AWARD NUMBER: W81XWH-18-2-0045

TITLE: Does venous thrombosis chemoprophylaxis (VTC) adversely affect the hemorrhagic lesion in a preclinical model of penetrating traumatic brain injury?

PRINCIPAL INVESTIGATOR: CAPT Randy Bell, MC, USN

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Does venous thrombosis chemoprophylaxis (VTC) adversely affect the hemorrhagic lesion in a preclinical model of penetrating traumatic brain injury?

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

Walter Reed Army Institute of Research
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503 Robert Grant Avenue
Silver Spring, MD 20910

8. PERFORMING ORGANIZATION REPORT NUMBER

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

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

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

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Severe penetrating traumatic brain injury (TBI) patients represent the highest risk for complication associated with thromboembolism formation and hematoma expansion. Previous clinical studies demonstrated that the early use of VTC in these patients did not exacerbate intracerebral hematoma while reducing the risk of thromboembolism. Despite these findings, there is no consensus among neurosurgeons regarding its use in the presence of TBI, the appropriate timing or the dosing regimen. To address these issues, we propose to investigate the safety of VTC with regard to adversely affecting intracerebral hemorrhage in a rat model of penetrating TBI. The results of this study will potentially provide the scientific basis for modifying the clinical practice guidelines regarding the use of heparinoids for severe TBI in both civilian and military populations.

15. SUBJECT TERMS
Traumatic brain injury (TBI), Venous thrombosis chemoprophylaxis (VTC), hemorrhagic lesion, thromboembolism formation and hematoma expansion

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	17	**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.*

Recent conflicts have shown that the incidence of thrombotic and embolic complications in combat casualties is disproportionally higher than in civilian casualties, thus leading to the practice among trauma surgeons to use Venous Thrombosis Chemoprophylaxis (VTC), i.e. heparin. This however meets reluctance among neurosurgeons whose concern is that this could potentially increase cerebral hemorrhage.
We hypothesize that prophylactic doses of heparin in a traumatic brain injury (TBI) patient will not exacerbate cerebral contusion or hemorrhage. We plan to test this hypothesis in a rat survival model of penetrating TBI.
Specific Aims: (1) Establish the dose-response relationship between heparinoid administration at 24h post-injury and intracerebral hemorrhage. (2) Define the time window for administration of heparinoid following PBBI.
Data from this study could potentially aid in the expansion of the safety profile for the use of VTC in TBI in an effort to reduce morbidity and mortality of combat casualties.

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

Traumatic Brain Injury, penetrating head injury, hemorrhage, venous thrombosis chemoprophylaxis, heparinoids

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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.

See next page.

	Timeline (months)	Task	Status
Task 1. IACUC/ACURO approval for animal studies and procurement of equipment:			
Subtask 1. Write IACUC protocol.	1	Statistics/ Writing	Completed
Subtask 2. IACUC review and approval.	1	Writing	Completed
Subtask 3. ACURO review and approval.	2-3	Writing	Completed
Subtask 4. Procurement/set-up of equipment.	1-3	Preparation	Completed
Specific Aim 1: Dose escalation study to determine optimal dose of heparin.			
Task 2. Perform animal experiments in Phase 1:			
Subtask 1. Complete instrumented, physiological experiments in 80 rats.	4-11	Animal experiments	In process
Subtask 2. Hematologic analysis of blood samples.	4-11	Hematology	In process
Subtask 3. Necropsy, gross pathology of 80 rats.	4-11	Pathology	
Specific Aim 2: Characterize responses to different doses and timing of heparin administration.			
Task 3. Perform animal experiments in Phase 2:			
Subtask 1. Complete instrumented, physiological experiments in 140 rats.	11-22	Animal experiments	
Subtask 2. Hematologic analysis of blood samples.	11-22	Hematology	
Subtask 3. Necropsy, gross pathology of 140 rats.	11-22	Pathology	
Task 4. Histopathologic analysis:			
Subtask 1. Complete histopathologic analysis.	20-22	Histo-pathology	
Subtask 2. Final pathology report.	23	Writing	
Task 5. Lock database, quality control, data analysis, report:			
Subtask 1. Quality control of databases and lock databases.	22	QC	
Subtask 2. Statistical analysis.	22-24	Statistics	
Subtask 3. Final Study report preparation and submission.	22-24	Writing	
Subtask 4. Manuscript preparation and submission to peer-reviewed journal.	24	Writing	

What was accomplished under these goals?*For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

During this reporting period animal use protocol was approved by the WRAIR/NMRC institutional IACUC and ACURO (IACUC protocol 19-PN-01S approval 23JAN19 and ACURO 14FEB19) and an amendment using Magnetic Resonance Imaging as a replacement to micro-CT and for transporting animals to the Uniformed Services University, Center for Neuroscience and Regenerative Medicine, Translational Imaging Facility was approved. We confirmed that the MRI will provide the necessary imaging modality to track temporal changes in intracerebral hemorrhage and edema. We initiated Phase 2 of the study.

