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TITLE: Apyrase: A Portable Treatment to Prevent Burn Progression and Infection

PRINCIPAL INVESTIGATOR: Stewart C. Wang, MD, PhD

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Definitive treatment of burns often requires surgical excision and grafting. However, the facilities and personnel needed for this may not be acutely available in the combat casualty care arena. This creates the need for interim care strategies that would promote healing and prevent infection until more definitive treatment can be provided. Topical apyrase, an adenosine triphosphate (ATP) hydrolyzing enzyme, has local anti-inflammatory and anti-microbial characteristics that proved beneficial in our preliminary studies. We hypothesize that topical application of apyrase to burn wounds will reduce inflammation, minimize wound progression,
and eliminate infection without local toxicity. In the first aim of the study, we developed a porcine model of partial thickness burn injury to compare the effectiveness of two dosages of apyrase with a standard method of treatment and the in vivo work was finished in this annual reporting period. Serial biopsies, wound measurements and photographs were taken over time to assess inflammation and healing responses. Final results are pending. Work on Specific Aim II involving infected burn wounds began in this reporting period as well. Optimal bacterial growth conditions were defined and inoculum size determined for the infection experiments. Wounds were infected and treatments applied one day after burn and then daily for 4 days. Most notably, blinded, assessments of wound characteristics suggest that infected wounds treated with apyrase more closely resemble uninfected wounds at Day 3 post burn than do infected burns treated with either saline of sulfamylon. The results of culture and biofilm studies will provide more quantitative assessments and will be available at the conclusion of the in vivo studies.

15. SUBJECT TERMS
Inflammation, Thermal, Healing, Antimicrobial, Enzyme, Infection, Biofilm

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

In the management of thermal injury, the major goals of initial, non-surgical treatment are reduction of local inflammation, prevention of wound progression, and inhibition of bacterial infection. However, at this time, there are no effect treatments to reduce wound progression and the emergence of resistant bacteria has threated the efficacy of antibiotic therapy. In this study, we propose that apyrase, an ATP hydrolytic enzyme, would fulfill the unmet need for an effective topical treatment for burn injury. Excessive extracellular ATP (eATP) released from injured tissues acts as a danger-associated molecular pattern, triggering inflammatory responses, and eATP also promotes biofilm formation in several strains of bacteria. Apyrase hydrolyzes ATP to ADP and phosphate which has effectively controlled the inflammatory response in our previous work in mouse models of thermal injury and associated complications. This current study will further examine the use of apyrase in a relevant porcine model. The study is designed to quantify healing, inflammation, and bacterial infection of burn wounds to compare the efficacy of apyrase with that of controls and standard of care topical therapy. The application of apyrase will be tested in partial thickness burns and repeated in burns with concurrent bacterial contamination. Outcome measures to be assessed include gross wound characteristics, histology, inflammation, and biofilm content. These measures will be used to assess the known anti-microbial, anti-inflammatory, and pro-healing effects of apyrase and act as a step towards translation of this treatment into burn wound therapy. Ultimately the goal is to improve recovery time, reduce costs, and improve outcomes for many burn patients.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Inflammation, Thermal, Healing, Antimicrobial, Enzyme, Infection, Biofilm

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 1: Demonstrate that topical apyrase decreases inflammation and wound expansion of partial thickness burns.
Subtask 1: UCUCA Approval: Completed
Subtask 2: Burn analysis for inflammation and progression: pending
Subtask 3: Conduct design of experiments analysis using pig model: Completed
Aim 2: Validate the anti-microbial properties of topical apyrase in partial-thickness burns.
Subtask 1: Perform burn model with gram negative and gram positive infection: 40% completed
Subtask 2: Burn analysis of inflammation and progression: pending
Subtask 3: Quantify bacterial load, biofilm and data analysis:pending

What was accomplished under these goals?

