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: MaineHealth, Portland, ME

REPORT DATE: July 2022

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

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1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2022	Annual	15Jun2021-14Jun2022

4. TITLE AND SUBTITLE

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

5a. CONTRACT NUMBER
W81XWH-20-1-0474

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Damien Carter, MD

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

MaineHealth
22 Bramhall Street
Portland, ME 04102

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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

10. SPONSOR/MONITOR'S ACRONYM(S)
USAMRDC

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The purpose of the experiments performed during the first 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 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. In the second year of the project we completed experiment two – a comb burn model in the setting of a 30% TBSA burn injury. Similarly, we confirmed that the beneficial effects on burn wound conversion persist even in the more severe burn injury model (see appendix). Additionally, we evaluated the effect of TXA on tissue repair in the burn wound conversion zones in the severe burn model and found that TXA accelerated wound healing. Finally, we evaluated capillary leak, immune cell infiltration in the lungs as well as systemic cytokine and DAMP expression and found that all were reduced with TXA treatment (see appendix). The third year will focus on mitochondrial effects of TXA in a 60% TBSA burn model.

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
					USAMRDC
a. REPORT	b. ABSTRACT	c. THIS PAGE	Unclassified	15	19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified			

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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, capillary leak, 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 conversion (months 1-21)

- STATUS: started Y1Q1, completed Y1Q4.

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

- STATUS: Started Y2Q1, Completed Y3Q1.
- All experiments completed for this aim as of July 10, 2022. The remainder of tissue processing and analysis will be completed by the end of Y3Q1.
- We have also begun the acquisition of materials for the third experiment (60% TBSA scald injury). This includes updates to the MRI scanner for perfusion studies. This model will address specific Aim #3.

Specific Aim 3: Determine the effects of TXA on burn induced SIRS and assess its ability to improve mitochondrial function after burn (months 23-36)

- 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 will combine this data with the findings of experiment #2 and begin writing a manuscript for publication in a major journal in Fall 2022.

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

All experiments completed for experiment #2 which addresses specific aim 2 and partially specific aim #3. We are analyzing this data and plan to combine with our year 1 data set to present our unified findings related to TXA and its ability to reduce burn wound conversion. We plan to publish these results in Fall 2022. Preliminary data presented as an appendix to this report. Briefly, TXA effectively reduced burn wound conversion even in this more severe injury model. IHC evaluation of the zone of stasis exhibited significantly more evidence of tissue repair as measured by proliferation and regeneration markers. Finally, TXA clearly reduces both lung tissue edema and capillary leak.

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?

We anticipate drafting and submission of a manuscript based on this completed work in Fall 2022. Additionally, we have done oral presentations of this data at the Shock society annual meeting (June 2022) and the North American Burn Society annual meeting (February 2022)

Plans for the next reporting period to accomplish the goals

We plan to begin experiment #3 (60% TBSA scald burn) in the latter part of Y3Q1 after acquisition of new custom immersion mold and other critical materials. This experiment is novel and will evaluate TXA impact on mitochondrial biogenesis, mitophagy and mitochondrial dysfunction in the setting of a severe burn injury. Preliminary findings for experiment # 3 are anticipated in Y3Q2. Final publishable results anticipated to be completed by end of project Y3Q4. dca

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 have significant clinical effect beyond the well-known anti-fibrinolytic properties of this drug and include burned-tissue repair, reductions in the SIRS response and reduced capillary leak. Further studies will elucidate the mechanism of action in addition to the impact on mitochondrial biogenesis and dysfunction.

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

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
2. Carter D, Prudovsky I, Kacer D, Sheppard F. Feb 2022. *Tranexamic Acid Reduces Burn conversion in a severe burn model*. North American Burn Society Annual meeting, Telluride CO. Invited talk (Oral

presentation). Directly related to SOW, specific aims 1&2. DOD funding acknowledged.

3. Carter D, Prudovsky I. June 2022. *Tranexamic Acid and Thermal Injury*. Shock Society annual meeting, Toronto (ON), Canada. Plenary presentation (Oral presentation and panelist). Directly related to SOW, specific aim 1&2.

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: .50 (Based on salary cap of)
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: 2.4
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: 1.68
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.

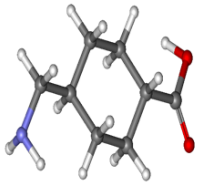
Aims:.1. Determine the effect of TXA on burn wound conversion; 2. Assess the capacity of TXA to attenuate burn-induced tissue edema; 3. Determine the effects of TXA on burn-induced SIRS and mitochondrial dysfunction.

