AWARD NUMBER: W81XWH-20-1-0474

TITLE: Use of Tranexamic Acid to Reduce Tissue Edema and Prevent Burn Wound Conversion

PRINCIPAL INVESTIGATOR: Damien Carter, MD

CONTRACTING ORGANIZATION: Maine Health, Portland, ME

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TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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Use of Tranexamic Acid to Reduce Tissue Edema and Prevent Burn Wound Conversion

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Damien Carter, MD

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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Approved for public release; distribution is unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The purpose of the experiments performed during the past year was to elucidate the effect of tranexamic acid (TXA) on burn wound conversion in a rat burn wound conversion model. The comb burn injury model is well validated in the literature for this purpose – particularly at 7 days post injury which represents the gold standard for this experiment. Our studies clearly demonstrated that burn wound conversion was reduced by treatment of rats with intraperitoneal (IP) TXA at this time point. Furthermore, TXA demonstrated efficacy at 24, 48 and 72 hours after comb burn injury. Additionally, we were able to demonstrate that less mitochondrial DNA – an established marker of tissue damage – was reduced at 24 and 48 hours. This finding indicates that TXA has significant cell protecting activity.

These findings clearly demonstrate that Tranexamic acid reduces burn wound conversion and has cell protective properties in the rat burn wound conversion model when compared to no treatment. Future experiments will evaluate these properties in the context of severe burn injury. Current findings suggest TXA may be an ideal therapy for burn injured warfighters in the forward deployed setting.

15. SUBJECT TERMS
Tranexamic acid, Burn wound conversion, comb burn, wound healing, zone of stasis.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
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Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

1. INTRODUCTION

Burn wound conversion is the process where superficial partial thickness burns convert into deep partial and full thickness burn injuries. This conversion process increases morbidity and often requires surgical intervention to achieve timely wound healing. Thus, therapeutic interventions that may prevent secondary progression and cell death in burn-injured tissue is desirable. Recent work by our group and others has established that tranexamic acid (TXA) has significant anti-inflammatory properties that may ameliorate the root causes of burn wound conversion. **Hypothesis**: TXA treatment of burn injuries will reduce burn wound progression and cutaneous tissue and organ edema.

2. KEYWORDS

Burn wound conversion, Tranexamic acid, SIRS, comb burn, tissue edema

3. ACCOMPLISHMENTS

What were the major goals of the project? (Goals to be accomplished and status.)

Specific Aim 1: Determine the effect of TXA on burn wound (months 1-21)

- STATUS: started Y1Q1, completed Y1Q3.
- During Y1Q4, we completed tissue processing with multiple time points demonstrating efficacy of TXA to attenuate burn wound conversion. We have also begun the acquisition of materials for the second experiment (30% TBSA burn + comb burn). This includes the custom production and acquisition of larger brass combs. This model will address specific Aims 2 & 3 over the next 2 years of the study.

Specific Aim 2: Assess the capacity of TXA to attenuate burn-induced tissue edema (months 13-30)

- STATUS: yet to start

Specific Aim 3: Determine the effects of TXA on burn induced SIRS and assess its ability to improve mitochondrial function after burn (months 13-36)

- STATUS: yet to start

What was accomplished under these goals? (Detailed progress and results.)

Specific Aim 1: Determine the effect of TXA on burn wound (months 1-21)

All experiments were completed and reported as preliminary data in the Y1Q3 report. During Q4, We completed processing of harvested tissues and completed data analysis. This more complete data set confirmed our preliminary findings. Our data demonstrate a benefit of intraperitoneal administered TXA in ameliorating burn wound conversion in the comb burn model at 48hrs, 72 hrs and 7 days (figure 1) post-injury when compared to no treatment.

Key Findings or Accomplishments:

Findings: Briefly, based on H&E and photographic evaluation of the ischemic zones, there appears to be a benefit to TXA administration. Ischemic zones had less evidence of necrosis visually and microscopically. During Q4, we also found that IHC evaluation confirmed these results. We have submitted an abstract to the 2021 Military Health System Research Symposium which was accepted for oral presentation.

