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TITLE: Application of Combined Cardioprotective Agents to Preserve Organ Function and Improve Survival during Experimental Hemorrhagic Shock

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14. ABSTRACT We compared the effects of different anesthesia regimen (with either intraperitoneal ketamine/xylazine or with isoflurane) on the survival rate at 6 weeks after hemorrhagic shock in rats, and determined the survival rates of rats that were subjected to 30 mmHg of hypotension for 10, 20, 30, 35, 45, or 60 minutes after hemorrhagic shock. The results showed that there was a 25.6 % survival rate in rats that were anesthetized with ketamine/xylazine and were subjected to 30 mmHg of hypotension for 30 minutes. Therefore we use this setting as hemorrhagic shock model for the further studies. We demonstrated that remote ischemic preconditioning improved recovery of blood pressure and maintained more circulating intravascular blood volume in the early phase of resuscitation, improved BUN, and markedly and significantly improved short and long term survival in rats subjected to hemorrhagic shock. However, mitochondrial protective agent SBT-0100-05 did not affect blood pressure and cardiac function during hemorrhagic shock and acute resuscitation phase, and did not improve long term survival in rats subjected to hemorrhagic shock.					
15. SUBJECT TERMS Hemorrhagic shock, remote ischemic preconditioning, fixed pressure hemorrhage, isoflurane					
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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Hemorrhagic shock remains a major cause of mortality and morbidity on the battlefield. Even with restoration of blood volume, organs subjected to hemorrhagic shock can develop ischemia/reperfusion injury and fail. This study aims to develop new therapeutic approaches to improve survival and protect vital organs during and after hemorrhagic shock. The therapies with proven cardio-protective properties include: (1) remote ischemic preconditioning, which could be given prophylactically (simple and cost-effective repetitive inflations and deflations of a blood pressure cuff on the arm) to soldiers prior to going into high risk combat situations; (2) the mitochondrial protective agent SS31, which could also be administered prophylactically; and (3) therapeutic hypothermia, which could be produced with a ThermoSuit device (already FDA approved for hyperthermia) to rapidly cool the body and protect vital organs in case the injury occur. These therapies, alone or in combination, will be investigated to improve overall survival and protect vital organs from ischemia/reperfusion injury of hemorrhagic shock (vs. placebo) in a standardized experimental model of fixed pressure hemorrhage in male and female adult Sprague Dawley rats.

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

Hemorrhagic shock; remote ischemic preconditioning; mitochondrial protective agent; therapeutic hypothermia; fixed pressure hemorrhage.

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: To test the hypothesis that prophylactic remote ischemic preconditioning improves long term survival and the function and structure of the vital organs (heart, brain, lung, kidney and liver) after experimental hemorrhagic shock, created by withdrawing blood and later reinfusing it, in Sprague Dawley rats of both sexes. **The major tasks** are: (1) from 1 to 6 months, instrument rats and perform studies for Specific Aim 1 (n = ~90 rats); (2) from 7 to 8 months, collect and collate data; (3) in 9th month, analyze data to assess survival, organ function and structure and mitochondrial function. **Milestone(s) Achieved** – Determine whether remote ischemic preconditioning improves long term survival and improves the function and structure of the vital organs (heart, brain, lung, kidney and liver) after an episode of hemorrhagic shock in an experimental rat model. This study is 100% completed.

Specific Aim 2: To test the hypothesis that prophylactic administration of mitochondrial protective agent, SS31, improves long term survival and the function and structure of the vital organs (heart, brain, lung, kidney and liver) after an episode of hemorrhagic shock in an experimental rat model. **The major tasks** are: (1) from 10 to 15 months, instrument rats and perform studies for Specific Aim 2 (n = ~90 rats); (2) from 16 to 17 months, collect and collate data; (3) in 18th month, analyze data to assess survival, organ function and structure and mitochondrial function. **Milestone(s) Achieved** – Determine whether prophylactic administration of mitochondrial protective agent, SS31, improves long term survival and improves the function and structure of the vital organs (heart, brain, lung, kidney and liver) after an episode of hemorrhagic shock in an experimental rat model. This study is 100% completed.

Specific Aim 3: To test the hypothesis that moderate hypothermia administered in the setting of hemorrhagic shock will improve long term survival and preserve the function and structure of the vital organs (heart, brain, lung, kidney and liver) after an episode of hemorrhagic shock in an experimental rat model. **The major tasks** are: (1) from 19 to 24 months, instrument rats and perform studies for Specific Aim 3 (n = ~90 rats); (2) from 25 to 26 months, collect and collate data; (3) in 27th month, analyze data to assess survival, organ function and structure and mitochondrial function. **Milestone(s) Achieved** – Determine whether moderate hypothermia administered in the setting of hemorrhagic shock will improve long term survival and preserve the function and structure of the vital organs (heart, brain, lung, kidney and liver) after an episode of hemorrhagic shock in an experimental rat model. We have started this study and will complete it in year 3.