The WRAIR site AI was changed from Dr. Lai Yee Leung to Dr. Zachary Bailey. We set up experimental study area and initiated the actual experiment work on this project. A research Associate dedicated to this project was hired and trained.

We obtained provide proof-of-concept evidence supporting the use of MRI following PBBI. Initiate Phase 2 experiments were initiated.

Proof-of-concept was successfully obtained. Dose escalation studies have been initiated with the following treatment groups: PBBI+Veh, PBBI+25 mg/kg LVX. For the initial dose escalation studies LVX or Veh administration was daily with the initial dose given at 24 hrs post-injury. The survival rate following drug/vehicle administration is currently 100%.

Proof-of-Concept: A proof-of-concept experiment was carried out in order to verify that MRI at USUHS would provide the necessary image resolution to visualize and quantify the progression of intracerebral hemorrhage. Figure 1 below demonstrates that following PBBI, we are able to visualize intracerebral hemorrhage and cerebral edema with the necessary resolution. We are also able to observe the progression over the initial 24 hours verifying that MRI will be a viable alternative to the micro-CT.

Weight Gain: Rats were weighed daily beginning immediately prior to injury and continuing for 7 days post-injury. Figure 2 demonstrates a decrease in weight over the first 24 hours following injury which is maintained until day 4 post-injury after which the weights begin to approach baseline values. The weight changes were similar between drug and vehicle groups.

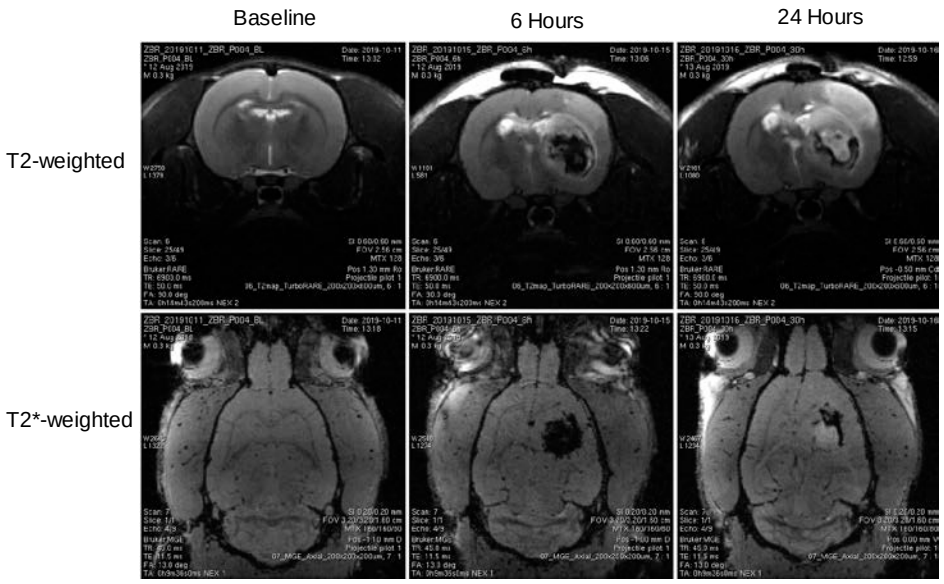


Figure 1. Representative MRI images demonstrating the development and progression of cerebral edema (top) and intracranial hemorrhage (bottom).