Specific Aim 1: Demonstrate that topical apyrase decreases inflammation and wound expansion of partial thickness burns.
During year 2, we completed all the in vivo work for this specific aim. Measurements of wound contraction and epithelialization were recorded and analyzed. Histology samples have been processed and final analysis is pending.

Description of methods used: Pigs (25-30kg, female Yorkshire-cross, pigs were acclimated to facilities for at least 5 days. The pigs were sedated with telazol/xylazine and maintained with isoflurane via face mask. Analgesia was provided with one pre-emptive buprenorphine injection and placement of a buprenorphine patch prior to procedure. Eight burn wounds were created with a heated metal block (5 x 5cm, 80°C). The corners of the burns were tattooed. Treatments were randomly assigned to wounds on either side of the animal and applied with a spray bottle (saline, sulfamylon, 0.5 Units apyrase and 1.0 Units Apyrase). The wounds were covered with telfa pads and Tegaderm followed by padding, protective bandaging and a jacket. Bandages were changed and treatments re-applied on post-burn days 1, 2, 3, 4, 7, and 14. On days 1,3,7, 14 and 21 post-burn, two (3mm) biopsies were obtained (one located centrally and one in a corner of the wound) and wounds were swabbed for ATP analysis. The animals were euthanized on Day21 and the entire wound resected for histology.

Key Findings or Accomplishments:
Wound size: Wounds edges were measured between tattoo marks at the corners of wounds. Measurements taken immediately after thermal injury and those taken on Day 21 were compared for each wound. The percent change in wound size was negligible over the time of the study and showed no difference between treatment groups. This was not surprising in that a significant amount of wound contraction was not necessarily expected from this particular depth of injury. on each wound.

Epithelialization: Photographs were obtained at each time point. Using Image J software, the burned area demarcated by the tattooed marks was outlined and the area of the wound determined. The area of open wound was then determined by outlining the demarcation between ingrowing epithelium and granulation tissue. The difference between the two areas was determined and expressed as a percentage of the burned area. The mean percent change in wound covered by epithelium was slightly higher in apyrase treated wounds compare to those treated with sulfamylon; however, this difference was not significant (Figure 1). It should be noted that this measurement may be confounded by the tendency of the fragile cell layer to be disrupted during bandage removal.

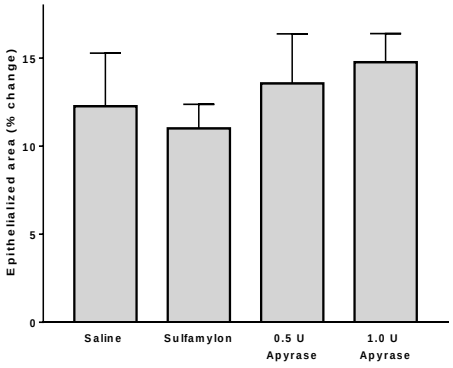


Figure 1. Assessment of epithelialization. The difference between total wound area and wound uncovered by epithelium was determined with Image J software. The difference was then expressed as a percentage of the total wound area.

Histology: Final histology results are pending. The analysis will include routine histologic evaluation as well as special staining for cell proliferation.
Dosing: All preliminary indications suggest no differences in results from the use of either the high or low dose of apyrase. Therefore, the lower dose will be evaluated in the studies of infected wounds.

Aim 2: Validate the anti-microbial properties of topical apyrase in partial-thickness burns. In year two, we began the studies in this aim to determine the effects of apyrase on infected wounds. In vitro studies were performed to determine the log phase growth of the bacteria and thus the best culture condition. Since these studies are ongoing, the results of culture, biofilm and histology analysis are pending.

Description of methods used: The methods for these studies were identical to those above with some exceptions. After initial burn wound, the wounds were dressed without treatment. The following day, either an inoculum of log phase bacteria or equal volume of saline was placed on the surface of the wound. The surface of the wound was allowed to dry for 15 minutes followed by application of randomized treatments as described above. On days designated for biopsy harvest, a total of three, 3mm biopsies were obtained, one from the corner of each wound was placed in formalin for histology, two from the center of each wound were placed in sterile vials for quantitative culture and biofilm analyses.