Specific Aim 2: Assess the capacity of TXA to attenuate burn-induced tissue edema (months 13-30)

N/A

Specific Aim 3: Determine the effects of TXA on burn induced SIRS and assess its ability to improve mitochondrial function after burn (months 13-36)

N/A

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

Nothing to report.

How were the results disseminated to communities of interest?

Presentation of these results is forthcoming at the MHSRS meeting in August. We anticipate drafting and submission of a manuscript based on this work in September.

Plans for the next reporting period to accomplish the goals

We plan to begin experiment #2 (30% TBSA burn + comb burn) in the latter part of Y2Q1 after acquisition of new custom brass combs and other critical materials. This experiment is novel and will evaluate TXA impact on burn wound conversion in the setting of a severe burn injury. Preliminary findings for experiment #2 (addressing specific aims 2 and 3) are anticipated in Y2Q2 and Y2Q3.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Our findings provide further evidence of the anti-inflammatory properties of tranexamic acid. Additionally, these properties may have significant clinical effect beyond the well-known anti-fibrinolytic properties of this drug. Examination of TXA effects in more severe burn injuries is forthcoming.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS

IMPORTANT REMINDER – Award recipient organization is required to obtain prior written approval from the awarding agency Contracting/Grants Officer whenever there are significant changes in the project or its direction such as significant change in scope or the Statement of Work (e.g. removal, change, or addition of aims/tasks or animal model change), change in PI or key personnel, reduction of 25% FTE, or significant change in budget.

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

We are currently on track with our planned progress and statement of work.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects

Not applicable.

Significant changes in use or care of vertebrate animals

TOTAL PROTOCOL(S): 1

PROTOCOL (X of Y total):
IACUC Protocol Number: 2001
ACURO Protocol Number: MB190064.e001
Protocol PI: Damien Carter, MD
Protocol Site: MaineHealth
Protocol Title: Use of Tranexamic Acid to reduce tissue edema and prevent burn wound conversion
Number of Animals Approved for Use: Write Number
IACUC INITIAL APPROVAL DATE: 03/13/2020 (expires 03/13/2023)
ACURO INITIAL APPROVAL DATE: 05/13/2020
RENEWAL APPROVAL DATES:
- Due 03/13/2023

AMENDMENTS:
- Amendment 1 approved 05/13/2020 changed (please complete)

ADVERSE EVENTS OR UNANTICIPATED PROBLEMS:
- None.

Significant changes in use of biohazards and/or select agents

Not applicable.

6. PRODUCTS

Journal publications

Nothing to Report.

Books or other non-periodical, one-time publications

Nothing to Report.

Other publications, conference papers, and presentations

1. Carter D, Prudovsky I, Kacer D, Rappold J, Sheppard F. Aug 2021. *Tranexamic Acid Reduces Burn Wound Conversion in a Murine Comb Burn Model*. Military Health System Research Symposium, Kissimmee, FL.
    a. Invited talk (oral presentation)
    b. Accepted
    c. Directly related to SOW, specific aim 1
    d. DoD funding acknowledged

Website(s) or other Internet site(s)

Nothing to Report

Technologies or techniques

Nothing to Report

Inventions, patent applications, and/or licenses

Nothing to Report.

Other Products

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Damien Carter
Project Role: PD/PI
Researcher Identifier:
Nearest person month worked: .43 (Based on salary cap)
Contribution to Project: Dr. Carter supervises the entire project, designs the studies and performs animal experiments.

Name: Igor Prudovsky
Project Role: Co-PD/PI
Researcher Identifier:
Nearest person month worked: 1.13
Contribution to Project: Dr. Prudovsky is responsible for design and supervision of biochemical and histological studies. He performed animal experiments together with Dr. Carter.

Name: Doreen Kacer
Project Role: Research Associate
Researcher Identifier:
Nearest person month worked: 1.75
Contribution to Project: Responsible for performing biochemical and histological studies.

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

QUAD CHART

Convert this report to a PDF file and append updated quarterly Quad Chart in PDF as an appendix.