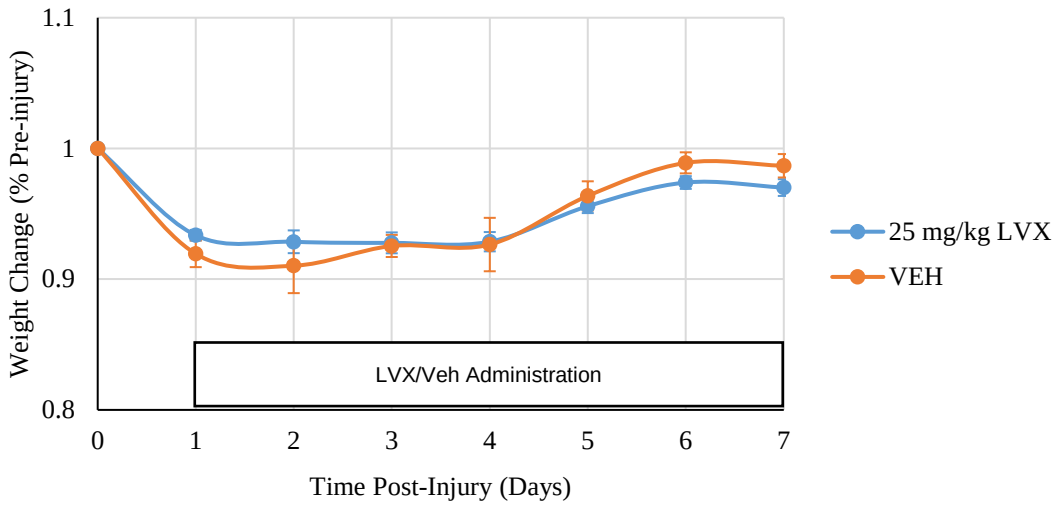


Figure 2. Weight changes following injury. Data expressed as mean±SEM.

Neurological Assessment: Modified Bederson's neurological deficit criteria was used to assess neurological deficits daily following injury. The modified criteria is a 12-point scale ranging from 0 (normal) to 12 (impaired). The scores were agreed upon by 1-2 investigators blind to drug treatment based on contralateral forelimb flexion, shoulder abduction, open field pivot, and resistance to lateral push. Deficits are shown in Figure 3 following LVX/Veh administration. Moderate impairments were observed following PBBI which last until the 7 day endpoint for both groups.

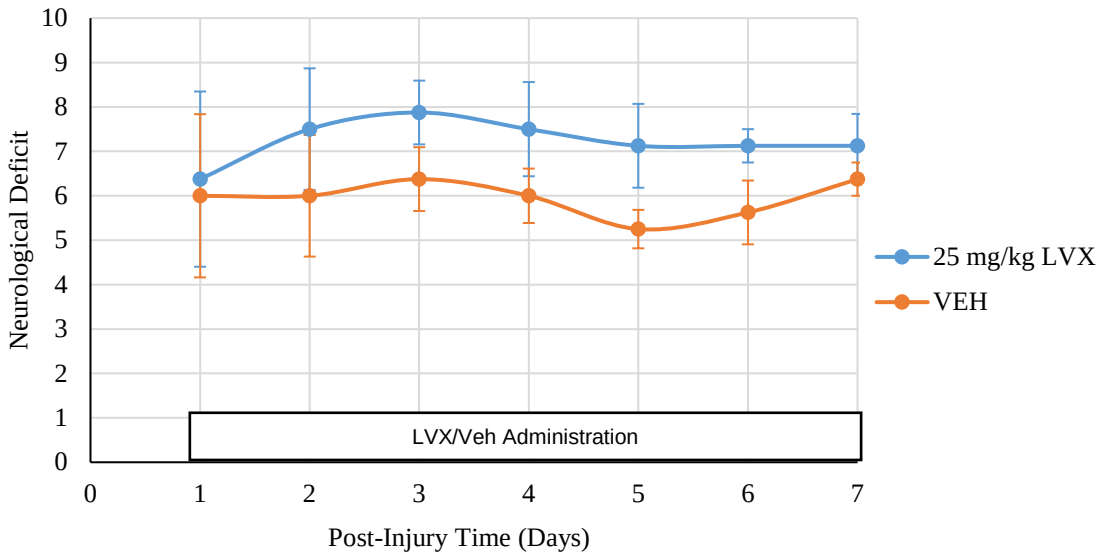


Figure 3. Neurological deficit following injury. Data expressed as mean±SEM.

Thromboelastography: At the 7 day endpoint, blood was collected for thromboelastgraphy to evaluate the coagulation dynamics. Figure 4 demonstrates the changes in clot formation evaluate 30 minutes after drug administration (LVX/Veh). The animals receiving Veh demonstrated a slightly hypocoagulable state compared to the reference tracing provided. Animals receiving LVX at 25 mg/kg demonstrated no signs of coagulation or clot formation indicating the effectiveness of the dose administered.