Specific Aim 4: To test the hypothesis that a combination of promising therapies tested in aims 1, 2, and/or 3, which have been shown to have a positive effect, will have additive (or synergistic) effects on survival and organ preservation in the setting of hemorrhagic shock. **The major tasks** are: (1) from 28 to 33 months, instrument rats and perform studies for Specific Aim 4 (n = ~90 rats); (2) from 34 to 35 months, collect and collate data; (3) in 36th month, analyze data to assess survival, organ function and structure and mitochondrial function. **Milestone(s) Achieved** – Determine whether a combination of promising therapies tested in aims 1, 2, and 3 have additive (or synergistic) effects on survival and organ preservation in the setting of hemorrhagic shock. Study to start in year 3.

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.

This annual report covers 1 Sep 2017 – 30 Aug 2018, and focuses on the major goals listed in the **Specific Aim 1 and 2**; as well as another 2 studies in the amendment to this protocol.

In the study of **Specific Aim 1**, the hypothesis that prophylactic remote ischemic preconditioning (RIPC) improves long term survival and the function and structure of the vital organs (heart, brain, lung, kidney and liver) after experimental hemorrhagic shock was investigated and completed. Sprague-Dawley rats (both genders) were randomly assigned to RIPC (n = 26; RIPC was induced by inflating small bilateral pressure cuffs to 200 mmHg around the femoral arteries for 5 minutes, followed by 5 minute release of the cuffs, repeated 4 times prior to shock inducing) or control group (n = 27; the blood pressure cuffs were inflated only to 30 mmHg). Fixed-pressure hemorrhagic shock was induced in the rats. The rats were anesthetized with intraperitoneal ketamine and xylazine (90mg/kg and 10mg/kg), and were heparinized with 500 U/kg heparin. Hemorrhagic shock was induced by removing blood via the carotid artery catheter to attain a mean blood pressure of 30 mm Hg. Mean blood pressure was maintained at 30 mm Hg for 30 minutes and then the collected blood was returned over the next 30 minutes. At 60 minutes after returning the shed blood and closely monitoring the animals, the catheters were removed from the blood vessels and the rats were allowed to recover from anesthesia; and then allowed to survive for 6 weeks. The percentage of estimated total blood volume withdrawn to maintain a level of 30 mmHg was similar in the RIPC group ($41.7 \pm 1.0 \%$) and control group ($41.9 \pm 1.0 \%$). Recovery of blood pressure during the early resuscitation phase was significantly improved in the RIPC group. The diastolic internal dimension of the left ventricle (echocardiogram), which indicates circulating intravascular blood volume, was significantly larger in the RIPC group at 1 hour after initiation of shed blood reinfusion (5.8 ± 0.1 mm) compared to 5.4 ± 0.1 mm in the control group ($p=0.04$). Left ventricular fractional shortening was comparable between RIPC ($50.9 \pm 1.9 \%$) and control group ($49.6 \pm 1.8 \%$; $p=0.64$) at 1 hour after initiation of resuscitation. At 48 hours after shock, BUN was within normal range in the RIPC group (17.3 ± 1.2 mg/dl); but elevated in the control group (22.0 ± 1.7 mg/dl). At 72 hours after hemorrhagic shock injury, 6 of 27 (22.2 %) rats in the control group and 13 of 26 (50 %, $p = 0.047$) rats in the RIPC group survived. At 6 weeks, 5 of 27 (18.5 %) rats in the control group and 13 of 26 (50 %; $p = 0.021$) rats in the RIPC group survived. RIPC significantly increased survival rate at both 72 hours and 6 weeks. In conclusion, RIPC improved recovery of blood pressure and maintained more circulating intravascular blood volume in the early phase of resuscitation, improved BUN, and markedly and significantly improved short and long term survival in rats subjected to hemorrhagic shock.

We have completed the study to determine whether prophylactic administration of a mitochondrial protective agent could improve long term survival and the function and structure of the vital organs (heart, brain, lung, kidney and liver) after an episode of hemorrhagic shock in an experimental rat model in **Specific Aim 2**. . Sprague-Dawley rats (both genders) were anesthetized with intraperitoneal ketamine and xylazine (90mg/kg and 10mg/kg), and were randomly assigned to SBT-0100-05 (n = 10) or saline control group (n = 10), thirty minutes prior to bleeding, rats receiving the mitochondrial targeted agent SBT-0100-05 were initially given the drug IV (1 mg/kg/hr) for 2 hours followed by 3mg/kg/day for 6 weeks (given by an osmotic pump implanted through a small cut-down made between the scapulae just after implanting the jugular and carotid catheters during the initial surgical

procedure). In the control group, the same volumes of saline were given. After administering 500 U/kg heparin, hemorrhagic shock was induced by removing blood via the carotid artery catheter to attain a mean blood pressure of 30 mm Hg. Mean blood pressure was maintained at 30 mm Hg for 30 minutes and then the collected blood was returned over the next 30 minutes. At 60 minutes after returning the shed blood and closely monitoring the animals, the catheters were removed from the blood vessels and the rats were allowed to recover from anesthesia; and then allowed to survive for 6 weeks. The percentage of estimated total blood volume withdrawn to maintain a level of 30 mmHg was similar in the SBT group ($38.7 \pm 2.6 \%$) and control group ($40.5 \pm 2.7 \%$; $p = 0.63$). Mean blood pressure was similar in both groups during baseline, shock phase and resuscitation phase. The diastolic internal dimension of the left ventricle (echocardiogram), which indicates circulating intravascular blood volume, was comparable in the SBT group at 1 hour after initiation of shed blood reinfusion (5.5 ± 0.2 mm) compared to 5.0 ± 0.3 mm in the control group ($p=0.14$). Left ventricular fractional shortening was comparable between SBT ($47.9 \pm 3.4 \%$) and control group ($54.9 \pm 2.7 \%$; $p=0.13$) at 1 hour after initiation of resuscitation. At 6 weeks, 2 of 10 (20 %) rats in the control group and 2 of 10 (20 %; $p = 1.0$) rats in the SBT group survived. In conclusion, SBT did not affect blood pressure and cardiac function during hemorrhagic shock and acute resuscitation phase, and did not improve long term survival in rats subjected to hemorrhagic shock.

