºC (see diagram h).
- After incubation, zones of bacterial growth inhibition were measured using ImageJ 1.41o software, below example (g).



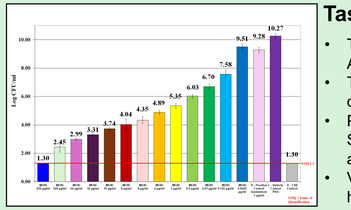


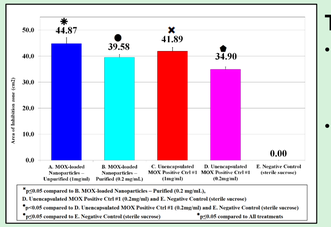
6. Statistical analysis

- Statistical analysis was conducted using one way ANOVA IBM SPSS v29 was performed in Task#2 and Task#3. For Tasks 1 and 4 statistical analysis was not performed.

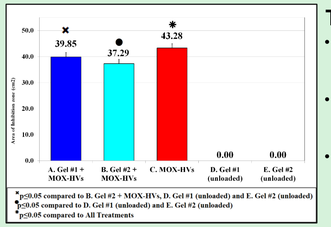
Results

MRSA USA300

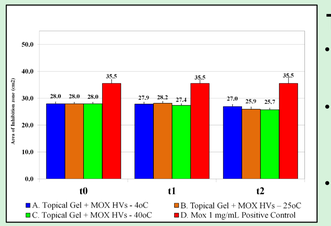




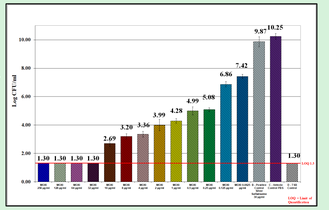
*p≤0.05 compared to B. MOX-loaded Nanoparticles – Purified (0.2 mg/mL),
D. Unencapsulated MOX Positive Ctrl #1 (0.2mg/ml) and E. Negative Control (sterile sucrose)
*p=0.05 compared to D. Unencapsulated MOX Positive Ctrl #1 (0.2mg/ml) and E. Negative Control (sterile sucrose)
*p≤0.05 compared to E. Negative Control (sterile sucrose)
*p≤0.05 compared to All treatments

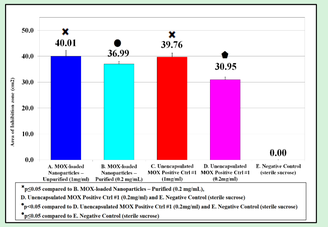


*p≤0.05 compared to B. Gel #2 + MOX-HVs, D. Gel #1 (unloaded) and E. Gel #2 (unloaded)
*p≤0.05 compared to D. Gel #1 (unloaded) and E. Gel #2 (unloaded)
*p≤0.05 compared to All Treatments

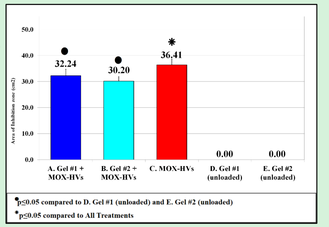


AB19606

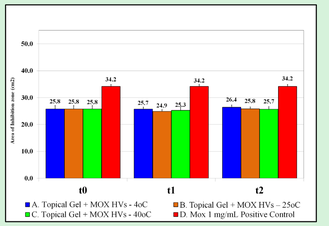




*p≤0.05 compared to B. MOX-loaded Nanoparticles – Purified (0.2 mg/mL),
D. Unencapsulated MOX Positive Ctrl #1 (0.2mg/ml) and E. Negative Control (sterile sucrose)
*p=0.05 compared to D. Unencapsulated MOX Positive Ctrl #1 (0.2mg/ml) and E. Negative Control (sterile sucrose)
*p≤0.05 compared to E. Negative Control (sterile sucrose)



*p≤0.05 compared to D. Gel #1 (unloaded) and E. Gel #2 (unloaded)
*p≤0.05 compared to All Treatments



Task #1 MIC Results

- The MIC for unencapsulated MOX against MRSA was 256 µg/mL. Against AB19606, the MIC was 32 µg/mL.
- The MICs observed reduced bacterial growth below the limit of quantification.
- Positive Controls, Gentamicin (1 µg/mL) against MRSA USA 300 or Silver Sulfadiazine (30 µg/mL) against AB19606, showed 9.28 ± 0.18 and 9.87 ± 0.35 Log CFU/mL, respectively.
- Vehicle control against MRSA USA300 and AB19606 exhibited the highest bacterial 10.27 ± 0.17 and 10.25 ± 0.20 Log CFU/mL, respectively.

Task #2 Encapsulation Results

- Against MRSA, the encapsulated or MOX-loaded nanoparticles produced inhibition zones at both concentrations tested that were significantly ($p \leq 0.05$) greater in diameter compared to zones of unencapsulated MOX.
- The MOX-loaded Nanoparticles at 0.2 mg/mL had significantly ($p \leq 0.05$) larger zones of inhibition (ZOI) against AB19606 than the unencapsulated MOX at 0.2 mg/mL. The greatest inhibition was observed against MRSA with the 1 mg/mL MOX-loaded nanoparticles producing a ZOI of 44.87 cm^2.

Task #3 Gel Vehicle Results

- The MOX-loaded hybrid vesicles (MOX-HVs) in Gel #1 against MRSA produced ZOIs that were significantly ($p \leq 0.05$) larger than those for Gel #2 with Mox-HVs and the Vehicle Control Gels #1 and #2.
- The MOX-HVs in Gel #1 had ZOIs against AB19606 that were larger than those for MOX-HVs in Gel #2 though this difference was not significant.
- The largest ZOIs against both pathogens was observed for treatment group C, with ZOIs of 43.28 cm^2 against MRSA and 36.41 cm^2 against AB19606. These were statistically significant ($p \leq 0.05$) compared to all other treatment groups for both pathogens.

Task #4 Storage Viability Results

- The ZOIs produced by the MOX HVs in Topical Gel at time point 2 (t2 or two weeks) and stored at 4ºC were similar to those produced at t0 also stored at 4ºC against both pathogens.
- The ZOI for the storage at 40ºC was smaller (25.7 cm^2) at t2 against MRSA USA300 compared to storage at 4ºC (28.0 cm^2) but against AB19606, the ZOI after 40ºC at t2, 25.7 cm^2, is more similar to t0 (25.8 cm^2).
- Positive Control MOX 1 mg/mL showed against both organisms similar ZOI (35.5 and 34.2 cm^2, respectively).

Conclusions

- The loading of MOX into exosomes enhanced the antimicrobial efficacy of the antibiotic. The MOX-loaded nanoparticles produced greater zones of inhibition than unencapsulated MOX at identical concentrations and remain effective in a topical gel vehicle or at various storage timepoints and temperatures.
- MOX-loaded hybrid vesicles are a promising treatment for wound infections by both Gram-positive and Gram-negative pathogens.

Acknowledgements

This material is based upon work supported by the Defense Health Agency (DHA) Small Business Innovation Research (SBIR)/Small Business Technology Transfer (STTR) Programs under U.S. Army Medical Research Acquisition Activity (USAMRAA) Contract No. HT9425-24-P-0035. Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the Department of Defense, DHA, SBIR/STTR Programs, or USAMRAA.