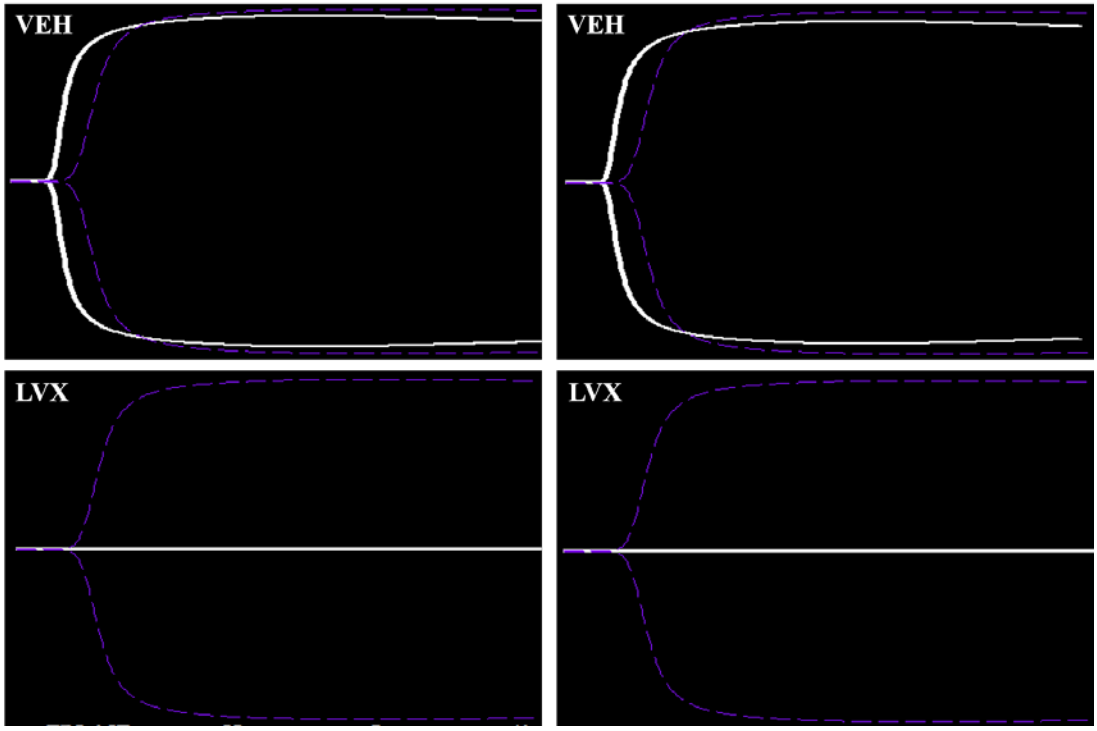


Figure 4. Thromboelastography tracings at 7 days post-injury. Purple dotted tracing represents approximately normal curves. Horizontal axis: time. Vertical axis: clot strength.

MRI: MRI procedures (T2-weighted, T2*-weighted, Diffusion Tensor Imaging, and Function MRI) were carried out prior to injury and 1, 2, 3, and 7 days following injury at the Translational Imaging Facility at USUHS. Selected images are included in Figure 5. The T2-weighted images (top) demonstrate the cerebral edema (hyperintense regions within brain) development and progression following PBBI and over the course of the treatment regimen. The T2*-weighted images (bottom) demonstrate the development and progression of intracerebral hemorrhage (hypointense regions within brain). Despite dramatic effects on coagulation of LVX at 25 mg/kg, these preliminary images do not appear to suggest any significant exacerbation of neuropathology. Definitive analysis and comparisons between treatment groups is pending region of interest selection and volume reconstruction/analysis.