Use of Tranexamic Acid to Reduce Tissue Edema and Prevent Burn Wound Conversion

Proposal # MB190064, Award # W81XWH-20-1-0474

PI: Damien Wilson Carter, MD **Org:** MaineHealth **Award Amount:** $500,000

Study/Product Aim(s)

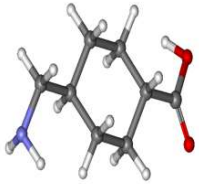
• Burn wound conversion is the process where superficial partial thickness burns convert into deep partial and full thickness burn injuries. This conversion process increases morbidity and often requires surgical intervention to achieve timely wound healing.
• Thus, therapeutic interventions that may prevent secondary progression and cell death in burn-injured tissue is desirable.
•Recent work by our group and others has established that tranexamic acid (TXA) has significant anti-inflammatory properties that may ameliorate the root causes of burn wound conversion.
Hypothesis: TXA treatment of burn injuries will reduce burn wound progression and cutaneous tissue and organ edema.
Aims:.1. Determine the effect of TXA on burn wound conversion; 2. Assess the capacity of TXA to attenuate burn-induced tissue edema; 3. Determine the effects of TXA on burn-induced SIRS and mitochondrial dysfunction.

Approach

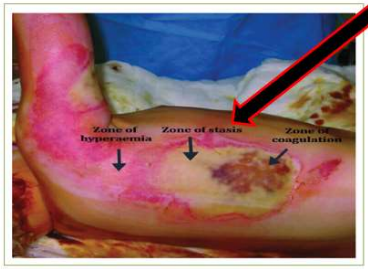
Wild type Sprague-Dawley rats will be subjected to the following types of burn: 1. comb burn injury; 2. comb burn injury + an adjacent 30% TBSA burn; 3. 60% TBSA scald burn injury. **For each type of burn, four experimental groups will be studied: (i) sham; (ii) burn, no treatment; (iii) burn, TXA given once post-injury, and (iv) burn, TXA given daily until sacrifice**. Tissues will be analyzed by histology and immunohistochemistry to determine if TXA effectively reduces burn wound conversion. Additional analysis will evaluate TXA effect on tissue edema formation, mitochondrial health and burn-induced systemic inflammation.

Experimental Groups	No treatment (#)	Single dose TXA(#)	Daily TXA(#)
Sham	(6)	(6)	(6)
Comb burn injury	(6)	(6)	(6)
30%TBSA Burn + Comb burn injury	(6)	(6)	(6)
60%TBSA Burn	(6)	(6)	(6)

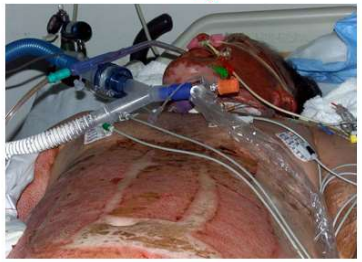
Sacrifice at 12h, 24h, 72h, 7days



Tranexamic Acid



Preserves the zone of stasis



Reduces edema and burn-induced SIRS

Timeline and Cost

Activities CY	20	21	22	23
Approval of animal procedures Preparation for experiments				
Study of TXA effects on comb burn injury				
Study of TXA effects on comb burn + adjacent 30% TBSA injury				
Study of TXA effects on 60% TBSA scald burn injury				
Estimated Budget ($500K)	**$166,538**	**$166,667**	**$166,795**	

Updated: 7/4/2021

Goals/Milestones

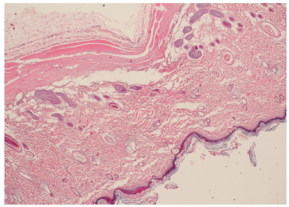
CY20 Goal – Preparation for experiments, and initial studies
CY21 Goals –Elucidation of TXA effects on animals with light and intermediate burns
CY22 Goal – Elucidating TXA effects in animals with intermediate and severe burns
CY23 Goal –Finalizing the study and preparing the report and publications
☐Finish the studies of TXA effects in animals with severe burns
☐Prepare the final report
☐Prepare the application for clinical studies of TXA effects on burn.
☐**Comments/Challenges/Issues/Concerns**

- Experiment #1 Completed. Manuscript preparation underway.
- Abstract accepted for oral presentation (MHSRS; Aug 2021).