In the amendment to this protocol, we requested to compare the effects of a different anesthesia regimen on the survival rate after hemorrhagic shock, and this investigation has been completed. Sprague Dawley rats (both genders) were anesthetized with either intraperitoneal ketamine/xylazine (K/X, 90 mg/kg and 10 mg/kg; $n=12$), or with isoflurane (5% isoflurane induction and 2% maintenance in room air; $n=12$). Rats were intubated and ventilated with room air, heparinized, and hemorrhagic shock induced by withdrawing blood from the left carotid artery to a fixed mean blood pressure of 30 mm Hg for 60 minutes, followed by 30 min of resuscitation with shed blood. Rats remained anesthetized for one hour during which hemodynamics were monitored (femoral artery catheter); and then allowed to survive for 6 weeks. During the shock phase, the total withdrawn blood volume (expressed as % of estimated total blood volume) to maintain MBP at 30 mmHg was significantly higher in the isoflurane group ($51 \pm 1.5 \%$) compared to the K/X group ($45.3 \pm 1.8 \%$; $p=0.023$). The diastolic internal dimension of the left ventricle, which indicated circulating intravascular blood volume, was significantly larger in the isoflurane group at the end of 1 hour of the shock phase (4.5 ± 0.2 mm compared to 3.5 ± 0.2 mm in K/X group; $p=0.0003$) and at 1 hour after initiation of shed blood reinfusion (6.3 ± 0.2 mm compared to 5.3 ± 0.3 mm in K/X group; $p=0.014$). Recovery of blood pressure during the resuscitation phase was significantly improved in the isoflurane group compared to the K/X group. The survival rate at 6 weeks was 1 of 12 (8.3%) in rats receiving K/X and 10 of 12 (83.3%) in rats receiving isoflurane ($p < 0.001$). Histology demonstrated brain infarction in the 1 surviving rat receiving K/X; no brain infarction in the 10 surviving rats that received isoflurane at 6 weeks. No infarction was detected in heart, lung, liver or kidneys in all surviving rats. In summary, these results suggest that isoflurane stabilizes the cardiovascular response to acute blood loss and benefits the perfusion of tissue, which resulted in significantly higher long term survival rate and improved blood pressure response to resuscitation, without end-organ infarction.

We determined how long of hypotension (30 mmHg) could be tolerated before death was inevitable; that is a dose finding study in which survival went out to 6 weeks. Sprague Dawley rats (both genders) were anesthetized with intraperitoneal ketamine and xylazine (90mg/kg and 10mg/kg), intubated and ventilated with room air. Rats were heparinized and subjected to blood withdrawal via catheter implanted in the left carotid artery in order to establish hypotension at a level of 30 mmHg. Rats were subjected to this level of hypotension for 10, 20, 30, 35, 45, or 60 minutes and then the shed blood was reinfused within 30 min (n=6 in the 10, 20, 35, 45, or 60 minutes groups and in the 30 minutes group). The rats were monitored under anesthesia for one hour and then allowed to survive for 6 weeks. Rats exposed to 10 minutes of 30 mmHg had a 6 week survival rate of 5/6 (83.3%); Rats exposed to 20 minutes of 30 mmHg had a 6 week survival rate of 4/6 (66.7%); rats exposed to 30 minutes of 30 mmHg had a 6 week survival rate of 11/43 (25.6%); rats subjected to 35 minutes of 30 mmHg had a 6 week survival rate of 1/6 (16.7%); rats subjected to 45 minutes of 30 mmHg had a 6 week survival rate of 0/6 (0%); rats subjected to 60 minutes of 30 mmHg had a 6 week survival rate of 0/6 (0%) ($p < 0.01$ by Fisher Exact Test). There is a critical period between 30 minutes and 35 minutes of hemorrhagic shock in which survival plummets from 25.6 % down to 16.7%. Based on these data, we decided to use this protocol to investigate whether prophylactic remote ischemic preconditioning could improve long term survival and the function and structure of the vital organs (heart, brain, lung, kidney and liver) after experimental hemorrhagic shock in the setting of mean blood pressure 30 mmHg maintained for 30 minutes.