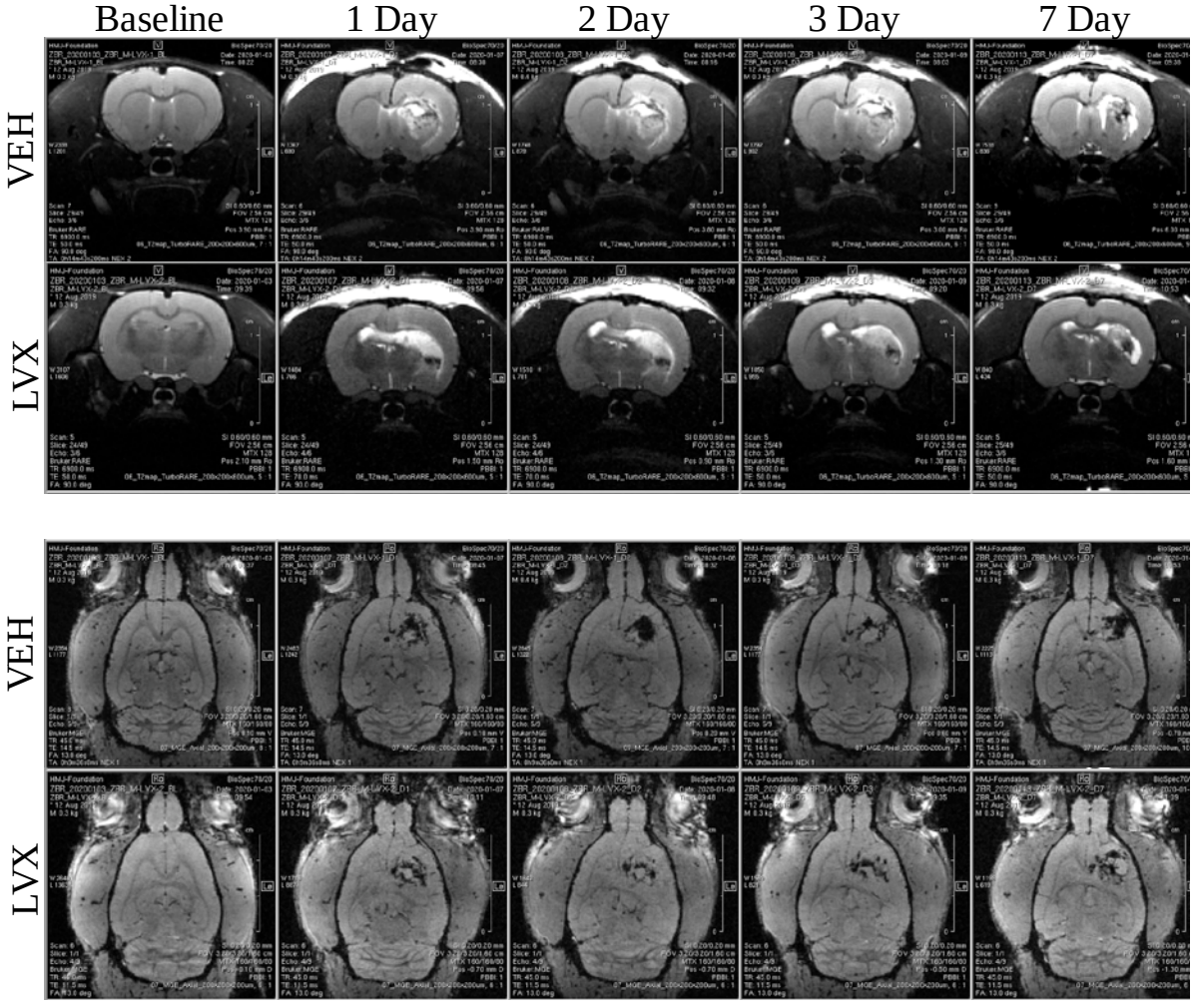


Figure 5. Selected T2-weighted (top) and T2*-weighted (bottom) images demonstrating the development and progression of intracranial hemorrhage and cerebral edema.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

This project so far provided several one-on-one training activities for new employees who will work as junior scientists or research assistants on this project. Through literature search and regular discussion groups within our team we were able to significantly increase their knowledge platform in regards to battlefield care, physiology and damage

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."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and
objectives.

Continue animal experiments.

4. **IMPACT:** D*escribe 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."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Data so far encourages the notion that high dose of heparin in the presence of TBI does not increase mortality.

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."
Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Combat casualties are at risk of venous thromboembolism due to their initial injury severity followed by multiple, long distance air evacuation (intubated, sedated, lying flat at altitude) to hospitals where definitive care can be rendered. The use of heparinoids as prophylaxis for venous thromboembolism in the presence of TBI remains controversial among neurosurgeons and may exacerbate intracerebral hemorrhage. The proposed study aims at answering this important question: does chemical VTC worsen hemorrhage in underlying brain injury? The answers to this question, whether for or against the use of heparinoids, is of critical importance to the injured active duty warfighter and is directly translatable from the benchtop to the bedside. Most certainly, the data will be used to further improve the military clinical practice guidelines for the management of severe TBI.

