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TITLE: Impact of Analgesics on Cardiovascular and
Respiratory Responses After Trauma and Hemorrhage

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CONTRACTING ORGANIZATION: U.S. Army Institute of Surgical Research (USAISR)

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14. ABSTRACT Blood loss is the leading cause of death on the battlefield, and it is important that pain medications used on the battlefield not interfere with the body's natural responses during bleeding that protect against death. The proposed studies will evaluate alternatives to existing pain medications to identify better options for battlefield pain management of the bleeding, injured Service Member. During the first year, the following were completed: --Major Task 1, Subtask 1: The animal protocol for Specific Aim 1 received IACUC and ACURO approval. --Major Task 1, Subtask 2: The estimate blood volume in a rat will be $6.84 \pm .67$ ml/100 g body weight. --Major Task 1, Subtask 3: The doses of ketamine (50mg/kg) and midazolam (5mg/kg) were determined for use in the study. The following were initiated, and studies are ongoing: --Major Task 1, Subtask 4: Effects of ketamine sedation with/without midazolam on compensatory responses after hemorrhage --Major Task 2, Subtask 1: Effects of ketamine sedation with/without midazolam on survival rate after decompensated hemorrhage with 55% blood volume loss									
15. SUBJECT TERMS hemorrhage, trauma, analgesia, ketamine, sedation, blood pressure, heart rate, respiration, survival, rat									
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1. **INTRODUCTION:** Pain management on the battlefield is challenging because the choice of analgesic medications is based on the presence of, or the potential to develop hemorrhagic shock or respiratory distress. Analgesia administration in hemorrhaging patients should not compromise cardiovascular and respiratory function, which could diminish the patient's ability to tolerate or survive a hemorrhage. The studies described in this proposal will evaluate ketamine-induced dissociative sedation and novel analgesics for their effects on the physiological compensatory responses during compensated hemorrhage, and survival time during decompensated hemorrhage. The hypothesis is that pain management options to be used on the battlefield do not affect physiological compensatory responses and survival following traumatic injury and hemorrhage. Analgesic options will be evaluated in a conscious rat model of battlefield-relevant traumatic hemorrhage while monitoring cardiovascular and respiratory function before and during 4 hours after analgesia. Animals will be subjected to a moderate blood volume hemorrhage to evaluate the effects of ketamine-induced sedation or novel analgesics on the compensatory responses to hemorrhage. In separate studies, animals will be subjected to a severe blood volume hemorrhage to evaluate the effects of ketamine-induced sedation or novel analgesics on survival after hemorrhage.

2. **KEYWORDS:** hemorrhage, trauma, analgesia, ketamine, sedation, blood pressure, heart rate, respiration, survival, rat

3. **ACCOMPLISHMENTS:** Months 1-12:

◦ **What were the major goals of the project? See table below**

	Proposed Completion Months	REVISED Completion Months	Actual Completion Months	Status
Specific Aim 1: To evaluate ketamine-induced sedation with and without midazolam				
Major Task 1: Assess effects of ketamine-induced sedation with and without midazolam during compensated hemorrhage				
Subtask 1: Submit animal use protocol for assessment of ketamine sedation with and without midazolam on compensated and decompensated hemorrhage in rats with extremity trauma for approval by USAISR Institutional Animal Care and Use Committee (IACUC) and USAMRDC Animal Care and Use Review Office (ACURO).	1-2		2	100% Completed
<i>Milestone Achieved: Obtain IACUC & ACURO approval</i>	2		2	Achieved
Subtask 2: Complete blood volume determination (n=6).	2-4		Used previous data	100% Completed
Subtask 3: Identify sedation dose of ketamine (n=6).	4-6		7	100% Completed
<i>Milestones Achieved: Identify blood volumes that equate to 40% and 55% total blood volume; identify the dose of ketamine that produces sedation in rats</i>	6		7	100% Completed

Subtask 4: Conduct experiments to determine the effects of ketamine sedation with and without midazolam on compensatory responses in conscious rats by monitoring blood pressure, heart rate, respiration, blood gases, and biochemical measurements indicative of tissue oxygen perfusion after compensated hemorrhage with 40% blood volume loss (n=60 rats).	7-12	7-18		Initiated 50% completed
<i>Milestone Achieved: Complete experiments of ketamine sedation with and without midazolam on compensatory responses after compensated hemorrhage</i>	12	18		
<i>Milestone Achieved: Submit annual report for Year 1</i>	12	18		
Major Task 2: Assess effects of ketamine-induced sedation with and without midazolam during decompensated hemorrhage				
Subtask 1: Conduct experiments to determine the effects of ketamine sedation with and without midazolam in conscious rats by monitoring survival rate after decompensated hemorrhage with 55% blood volume loss (n=60).	13-18	7-18		Initiated 50% completed
<i>Milestone Achieved: Complete experiments of ketamine sedation with and without midazolam on compensatory responses after decompensated hemorrhage</i>	18			
Subtask 2: Analyze data and prepare presentations and manuscripts on the effects of ketamine sedation with and without midazolam on compensated and decompensated hemorrhage.	19-24			Not Started
<i>Milestone Achieved: Complete the data evaluation and manuscript preparation of ketamine sedation with and without midazolam on survival rate after compensated and decompensated hemorrhage</i>	24			
Specific Aim 2: To evaluate two novel analgesics				
Major Task 3: Assess effects of two novel analgesics during compensated hemorrhage				
Subtask 1: Submit animal use protocol for assessment of two novel analgesics on	18-19			Not Started