Key Findings or Accomplishments:
Visual assessment of wound isolation: Wounds were covered by individual pieces of tegaderm then covered by larger pieces of overlapping tegaderm. Assessment of the wound coverings were assessed at each bandage change. It appeared that individual, small pieces of tegaderm remained in place over each wound and wounds were isolated during the initial infective stage. As more randomized sets of wounds are performed, the impact of randomization of infected and uninfected wounds will be further evaluated to determine if and when cross contamination would be evident.

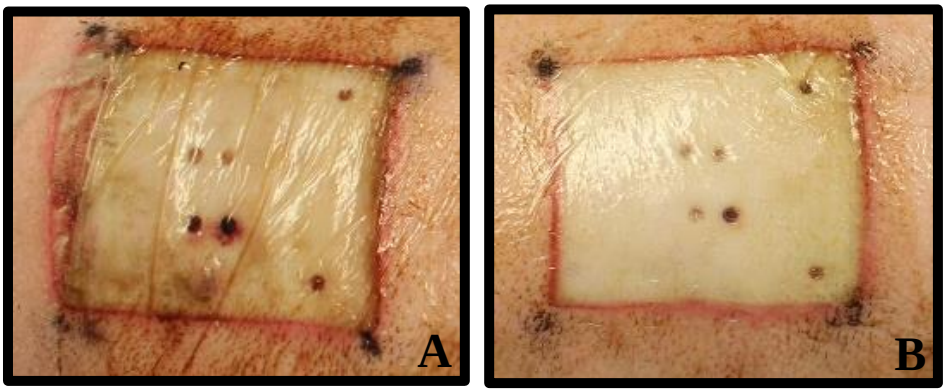


Figure 2: Visual characteristics of infected wounds. On day 1 post-burn, wound surfaces were exposed to an inoculum of Staph. aureus, followed by treatment with topical sprays. Representative wounds from Day 4 post-burn: (A) Wound treated with sulfamylon (B)Wound treated with 0.5U Apyrase.

Preliminary results: The infected wound studies are ongoing and the definitive results of biofilm and histology assays are pending. However, wound characteristics were assessed by blinded observers. In particular, the amount of erythema, discoloration, discharge and overall appearance of the wounds were observed. This qualitative evaluation suggests that the size of inoculum of bacteria (Staph aureus, 1 $x10^6$) was effective in creating a wound infection. Compared to uninfected wounds, the infected wounds were characterized by greater erythema at the wound margin, fragile tissue and greater bleeding during biopsy and brownish discharge on the surface. Infected wounds treated with sulfamylon appeared similar to those treated with saline (Figure 2). However, wounds treated with apyrase had less discharge and less erythema at the edges of the burn. Differences in wound characteristics were particularly evident on Day 4 post-burn (3 days after infection and first application treatment). Some differences were still noted on Day 7 but wounds appeared similar on Days 14 and 21. This suggests that the effects of apyrase on wound infection may be greatest with early application and that continued daily application may be beneficial.

Goals not met:
We had hoped to have one cohort of infection studies completed by the end of year two. Renovations to animal housing in order to expand the ABSL2 large animal housing and procedure space delayed progress. However, the expansion and addition of procedure space will allow more rapid progress and efficient completion of the in vivo work.

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

In the final year of these studies, we will finish the histology and reporting of result for Specific Aim 1. The major thrust of the work will be to complete Specific Aim 2: Validate the anti-microbial properties of topical apyrase in partial-thickness burns. This will entail finishing the animal studies of wounds infected with Staph aureus and with A. baumanni. Wound assessments

include: observational, histological and biofilm assessment. The methods for these studies have been confirmed and therefore an uncomplicated completion is anticipated.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Preliminary results suggest that apyrase may be most effective at reducing infection in contaminated wounds. This could impact the early treatment of wounds under austere conditions when definitive treatment is delayed.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Some delays in animal experiments were described in the previous annual report. Those experiments were completed in year two. The work anticipated for year two had some delay due to renovation of appropriately classified animal housing. No further delays are expected.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**

None

Books or other non-periodical, one-time publications.