We have performed pilot study of therapeutic hypothermia in 4 rats. Fixed-pressure hemorrhagic shock was induced in Sprague-Dawley rats (mixed gender). The rats were anesthetized with intraperitoneal ketamine and xylazine, and were heparinized with 500 U/kg heparin. Hemorrhagic shock was induced by removing blood via the left carotid artery catheter to attain a mean blood pressure of 30 mm Hg. Therapeutic hypothermia was instituted by using the Thermosuit starting 5 minutes after bleeding. Core temperature was maintained at $\sim 32^{\circ}\text{C}$ until blood volume was fully restored, after which the rats were allowed to warm back to normal body temperature. Mean blood pressure was maintained at 30 mm Hg for 30 minutes and then the collected blood was returned over the next 30 minutes. At 60 minutes after closely monitoring the animals and returning the shed blood, the catheters were removed from the blood vessels and the rats were allowed to recover from anesthesia, and then allowed to survive for 6 weeks. Three of 4 rats survived from the hemorrhagic shock, and the data suggested that therapeutic hypothermia is safe and doable in the setting of hemorrhagic shock. There is therefore a strong trend favoring improved survival in the therapeutic hypothermia treatment.

We have formally started to instrument rats and perform studies for Specific Aim 3. We are planning on continuing this study into the first few months of year three to achieve about 44 rats in each group which was our original estimate of number of animals needed.

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.

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.

During the year of 1 Sep 2017 – 30 Aug 2018, the present studies have resulted in 2 abstracts that are presented in 2018 Experimental Biology meeting, which was held in San Diego, CA, April 21-25, 2018

1. Wangde Dai, Jianru Shi, Juan Carreno, Sharon Hale, and Robert A. Kloner. Remote limb ischemic preconditioning improves post-resuscitation long term survival in a rat fixed pressure hemorrhagic shock model. FASEB J. 2018 April; Volume 32, Issue 1-supplement.
2. Jianru Shi, Wangde Dai, Juan Carreno, Sharon L. Hale, and Robert A. Kloner. Blood-based biomarkers as early predictor of mortality in experimental hemorrhagic shock. FASEB J. 2018 April; Volume 32, Issue 1-supplement.

And another 3 abstracts are accepted for the American Heart Association Scientific Sessions 2018 (ReSS), which will be held in Chicago, IL, November 10-11, 2018

1. Wangde Dai, Jianru Shi, Juan Carreno, Sharon Hale, and Robert A. Kloner. Remote limb ischemic preconditioning improves short and long term survival and maintains intravascular blood volume during resuscitation of hemorrhagic shock.

2. Wangde Dai, Jianru Shi, Juan Carreno, Sharon Hale, and Robert A. Kloner. Effects of anesthetic agents on the hemodynamic stabilization and long term survival in an experimental model of hemorrhagic shock.
3. Jianru Shi, Wangde Dai, Juan Carreno, Sharon L. Hale, Robert A. Kloner. Effects of anesthetic agents on blood parameters in rats with acute hemorrhagic shock.

In addition, we are in the process of writing up a full length manuscript based on the results of specific Aim 1 and hope to have it submitted to a peer review journal after months.

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.

During next reporting period, we will continue to complete the studies of therapeutic hypothermia in Specific Aim 3 in the first half of year 3. If we find a benefit, we will begin studies combining remote ischemic conditioning with hypothermia.

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."

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).

Results from our dose finding study using isoflurane suggest that this anesthetic agent may have protective effects on the whole body, as it was shown to have protective effects in previous studies of experimental myocardial infarction. Isoflurane markedly improved survival in our model of hemorrhagic shock, compared to standard ketamine and xylazine. Isoflurane showed better recovery of blood pressure early after transfusion/ resuscitation, as well as long term survival without end-organ infarction. This finding was unexpected but may have very important clinical implications for wounded soldiers suffering blood loss. Whether this beneficial effect is unique to only isoflurane or occurs with other anesthetics needs to be further investigated.

Remote ischemic preconditioning improves long term survival, preserves blood pressure and kidney function, and it should have a major clinical implication for the military. It suggests that a cost effective maneuver of inflating and deflating a blood pressure cuff on the arm of a combat soldier, prior to going into battle, could provide protection from dying from hemorrhagic shock. This study could form the basis of an important clinical trial, testing remote ischemic conditioning in soldiers.

Our dose finding study suggested that there is a critical period of time at which hemorrhagic shock at a level of 30 mmHg becomes irreversible – that is restoration of the shed blood still does not save the subject. It appears that 30-35 minutes of hypotension is a critical period.

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.

It could be that isoflurane and preconditioning work in this model that it might work in other types of shock that include not only hypotension but tissue trauma, septic shock, and other forms of ischemia/reperfusion injury to organs.

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.*

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.”

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.*

Because there was a significant difference in the survival rate under anesthesia with isoflurane versus ketamine/xylazine in rats that were subjected to hemorrhagic shock, the finding suggests that the type of anesthesia used during surgery in hemorrhagic shock situations may need to be re-evaluated. Isoflurane appears to be more protective than the ketamine/xylazine.

In addition we observed an improved survival with remote ischemic preconditioning. The results are suggestive of a benefit, and it would be relatively easy to translate this to a clinical study whereby soldiers going into battle precondition themselves beforehand using a blood pressure cuff and inflation over the brachial artery with 5 minutes of inflation and 5 minutes of deflation repeated 3-4 times.

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.

During the year of 1 Sep 2017 – 30 Aug 2018, we further explored the dose finding study and added whether 10 and 20 minutes of hypotension at 30 mmHg were associated with less irreversible shock in rats. We think it is important to determine the cut off for reversible shock (when blood can be returned and the animal survives), versus irreversible shock (when blood is returned but the animal dies, anyway.)