compensated and decompensated hemorrhage in rats with extremity trauma for approval by USAISR IACUC and USAMRDC ACURO.				
<i>Milestone Achieved: Obtain IACUC & ACURO approval</i>	19			
Subtask 2: Conduct experiments to determine the effects of two novel analgesics on compensatory responses in conscious rats by monitoring blood pressure, heart rate, respiration, blood gases, and biochemical measurements indicative of tissue oxygen perfusion after compensated hemorrhage with 40% blood volume loss (n=60).	19-24			Not Started
<i>Milestone Achieved: Complete experiments of two novel analgesics on compensatory responses after compensated hemorrhage</i>	24			
<i>Milestone Achieved: Submit annual report for Year 2</i>	24			
Major Task 4: Assess effects of two novel analgesics during decompensated hemorrhage				
Subtask 1: Conduct experiments to determine the effects of two novel analgesics in conscious rats by monitoring survival rate after decompensated hemorrhage with 55% blood volume loss (n=60).	25-30			Not Started
<i>Milestone Achieved: Complete experiments of two novel analgesics on survival rate after decompensated hemorrhage</i>	30			
Subtask 2: Analyze data and prepare presentations and manuscripts on the effects of novel analgesics on compensated and decompensated hemorrhage	31-36			Not Started
<i>Milestone Achieved: Complete the data evaluation and manuscript preparation of the effects of two novel analgesics on survival rate after compensated and decompensated hemorrhage</i>	36			
<i>Milestone Achieved: Submit final report</i>	36			

- **What was accomplished under these goals?**

- Major Task 1: Subtasks 1 and 2 were completed (see table above).

Major Task 1, Subtask 1: The animal protocol for this Specific Aim 1 was written and approved the institutional Animal Care and Use Committee (IACUC). In addition, the approved protocol was submitted to ACURO for their review and approval. ACURO approval was obtained prior to the initiation of animal studies.

Major Task 1, Subtask 2: Blood volumes in rats have been determined previously. The data from these previous studies will be used to calculate the blood loss volumes in the current project. The formula to estimate blood volume in a rat will be $6.84 \pm .67 \text{ ml}/100 \text{ g body weight}$.

Major Task 1, Subtask 3: The dose of ketamine to be used in this proposal was determined to be 50 mg/kg. This dose of ketamine induced a sedation time of 27 minutes (n=6). The dose of midazolam to be use in this proposal was determined to be 5 mg/kg. This dose of midazolam combined with ketamine (50mg/kg) induced a sedation time of 61 minutes (n=6).

Major Task 1, Subtask 4: Studies are 50% complete. Preliminary findings indicate that ketamine sedation alone or combined with midazolam are affecting cardiovascular and respiratory compensatory responses after moderate hemorrhage.

Major Task 2, Subtask 1: Studies are 50% complete. Preliminary findings indicate that ketamine sedation with or without midazolam will decrease survival after severe hemorrhage.

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

- Training: Nothing to Report.

- Professional Development: Dr. Hinojosa-Laborde attended the 2022 Experimental Biology Meeting on April 1-6, 2022 in Philadelphia, PA; and the 2022 Military Health System Research Symposium (MHSRS) on Sept. 19-23, 2022 in Orlando, FL. Dr. Pando attended the 2022 Military Health System Research Symposium (MHSRS) on Sept. 19-23, 2022 in Orlando, FL.

- **How were the results disseminated to communities of interest?**

- Nothing to Report.

- **What do you plan to do during the next reporting period to accomplish the goals?**

- During the next reporting period progress will continue on completing Major Task 1, Subtask 4 and Major Task 2, Subtask 1. The expected completion date for these goals is at the end of the 18th month of the project (Quarter 6).

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?**

- Nothing to Report.

- **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:** 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**

- Nothing to Report.

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

- Nothing to Report.