None

Other publications, conference papers, and presentations.

None

- **Website(s) or other Internet site(s)**

None

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

None

- **Inventions, patent applications, and/or licenses**

None

- **Other Products**

None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Stewart Wang, MD, PhD	No Change
Jean Nemzek, DVM, MS	No Change
Benjamin Levi, MD	No Change
Chuanwu Xi, MD	No Change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

Single PI report

9. APPENDICES

Appendix A: quad chart

Apyrase: A Portable Treatment to Prevent Burn Progression and Infection

MB150237
W81XWH-16-1-0574

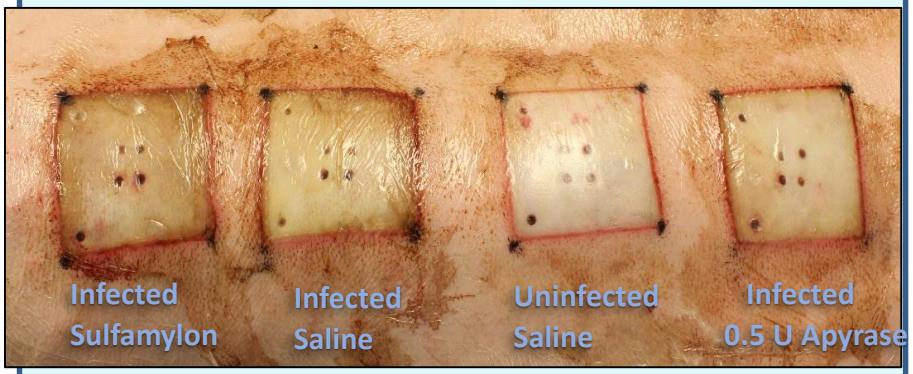
PI: Wang, Stewart C. **Org:** University of Michigan **Award Amount:** $750, 000

Study/Product Aim(s)

- Specific Aim I: Demonstrate that apyrase decreases inflammation and wound expansion of partial thickness burns without toxicity.
- Specific Aim II: Validate the anti-microbial properties of apyrase in partial-thickness burns.

Approach

Apyrase will be tested in a porcine model of multiple partial thickness burns produced by standardized thermal contact. First, an optimal dose will be determined by comparing inflammation and wound progression after treatment with apyrase. The optimal dose will be evaluated for evidence of local and systemic toxicity. Finally, the antimicrobial effects of apyrase will be tested in burns infected with bacteria.



Accomplishment: Representative wounds three days after infection with Staphylococcus aureus, treated with topical sprays of saline, sulfamylon or apyrase.

Timeline and Cost

Activities CY	16	17	18	19
Demonstrate effectiveness				
Validate anti-microbial properties				
Infection studies				
Data analysis & Preparation for possible clinical trials				
Estimated Budget ($K)	**$250**	**$250**	**$250**	

Updated: 03/16/2018

Goals/Milestones

CY17 Goal – Demonstrate topical apyrase decreases inflammation and wound expansion of partial thickness burns

100% Animal Use Approval (completed 11/16)
100% Design of experiment analysis using pig model
90% Burn analysis for inflammation progression

CY18 & Goal – Validate the anti-microbial properties of topical apyrase in partial-thickness burns

30% Perform burn model with gram negative/positive infection
20% Burn analysis of inflammation and progression

CY19 Goal – Infection studies and future directions

20% Quantify bacterial load, biofilm and data analysis

Comments/Challenges/Issues/Concerns

No budget or other concerns at this time

Budget Expenditure to Date

Projected Expenditure: $500,000.00
Actual Expenditure: $248,448.55