We increased n values in the isoflurane study to verify the reproducibility of the protective effects of isoflurane versus ketamine/xylazine in hemorrhagic shock by adding another 6 rats to each group.

The mitochondrial targeted agent that we initially planned to evaluate Elemipartide or SS 31, was no longer available from the company that was originally going to supply it. They supplied us with a mitochondrial protective agent SBT-0100-05 that has a similar mechanism of action and is more powerful than SS 31.

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 did have a delay in finding the correct dose and timing of hypotension to allow a death rate that was not so severe but not so low as to allow us to determine if remote ischemic conditioning would be of benefit on survival. During this dose finding study we made some unexpected observations and think that the findings with isoflurane may be even more important than the original therapies we suggested.

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.

None

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

Not applicable

Significant changes in use or care of vertebrate animals.

There were no significant changes in use or care of vertebrate animals.

Significant changes in use of biohazards and/or select agents

No change in biohazards. We used an alternative mitochondrial protective agent SBT-0100-05, rather than SS-31 as described above.

- 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).*

There are 2 abstracts that were published in the journal of FASEB J:

1. Wangde Dai, Jianru Shi, Juan Carreno, Sharon Hale, and Robert A. Kloner. Remote limb ischemic preconditioning improves post-resuscitation long term survival in a rat fixed pressure hemorrhagic shock model. FASEB J. 2018 April; Volume 32, Issue 1-supplement.
2. Jianru Shi, Wangde Dai, Juan Carreno, Sharon L. Hale, and Robert A. Kloner. Blood-based biomarkers as early predictor of mortality in experimental hemorrhagic shock. FASEB J. 2018 April; Volume 32, Issue 1-supplement.

There are another 3 abstracts that will be in press in the journal of Circulation.

1. Wangde Dai, Jianru Shi, Juan Carreno, Sharon Hale, and Robert A. Kloner. Remote limb ischemic preconditioning improves short and long term survival and maintains intravascular blood volume during resuscitation of hemorrhagic shock.
2. Wangde Dai, Jianru Shi, Juan Carreno, Sharon Hale, and Robert A. Kloner. Effects of anesthetic agents on the hemodynamic stabilization and long term survival in an experimental model of hemorrhagic shock.
3. Jianru Shi, Wangde Dai, Juan Carreno, Sharon L. Hale, Robert A. Kloner. Effects of anesthetic agents on blood parameters in rats with acute hemorrhagic shock.

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).*

None yet

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.*

During the year of 1 Sep 2017 – 30 Aug 2018, the present studies have resulted in 2 abstracts that are presented in 2018 Experimental Biology meeting, which was held in San Diego, CA, April 21-25, 2018

1. Wangde Dai, Jianru Shi, Juan Carreno, Sharon Hale, and Robert A. Kloner. Remote limb ischemic preconditioning improves post-resuscitation long term survival in a rat fixed pressure hemorrhagic shock model. FASEB J. 2018 April; Volume 32, Issue 1-supplement.
2. Jianru Shi, Wangde Dai, Juan Carreno, Sharon L. Hale, and Robert A. Kloner. Blood-based biomarkers as early predictor of mortality in experimental hemorrhagic shock. FASEB J. 2018 April; Volume 32, Issue 1-supplement.

And another 3 abstracts are accepted and will be presented in the American Heart Association Scientific Sessions 2018 (ReSS), which will be held in Chicago, IL, November 10-11, 2018

1. Wangde Dai, Jianru Shi, Juan Carreno, Sharon Hale, and Robert A. Kloner. Remote limb ischemic preconditioning improves short and long term survival and maintains intravascular blood volume during resuscitation of hemorrhagic shock.
2. Wangde Dai, Jianru Shi, Juan Carreno, Sharon Hale, and Robert A. Kloner. Effects of anesthetic agents on the hemodynamic stabilization and long term survival in an experimental model of hemorrhagic shock.
3. Jianru Shi, Wangde Dai, Juan Carreno, Sharon L. Hale, Robert A. Kloner. Effects of anesthetic agents on blood parameters in rats with acute hemorrhagic shock.

(The 5 abstracts are attached in Section 9 - **APPENDICES**)

- **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.

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.

Not applicable

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

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. 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.

Not applicable

- **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;*
- *biospecimen 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.*

OTHER: Reports at scientific sessions. 2 abstracts were presented in 2018 Experimental Biology meeting, which was held in San Diego, CA, April 21-25, 2018; another 3 abstracts are accepted and will be presented in the American Heart Association Scientific Sessions 2018 (ReSS), which will be held in Chicago, IL, November 10-11, 2018.

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: Mary Smith
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.

Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award).

1. Name: Robert A. Kloner
Project Role: Principle investigator
Nearest person month worked: 6 months (50% per year)
Contribution to Project: Dr. Kloner has performed work in the area of study design, data collection and analysis, report and manuscript writing.

2. Name: Wangde Dai
Project Role: Senior investigator
Nearest person month worked: 9.6 months (80% per year)
Contribution to Project: Dr. Dai has performed work in the animal studies, data collection and analysis, report and manuscript writing.

3. Name: Jianru Shi
Project Role: Senior investigator
Nearest person month worked: 5.4 months (45% per year)
Contribution to Project: Dr. Shi has performed work in the area of blood and tissue sampling, data collection and analysis, report and manuscript writing.