Approach

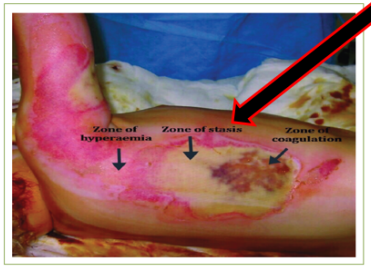
Wild type Sprague-Dawley rats will be subjected to the following types of burn: 1. comb burn injury; 2. comb burn injury + an adjacent 30% TBSA burn; 3. 60% TBSA scald burn injury. **For each type of burn, four experimental groups will be studied: (i) sham; (ii) burn, no treatment; (iii) burn, TXA given 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)

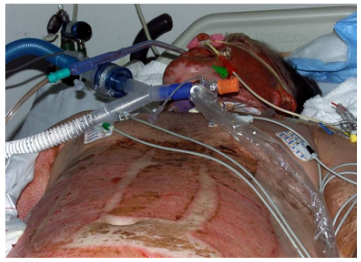
Sacrifice at 12h, 24h, 72h, 7days



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: 7/14/2022

Goals/Milestones

CY20 Goal – Preparation for experiments, and initial studies

CY21 Goals –Elucidation of TXA effects on wound conversion with animals and small burns

CY22 Goal – Elucidating TXA effects in animals with intermediate and severe burns in context of burn wound conversion

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. All tissue analysis completed.
- Experiment #2. Completed all experiments. Y2Q4. Draft manuscript by Y3Q1

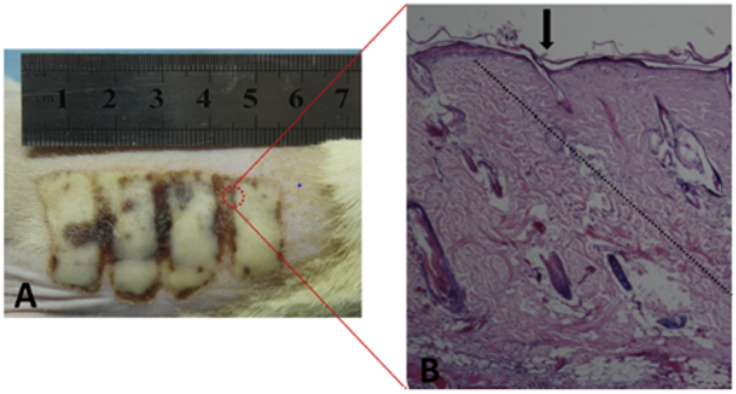
Budget Expenditure to Date

Projected Expenditure: $333,205

Actual Expenditure: $297,552.44

9. APPENDICES

Evaluation of burn wound conversion



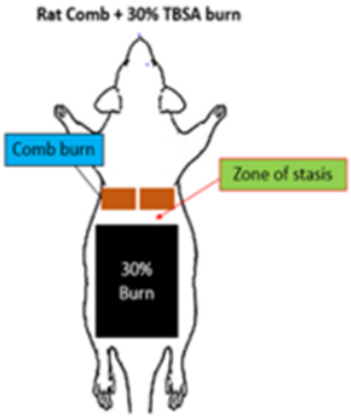
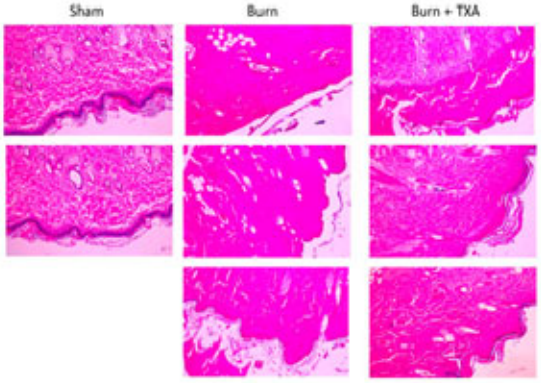
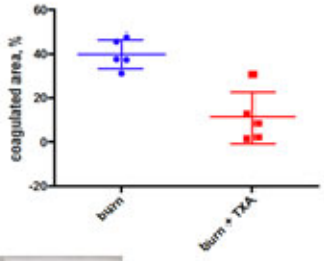