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."
Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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:*

Due to power limitations of the micro-CT x-ray source, our preliminary studies were unable to obtain the necessary soft tissue resolution/contrast to quantify intracerebral hemorrhage. In an attempt to improve image contrast, we administered Omnipaque intravenously during image acquisition but were still unable to obtain adequate soft tissue contrast.

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.

This issue will be resolved by employing Magnetic Resonance Imaging (MRI) as a replacement to micro-CT at the Uniformed Services University, Center for Neuroscience and Regenerative Medicine, Translational Imaging Facility.

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.

We have held a preliminary meeting with the Translational Imaging Facility to prepare for this change and have submitted the necessary IACUC protocol amendment approval. A memorandum of understanding between the WRAIR/NMRC and the USUHS IACUC allowing for the transfer of animals for the purpose of MRI imaging has also been put in place. Animal work will resume in October 2019.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Due to the above mentioned issues with imaging techniques, we had a delay in the start of animal work. This in turn resulted in a delay in actual expenditure. We do not foresee any problems with the actual expenditure by the end of the performance period of this grant.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals

N/A

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**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time*

conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  Nothing to report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*

- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Personnel	Role	Percent Effort
CAPT Randy Bell	PI	10
Dr. Anke Scultetus	Site-PI NMRC	5
CDR Carl Goforth	AI	5
Dr. Francoise Arnaud	Scientist	25
Dr. Yaron Dayani	Scientist	50
Dr. Melissa Mehalick	Scientist	35
Dr. Krystina Lavnik	Research Associate	100
Babita Karajuli	Technician	15
Eileen McDaniel	Program Manager	5
Dr. Deborah Shear	Site-PI, WRAIR	5
Dr. Zachary Bailey	AI	40
Dr.Teodoro Tigno	USUHS Coordinator	35

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

If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

The WRAIR Site AI, Dr. Lai Yee Leung left WRAIR and Dr. Zachary Bailey took over as AI.

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed. Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*
QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

Does venous thrombosis chemoprophylaxis (VTC) adversely affect the hemorrhagic lesion in a preclinical model of penetrating traumatic brain injury?
Log Number: BA160542

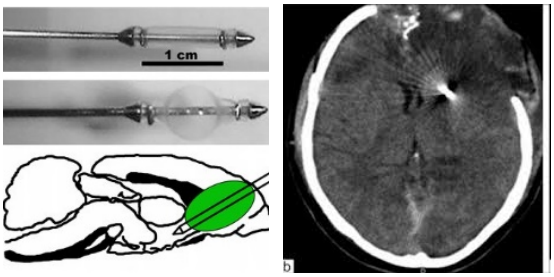
PI: CAPT Randy Bell **Org:** USUHS/NMRC/WRAIR **Award Amount(K):** $ 1,109

Study Aims

- We hypothesize that prophylactic doses of heparin in a traumatic brain injury (TBI) patient will not exacerbate cerebral contusion or hemorrhage. We plan to test this hypothesis in a rat survival model of penetrating TBI.

Approach

- Anesthetized rats will be exposed to a moderate level of a well-characterized penetrating brain injury.
- Starting 24 hours after injury, animals will be injected daily for 7 days with heparin for venous thrombosis chemoprophylaxis (VTC).
- Blood coagulation parameters, CT scan of brain and neuroscores will be assessed and data will be analyzed for evidence of increased brain contusion or hemorrhage.



A penetrating ballistic like brain injury model developed by co-investigators at WRAIR (left) will be used to simulate military relevant TBI (right) and evaluate possible adverse events with the administration of VTC to rats..

Timeline and Cost

Activities	Y1	Y2
IACUC/ACURO approval		
Animal experiments Phase 1		
Animal experiments Phase 2		
Pathology/hematology studies		
Data analysis, manuscript, final report		
Estimated Budget ($1,109K)	**$270,985**	**$838,276**

Updated: 07OCT2019

Goals/Milestones

Y1 Goals
☑ IACUC and ACURO approval
☐ Animal experiments Phase 1
Y2 Goals
☐ Animal experiments Phase 2
☐ Pathology and hematology analysis of blood and tissue samples
☐ Data Analysis
☐ Final report and manuscript

Comments/Challenges/Issues/Concerns: None

Budget Expenditure to Date
Projected Expenditure: $521K
Actual Expenditure: $271k

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*