4. Name: Sharon Hale
Project Role: Senior investigator
Nearest person month worked: 1 month (8.3% per year)
Contribution to Project: Mrs Hale has performed work in the area of study design, data collection and analysis, report and manuscript writing.

5. Name: Juan Carreno
Project Role: Senior investigator
Nearest person month worked: 0.6 month (5% per year)
Contribution to Project: Dr. Carreno has performed work in the area of animal handling and caring, data collection and analysis, report and manuscript writing.

6. Name: Jesus Chavez
Project Role: Histology technician
Nearest person month worked: 4.2 month (35 % per year)
Contribution to Project: Mr. Chavez has performed work in the area of tissue collection, processing, sectioning, and various staining.

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.

Nothing to Report

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.

None.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating 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.

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.

Below please find the 5 accepted abstracts for presentation at 2018 Experimental Biology meeting and the 2018 American Heart Association Meeting

Remote limb ischemic preconditioning improves post-resuscitation long term survival in a rat fixed pressure hemorrhagic shock model

Wangde Dai, Jianru Shi, Juan Carreno, Sharon Hale, and Robert A. Kloner
HMRI Cardiovascular Research Institute, Huntington Medical Research Institutes, 10 Pico Street, Pasadena, CA 91105, and
Division of Cardiovascular Medicine of the Keck School of Medicine, University of Southern California, Los Angeles, California
90017-2395.

Background: We investigated whether bilateral lower limb remote ischemic preconditioning (RIP) could improve long-term survival in a rat model of hemorrhagic shock/resuscitation.

Methods: Sprague Dawley rats (both genders) were anesthetized with intraperitoneal ketamine and xylazine, and were intubated and ventilated with room air. Rats were randomly assigned to RIP (n= 26; induced by inflating small bilateral pressure cuffs to 200 mmHg around the femoral arteries for 5 minutes, followed by 5 minute release of the cuffs, repeated 4 times) or control group (n= 27; the blood pressure cuffs are inflated only to 30 mmHg). After heparinization, hemorrhagic

shock was induced by withdrawing blood from the left carotid artery to a fixed mean blood pressure of 30 mmHg for 30 minutes, followed by 30 min of resuscitation with shed blood. Rats remained anesthetized for one hour during which hemodynamics were monitored (femoral artery catheter); and then allowed to survive for 6 weeks.

Results: The survival rate at 6 weeks was 5 out of 27 rats in the control group (18.5%), and 13 out of 26 rats in the RIP group (50%; $p < 0.05$ by Fisher's exact test). Recovery of blood pressure during the early resuscitation phase was significantly improved and the heart rate was significantly lower in the RIP group compared to the control group (Figure below). At 6 weeks, all of the surviving rats were neurologically intact. Histochemistry/histologic staining was performed in the tissues from 8 survived rats, and showed brain infarction in 1 out of 3 rats in control and 1 out of 5 rats in RIP group; no evidence of myocardial or kidney infarction were detected.

Conclusions: RIP markedly and significantly improved long term survival after experimental hemorrhagic shock/resuscitation and improved recovery of blood pressure in the early phase of resuscitation.

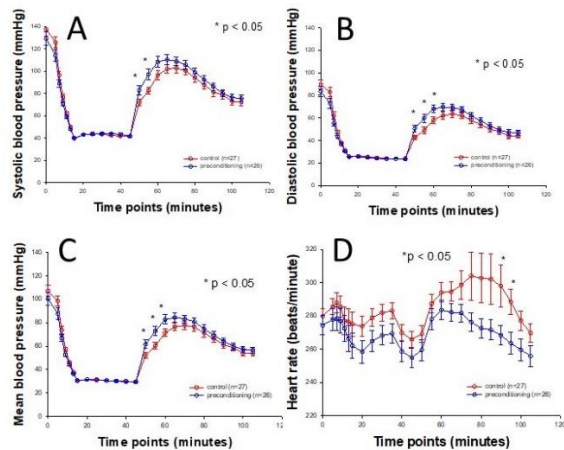


Figure: Blood pressure and heart rate during hemorrhagic shock and resuscitation. Red: control; blue: preconditioning group. (A) Systolic BP; (B) Diastolic BP; (C) Mean BP; (D) Heart rate.

Blood-based biomarkers as early predictor of mortality in experimental hemorrhagic shock

Jianru Shi, Wangde Dai, Juan Carreno, Sharon L. Hale, Robert A. Kloner
 HMRI Cardiovascular Research Institute, Huntington Medical Research Institutes, 10 Pico Street,
 Pasadena, CA 91105, and Division of Cardiovascular Medicine of the Keck School of Medicine,
 University of Southern California, Los Angeles, California 90017-2395.

Background: Hemorrhagic shock remains a major cause of mortality and morbidity on the battle field. Appropriate use of blood-based biomarkers is clinically important to manage patients and predict death versus survival in the early stage of hemorrhagic shock. In continuation of our previous study, blood-based early biomarkers were tested in 94 rats undergoing hemorrhagic shock.

Methods: Sprague Dawley rats (both genders) were anesthetized with either intraperitoneal ketamine and xylazine or with Isoflurane. Rats were intubated and ventilated with room air. After heparinization, hemorrhagic shock was induced by withdrawing blood to a fixed mean

blood pressure of 30 or 40mmHg for 30 minutes, 35 minutes, 45 minutes, or 60 minutes and then the shed blood was reinfused. Arterial blood samples were collected 1-3 hours after resuscitation with shed blood. The rats were allowed to survive for 6 weeks.