Figure 5. Schematic of rat comb injury with 30% TBSA burn

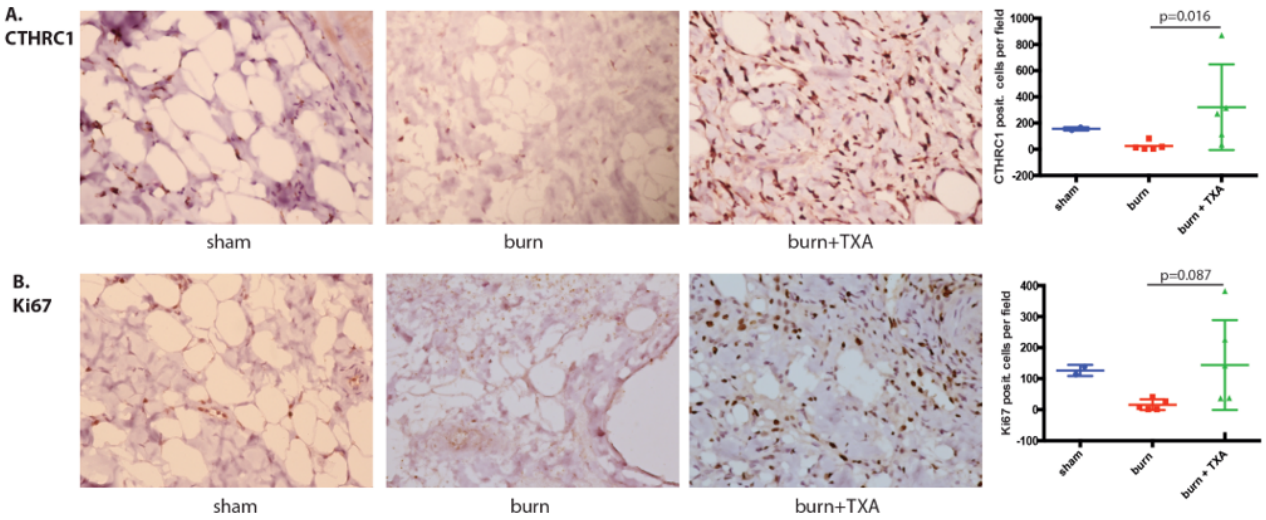
<u>Two experimental models</u>
10% TBSA comb burn
30% TBSA contact burn + comb burn

HE staining of skin sections demonstrates that TXA decreases burn conversion areas 7 days after 30% burn



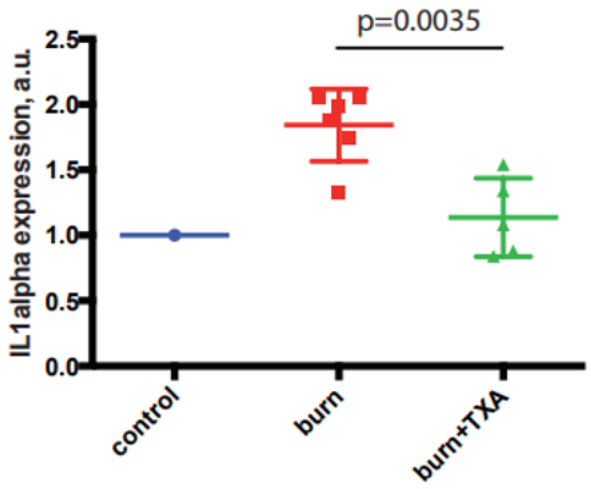


Daily TXA injections increase the presence in dermis of cells positive for CTHRC1 (regeneration marker) and Ki67 (proliferation marker).

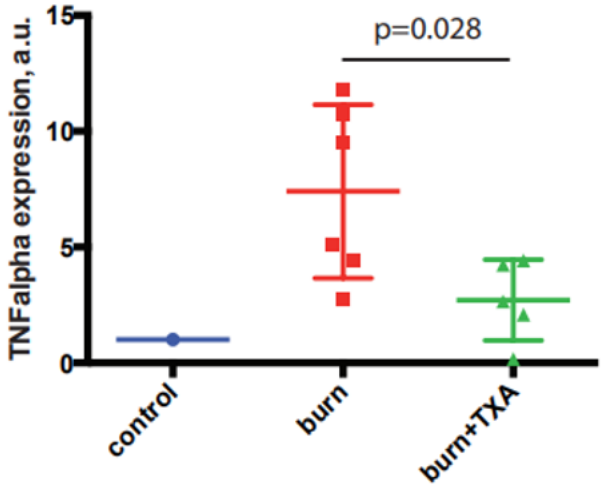


10% TBSA comb burn

TXA decreases the levels of pro-inflammatory cytokines TNFα and IL1α in rat hearts. 6h after 30% burn.