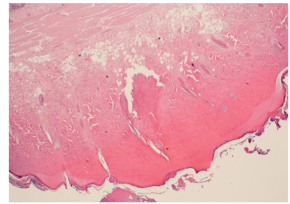
Budget Expenditure to Date
Projected Expenditure: $166,538.00
Actual Expenditure: $126,826.23

9. APPENDICES

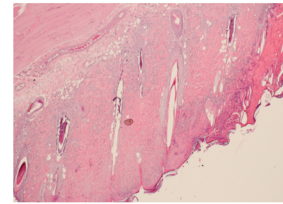
A



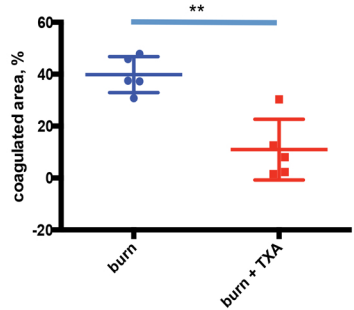
control



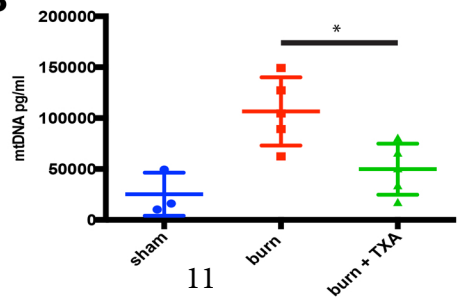
burn



burn + TXA



B



Tranexamic Acid Reduces Burn Wound Conversion in a Murine Comb Burn Model

***Background*:** Burn wound conversion is the observed process where superficial partial thickness burns convert into deep partial and even full thickness burn injuries. This conversion process often requires surgical excision to achieve timely wound healing. Unfortunately, the pathophysiology of this phenomenon is multifactorial and poorly understood. Thus, therapeutic interventions that may prevent secondary progression and cell death in burn-injured tissue is desirable. Recent work by our group and others has established that tranexamic acid (TXA) has significant anti-inflammatory properties in addition to its well-known anti-fibrinolytic effects. Interestingly, our research has shown that TXA improves mitochondrial energetics, which may reduce the loss of injured, but viable skin (zone of stasis). This study investigates tranexamic acid as a novel therapeutic treatment to mitigate burn wound conversion.

Methods: Sprague-Dawley rats were subjected to a hot comb burn contact injury using a 150-g brass comb preheated to 100°C, to create four rectangular burns, separated by three unburned interspaces. The interspaces represent the ischemic zones simulating the zone of stasis. The treatment group received IP injection of TXA (100mg/kg) immediately after injury and once daily until sacrifice. Sham animals underwent an identical procedure, with application of a room temperature comb. Animals were sacrificed at 24hr, 48hr, 72hr and 7 day time points. Photo images were obtained of the comb burn injury. Full-thickness biopsies from the interspaces were evaluated with Hematoxylin and Eosin staining and the percent of skin sections area showing tissue coagulation has been calculated. In addition, qPCR was used to determine the release of mitochondrial DNA (mtDNA), which is a major damage associated molecular patter, into plasma.

Results: The percentage of unburned interspaces undergoing necrosis was significantly reduced in the treatment group when compared with untreated burn controls by photographic image analysis. When compared with controls, the treatment groups had significantly less progression of interspaces to necrosis when assessed by standard microscopy. We also found that at early time points after burn TXA decreases the release of mtDNA to plasma indicating its cell protecting activity.

Conclusion: Animals treated with tranexamic acid demonstrated reduced burn wound conversion. TXA may be an ideal therapy for burn injured warfighters in the forward deployed setting.

Figure legends

A. TXA strongly decreases tissue coagulation in the skin of burned rats. Haematoxylin/Eosin stained sections of rat skin, 7 days after burn were studied. The percentages of coagulated tissue area were compared in mice daily treated and untreated with TXA.

B. TXA decreases the presence of mtDNA in the plasma of burned rats. mtDNA content was determined by qPCR in rat plasma, 24h after burn.