Results: The pH, bicarbonate (HCO_3), oxygen saturation ($\text{SO}_2\%$), base excess and calcium levels were significantly lower in rats that died (n=63) versus survivors (n=31). Rats that died had significantly elevated anion gap, potassium, sodium and chloride levels compared to rats that survived (Table). PO_2 , PCO_2 , hematocrit, hemoglobin, glucose and lactate levels are comparable between rats that died versus survived.

Conclusions: The low pH, HCO_3 , $\text{SO}_2\%$, base excess and calcium coupled with high anion gap and high potassium, sodium and chloride levels may help predict long term prognosis in hemorrhagic shock.

Table: Comparison of blood-based biomarker levels between death and survival group. Data are presented as mean $\pm$ SE.

	Death (n=63)	Survival (n=31)	P value
pH	7.34 $\pm$ 0.02	7.43 $\pm$ 0.01	<0.0001
HCO_3 (mmol/L)	16.58 $\pm$ 0.58	19.79 $\pm$ 0.81	0.002
Base Excess (mmol/L)	-7.47 $\pm$ 0.61	-2.98 $\pm$ 0.68	<0.0001
SO_2 %	90.35 $\pm$ 1.09	93.97 $\pm$ 0.97	0.0152

Remote limb ischemic preconditioning improves short and long term survival and maintains intravascular blood volume during resuscitation of hemorrhagic shock

Wangde Dai, Jianru Shi, Juan Carreno, Sharon Hale, and Robert A. Kloner

HMRI Cardiovascular Research Institute, Huntington Medical Research Institutes, 686 South Fair Oaks Avenues, Pasadena, CA 91105, and Division of Cardiovascular Medicine of the Keck School of Medicine, University of Southern California, Los Angeles, California 90017-2395.

Background: We further examined the protective effects of experimental bilateral lower limb remote ischemic preconditioning (RIPC) in rats undergoing experimental hemorrhagic shock.

Methods: Sprague Dawley rats (both genders) were randomized into RIPC (n= 26) or control group (n= 27), and anesthetized with intraperitoneal ketamine/xylazine. RIPC was induced by 4 cycles of inflating small bilateral pressure cuffs to 200 mmHg around the femoral arteries for 5 minutes, followed by 5 minute deflation of the cuffs. Hemorrhagic shock was induced by withdrawing blood from the carotid artery. Target mean blood pressure of 30 mmHg was maintained for 30 minutes followed by reinfusion of shed blood within the next 30 min. Rats remained anesthetized for another 30 min before recovery; endpoints were survival at 72 hours and 6 weeks.

Results: The % of estimated total blood volume withdrawn to maintain a level of 30 mmHg was similar in the RIPC group (41.7 $\pm$ 1.0 %) and control group (41.9 $\pm$ 1.0 %). Recovery of blood pressure during the early resuscitation phase was significantly improved in the RIPC group. The diastolic internal dimension of the left ventricle (echocardiogram), which indicates circulating intravascular blood volume, was significantly larger in the RIPC group at 1 hour after initiation of

shed blood reinfusion (5.8 ± 0.1 mm) compared to 5.4 ± 0.1 mm in the control group ($p=0.04$). Left ventricular fractional shortening was comparable between RIPC (50.9 ± 1.9 %) and control group (49.6 ± 1.8 %; $p=0.64$) at 1 hour after initiation of resuscitation. At 48 hours after shock, BUN was within normal range in the RIPC group (17.3 ± 1.2 mg/dl); but elevated in the control group (22.0 ± 1.7 mg/dl). At 72 hours after hemorrhagic shock injury, 6 of 27 (22.2 %) rats in the control group and 13 of 26 (50 %, $p = 0.047$) rats in the RIPC group survived. At 6 weeks, 5 of 27 (18.5 %) rats in the control group and 13 of 26 (50 %; $p = 0.021$) rats in the RIPC group survived. RIPC significantly increased survival rate at both 72 hours and 6 weeks.

Conclusions: RIPC improved recovery of blood pressure and maintained more circulating intravascular blood volume in the early phase of resuscitation, improved BUN, and markedly and significantly improved short and long term survival in rats subjected to hemorrhagic shock.

Effects of anesthetic agents on the hemodynamic stabilization and long term survival in an experimental model of hemorrhagic shock

Wangde Dai, Jianru Shi, Juan Carreno, Sharon Hale, and Robert A. Kloner

HMRI Cardiovascular Research Institute, Huntington Medical Research Institutes, 686 South Fair Oaks Avenues, Pasadena, CA 91105, and Division of Cardiovascular Medicine of the Keck School of Medicine, University of Southern California, Los Angeles, California 90017-2395.

Background: We investigated the cardiovascular responses to acute bleeding and shed blood restoration under different anesthetic agents, and their effects on long-term survival rate after hemorrhagic shock in rats, after our initial pilot study suggested differences between anesthetics.