- **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**

- *Nothing to Report.*
- **Significant changes in use or care of human subjects**
- *Not Applicable.*
- **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**
- *Nothing to Report.*
- **Journal publications.**
- *Nothing to Report.*
- **Books or other non-periodical, one-time publications.**
- *Nothing to Report.*
- **Other publications, conference papers, and presentations.**
- *Nothing to Report.*
- **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?**
- *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."*

Example:

Name: *Carmen Hinojosa-Laborde, PhD*

Project Role: *Principal Investigator*

Researcher Identifier (e.g. ORCID ID): *#0000-0001-7104-3305*

Nearest person month worked: *1.5*

Contribution to Project: *Dr. Hinojosa-Laborde wrote the animal protocol and completed the processes for approval by the IACUC and ACURO. She analyzed data produced from experiments.*

Name: *Kathy Ryan, PhD*

Project Role: *Co-Investigator*

Researcher Identifier (e.g. ORCID ID): *Not Applicable*

Nearest person month worked: *1.2*

Contribution to Project: *Dr. Ryan reviewed and revised the animal protocol as needed. She analyzed data produced from experiments.*

Name: *Miryam Pando, PhD*

Project Role: *Postdoctoral Fellow*

Researcher Identifier (e.g. ORCID ID): *Not Applicable*

Nearest person month worked: *0.5*

Contribution to Project: *Dr. Pando completed the training required to conduct animal studies on this proposal. She is responsible for the management of the protocol schedule and execution of experiments.*

Name: *Cassandra Rodriguez*
 Project Role: *Lead Laboratory Technician*
 Researcher Identifier (e.g. ORCID ID): *Not Applicable*
 Nearest person month worked: 3

Contribution to Project: *Ms. Rodriguez prepared the laboratory space to conduct experiments, and assisted with the writing and revisions of the animal protocol. She assists Dr. Pando with management, preparation, and execution of experiments.*

Name: *Roger Chavez*
 Project Role: *Laboratory Technician*
 Researcher Identifier (e.g. ORCID ID): *Not Applicable*
 Nearest person month worked: 1.5

Contribution to Project: *Mr. Chavez assisted with preparing the laboratory space to conduct experiments. He assisted Dr. Pando and Ms. Rodriguez with the execution of experiments.*

◦ **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

◦ **COLLABORATIVE AWARDS:** *Not applicable.*

◦ **QUAD CHARTS:**

Impact of Analgesics on Cardiovascular and Respiratory Responses After Trauma and Hemorrhage

Proposal Log Number: BA210031

Award number: CDMRPL-21-0-BA210031; Award Period of Performance: 3/7/22-3/6/25

PI: Hinojosa-Laborde, Carmen

Org: USAISR

Award Amount: \$2,110K



Study Aims

•**Aim 1:** To evaluate the effects of ketamine at doses that induce dissociative sedation, with and without midazolam, on the compensatory responses to a compensated hemorrhage in conscious rats. The effect of ketamine-induced dissociative sedation with and without midazolam on survival will also be evaluated after decompensated hemorrhage.

•**Aim 2:** To evaluate the effects of two novel analgesics on the responses to a compensated hemorrhage and survival after decompensated hemorrhage.

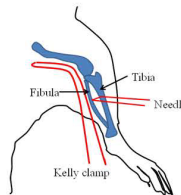
Hypothesis

Analgesics with the potential to be used on the battlefield do not affect physiological compensatory responses and survival during hemorrhage.

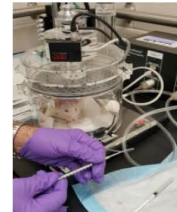
Approach

Using a battlefield relevant rodent model of trauma that is combined with either compensated or decompensated hemorrhage, analgesics will be administered to the conscious animal to determine how they affect cardiovascular and respiratory responses, and survival after hemorrhage.

Extremity Trauma



Conscious Hemorrhage



Compensated Hemorrhage (to assess compensatory responses)

Decompensated Hemorrhage (to assess survival)

Analgesics: Ketamine-induced Sedation, Ketamine-induced Sedation with Midazolam, and two Novel Analgesics

Accomplishment: Aim 1 - Ketamine Sedation Study in progress. Sedative doses of Ketamine and Midazolam are being tested after compensated and decompensated hemorrhage.

Timeline and Cost

Activities	CY	22	23	24	25
Ketamine-induced sedation after moderate and severe hemorrhage.		■	■		
Ketamine sedation with midazolam tested after hemorrhage.			■		
Two novel analgesics tested after compensated hemorrhage.				■	
Two novel analgesics tested after decompensated hemorrhage.					■
Estimated Budget (\$K)		\$200	\$868	\$736	\$306

Updated: (End Q4: March 6, 2023)

Goals/Milestones

CY22 Goal –Ketamine Sedation Study

- Identify Ketamine Sedation dose
- Initiate Ketamine Sedation with and without Midazolam study

CY23 Goal – Ketamine Sedation Study

- Complete Ketamine study with compensated and decompensated hemorrhage
- Initiate Novel Analgesics with compensated hemorrhage study

CY24 Goals –Ketamine Sedation and Novel Analgesics Studies

- Complete Novel Analgesics with compensated hemorrhage study
- Initiate Novel Analgesics with decompensated hemorrhage study

CY25 Goal – Novel Analgesics Study

- Complete Novel Analgesic with decompensated hemorrhage study

Comments/Challenges/Issues/Concerns

- If timelines change, comment here.
- If off by more than one quarter in spending, comment here.

Budget Expenditure to Date

Projected Expenditure: \$2,110K

Actual Expenditure: \$991K