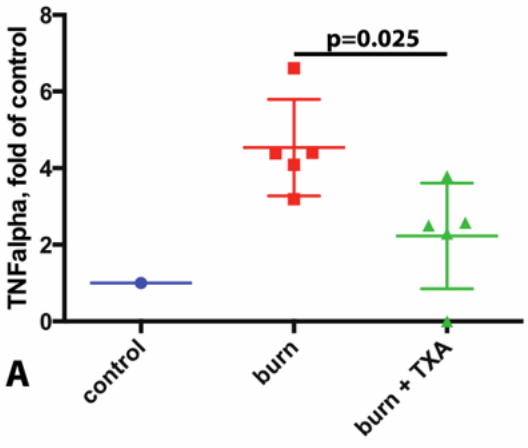


IL1alpha

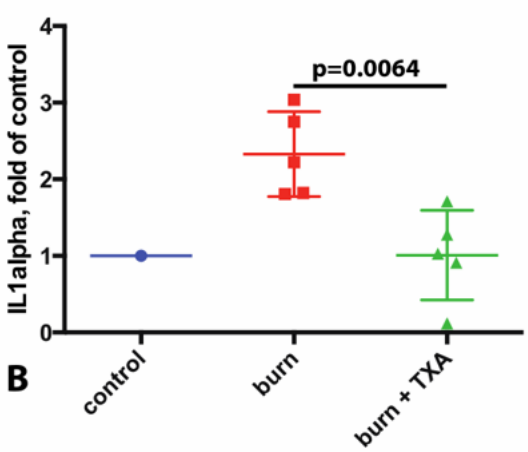


TNFalpha

TXA decreases the levels of TNFα in livers and IL1α in kidneys . 6h after 30% burn.

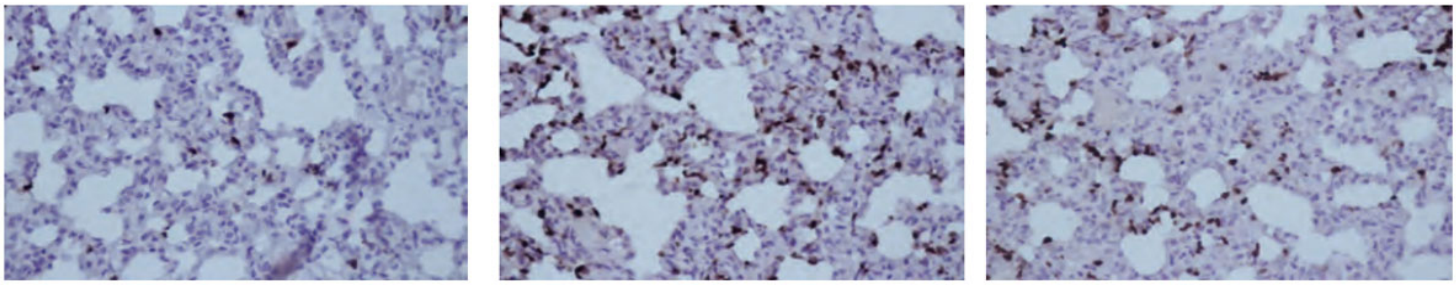


TNFα, liver



IL1α, kidney

TXA decreases the burn-induced invasion of neutrophils to lungs. 6h after burn.
Myeloperoxidase immunohistochemistry. Counterstaining with hematoxylin.



control burn burn+TXA

30% TBSA Burn model

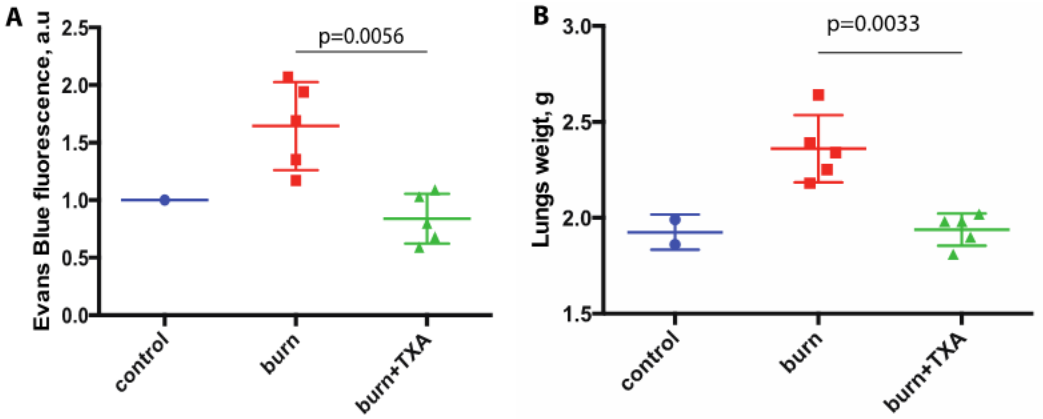


Figure 1. TXA treatment suppresses vascular leakage and edema caused by severe burn injury. Sprague-Dawley rats underwent 35% TBSA burn injury. In an experimental group the burn was immediately followed by the intraperitoneal injection of TXA (100 mg/kg weight). Six hours after burn, rats received the intravenous injection of Evans blue, followed in 30 min by sacrificing (isoflurane anesthesia and cardiac puncture) and perfusion of cardiovascular system and lungs. Lungs were removed, weighed, and Evans Blue was extracted from lungs with formamide. Evans Blue fluorescence was measured at 620 nm excitation and 670-690 nm emission. **A**. Evans Blue fluorescence. **B.** Lungs weight.