Methods: Sprague Dawley rats (both genders) were randomized to receive either intraperitoneal ketamine/xylazine (K/X, 90 mg/kg and 10 mg/kg; $n=12$), or isoflurane (5% isoflurane induction and 2% maintenance in room air; $n=12$) for anesthesia. Blood was withdrawn from the carotid artery to maintain mean arterial blood pressure (MBP) at 30 mm Hg for one hour, followed by 30 min of resuscitation with shed blood. Rats remained anesthetized for another 30 min before they were allowed to recovery and survive for 6 weeks. Hemodynamics were monitored during the surgical procedure.

Results: During the shock phase, the total withdrawn blood volume (expressed as % of estimated total blood volume) to maintain MBP at 30 mmHg was significantly higher in the isoflurane group (51 ± 1.5 %) compared to the K/X group (45.3 ± 1.8 %; $p=0.023$). The diastolic internal dimension of the left ventricle, which indicated circulating intravascular blood volume, was significantly larger in the isoflurane group at the end of 1 hour of the shock phase (4.5 ± 0.2 mm compared to 3.5 ± 0.2 mm in K/X group; $p=0.0003$) and at 1 hour after initiation of shed blood reinfusion (6.3 ± 0.2 mm compared to 5.3 ± 0.3 in K/X group; $p=0.014$). Recovery of blood pressure during the resuscitation phase was significantly improved in the isoflurane group compared to the K/X group. The survival rate at 6 weeks was 1 of 12 (8.3%) in rats receiving K/X and 10 of 12 (83.3%) in rats receiving isoflurane ($p < 0.001$). Histology demonstrated brain infarction in the 1 surviving rat receiving K/X; no brain infarction in the 10 surviving rats that received isoflurane at 6 weeks. No infarction was detected in heart, lung, liver or kidneys in all surviving rats.

Conclusions: These results suggest that isoflurane stabilizes the cardiovascular response to acute blood loss and benefits the perfusion of tissue, which resulted in significantly higher long term survival rate and improved blood pressure response to resuscitation, without end-organ infarction.

Effects of anesthetic agents on blood parameters in rats with acute hemorrhagic shock

Jianru Shi, Wangde Dai, Juan Carreno, Sharon L. Hale, Robert A. Kloner

HMRI Cardiovascular Research Institute, Huntington Medical Research Institutes, 686 South Fair Oaks Avenues, Pasadena, CA 91105, and Division of Cardiovascular Medicine of the Keck School of Medicine, University of Southern California, Los Angeles, California 90017-2395.

Background: Recent study indicates that isoflurane (ISO) has protective effects including improved survival in rats with hemorrhagic shock compared to ketamine and xylazine (K/X) anesthesia in our laboratory. The effect of these two anesthetic agents upon blood counts, gases and chemistries in the setting of hemorrhagic shock is unknown. The purpose of the present study was to examine the effects of these two commonly used anesthetic regimens on blood parameters in rats with acute hemorrhagic shock.

Methods and results: Sprague Dawley rats (both genders) were anesthetized with either intraperitoneal K/X (90mg/kg and 10mg/kg; n=12) or with isoflurane (5% isoflurane induction and 2% maintenance in room air; n=12). Rats were intubated and ventilated with room air. After heparinization, hemorrhagic shock was induced by withdrawing blood to a fixed mean blood pressure of 30 mmHg for one hour and then shed blood was reinfused. Arterial blood samples were collected at 1 hour after resuscitation with shed blood. We found that K/X was associated with lower PH and lower level of standard bicarbonate concentration (SBC) and oxygen saturation (SO₂%) and more negative base excess; and had a significantly elevated anion gap, potassium, sodium and chloride levels compared to isoflurane (Table). Platelet counts were preserved and there was less elevation of white blood cell (WBC) in ISO (Table). There were no significant differences in PO₂, PCO₂, hematocrit, hemoglobin, glucose and lactate levels between the two types of anesthesia.

Conclusions: The anesthesia influenced the levels of blood counts, gases and chemistries in rats with acute hemorrhagic shock, favoring ISO over K/X. Blood parameters remained essentially normal in ISO group, which may help explain the protective role of ISO in hemorrhagic shock.

Table: Comparison of blood parameters between K/X and ISO group. Data are presented as mean $\pm$ SE. p<0.05 vs ISO.

	K/X (n=12)	ISO (n=12)	P value
pH	7.32 $\pm$ 0.04	7.47 $\pm$ 0.02	0.0025
SBC (mmol/L)	18.22 $\pm$ 1.08	23.15 $\pm$ 0.87	0.0019
Base Excess (mmol/L)	-7.80 $\pm$ 1.45	-1.57 $\pm$ 1.02	0.0023
SO ₂ %	91.91 $\pm$ 1.66	96.57 $\pm$ 0.40	0.0177
AnionGap (mmol/L)	12.8 $\pm$ 1.07	8.81 $\pm$ 0.98	0.0119
Sodium (mmol/L)	137.82 $\pm$ 1.06	133.62 $\pm$ 0.71	0.0038
Potassium (mmol/L)	5.98 $\pm$ 0.31	4.82 $\pm$ 0.21	0.0064

Chloride (mmol/L)	114.33 ± 1.38	109.23 ± 0.85	0.0056
Platelet counts (K/uL)	441.08 ± 51.00	597.42 ± 34.59	0.0199
WBC (K/ul)	9.88 ± 0.80	7.23 ± 0.82	0.0304