AD_____

AWARD NUMBER: W81XWH-17-2-0053

TITLE: A Novel Advanced Resuscitation Fluid for Traumatic Brain Injury with Hemorrhagic Shock

PRINCIPAL INVESTIGATOR: Denis E. Bragin

RECIPIENT: University of New Mexico
Albuquerque, NM, 87131

REPORT DATE: October 2018

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE October 2018

2. REPORT TYPE Annual Report

3. DATES COVERED
15 Sep 2017-14 Sep 2018

4. TITLE AND SUBTITLE
A Novel Advanced Resuscitation Fluid for Traumatic Brain Injury with Hemorrhagic Shock

5a. CONTRACT NUMBER

5b. GRANT NUMBER W81XWH-17-2-0053

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S) Denis E Bragin

E-Mail: dbragin@salud.unm.edu

5d. PROJECT NUMBE

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

University of New Mexico Health Sciences Center
1 University of New Mexico, MSC 10 6515, Neurosurgery, Albuquerque, NM, 87131, USA

McGowan Institute for Regenerative Medicine, University of Pittsburgh
450 Technology Drive, Suite 300
Pittsburgh, Pennsylvania
15219, USA

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

10. SPONSOR/MONITOR'S ACRONYM(S)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Traumatic brain injury (TBI) is frequently accompanied by hemorrhagic shock (HS) which significantly worsens neurologic outcome, and increases mortality. Current resuscitation fluids (RF) for volume expansion after TBI with HS do not adequately ameliorate impaired microvascular cerebral blood flow (mvCBF). We suggested the addition of drag reducing polymers (DRP) to resuscitation fluid (DR-RF) for TBI with HS which will reduce the severity of brain injury, increase survival rate, improve neurologic recovery and will reduce the volume of resuscitation fluid required to prevent the transition to an irreversible stage and death or functional impairment of the brain. The purpose for the proposed research is to apply DRP as an additive to resuscitation fluids after TBI with HS, to determine which mechanisms are affected by DRP in the acute and late recovery phases and to define most effective parameters for application. During reported period we showed that colloid, hypertonic and colloid-based DRP-RF significantly improves cerebral regional and microvascular circulation and tissue oxygenation impaired by TBI/HS. Effect lasts at least 6 hours. Colloid-based DRP-RF was more effective than crystalloid and hypertonic—based DRP-RF tested. We have also done evaluation of TBI/HS-induced metabolic stress of mitochondria, hypoxia, neuronal survival and microthrombosis and beneficial effects of DRP-RF-vs. RF. Sub-Contractor performed experiments on DRP characterization and storage and drag reduction test circuit development. The results were presented on 4 conferences, one manuscript published, one accepted and two are in preparation.

15. SUBJECT TERMS
Traumatic Brain Injury with Hemorrhagic Shock, Resuscitation Fluid, Drag Reducing Polymers, Animal Models, Cerebral Microcirculation, Neuroprotection.

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	34	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

	Page No.
1. Introduction	1
2. Keywords	1
3. Accomplishments	1
4. Impact	11
5. Changes/Problems	12
6. Products	13
7. Participants & Other Collaborating Organizations	16
8. Special Reporting Requirements	18
9. Appendices	attached

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Traumatic brain injury (TBI) is frequently accompanied by hemorrhagic shock (HS) which significantly worsens neurologic outcome, and increases mortality. Current resuscitation fluids (RF) for volume expansion after TBI with HS do not adequately ameliorate impaired microvascular cerebral blood flow (mvCBF). In our previous studies in a rat TBI model, we have shown that nanomolar concentrations of intravascular blood soluble drag reducing polymers (DRP) significantly enhanced microvascular perfusion and tissue oxygenation in peri-contusional areas thereby protecting neurons. We hypothesized the addition of DRP to resuscitation fluid (DR-RF) for TBI with HS reduces the severity of injury, increases survival rate, improves neurologic recovery and will reduce the volume of resuscitation fluid required to prevent the transition to an irreversible stage and death or functional impairment of the brain. The purpose for the proposed research is to apply DRP as an additive to resuscitation fluids after TBI with HS, to determine which mechanisms are affected by DRP in the acute and late recovery phases and to define most effective parameters for application. The proposal fits well with all 3 Focus Areas of PFCRA: 1) Understand the clinical implications of PFC and pDCR, including "physiological parameters requiring intervention to reduce morbidity and mortality during the acute treatment of TBI and mitigation of the pathophysiology of prolonged hypotension"; 2) Develop next-generation resuscitation methods for PFC and pDCR, including "novel or improved methods for resuscitation and stabilization of TBI/HS, with or without other concomitant injuries; and 3) Develop enhanced treatment of injuries during PFC and pDCR, including "TBI treatments to reduce tissue loss, ischemia, secondary injury mortality and improve outcomes.

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

Traumatic Brain Injury with Hemorrhagic Shock, Resuscitation Fluid, Drag Reducing Polymers, Hemorheological Approach, Animal Models, Cerebral Microcirculation, Neuroprotection

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: Elucidate the major mechanisms and beneficial effects of DRP resuscitation fluid (DRP-RF) infused in animals at the "pre-hospital" acute phase up to 6 hours after traumatic brain injury with hemorrhagic shock (TBI/HS) compared to crystalloid or colloid fluids in controls and contrasted to HS or TBI only using sham as a control

Major Task 1: Evaluate effectiveness of DRP-RF in improvement of microvascular cerebral blood flow and preventing blood brain barrier degradation

Subtask 1. Laser speckle contrast imaging of changes in regional cerebral blood flow (rCBF) – 1-24 months – 45%

Subtask 2. Two-photon microscopy of changes in microvascular and quantitation of capillary density – 1-24 months – 40%

Major Task 2: Evaluate efficacy of DRP-RF in improving oxygen delivery to brain tissue after TBI/HS

Subtask 1. Multispectral optical intrinsic signal imaging of regional changes of oxy- and deoxy-hemoglobin concentration 1-24 months – 40%

Subtask 2. Two-photon microscopy of change in brain tissue oxygenation via nicotinamide adenine dinucleotide (NADH) fluorescence imaging 1-24 months – 50%

Major Task 3: Evaluate the effect of DRP-RF on oxidative stress and survival of neurons after TBI/HS

Subtask 1. 2PLSM imaging of i.v. injected hydroethidine to visualize superoxide – a major component of oxidative stress density – 1-24 months – 45%

Subtask 2. 2PLSM imaging of i.v. injected propidium iodide for visualization of dying neurons – 1-24 months – 40%

Major Task 4: Test the effect of DRP-RF in TBI/HS by physiological monitoring

Subtask 1. Monitoring of changes in physiological parameters including intracranial pressure, mean arterial pressure, pulse rate, cortical Doppler flow, and analysis of blood gases, electrolytes, hemoglobin, glucose/lactate, pH and coagulation – 1-24 months – 45%

Subtask 2. Evaluation of quantitative changes in cerebrovascular autoregulation with DRP-RF compared to crystalloid and colloid fluid resuscitation after TBI/HS– 1-24 months – 40%.

Sub Aim 1a: Optimization of the DRP-RF preparation process for combat casualty use

Major Task 1: Optimization of a process of preparation, sterilization, and storage conditions for creation of the concentrated DRP-RF which will be usable within a few minutes – 1-30 months – 30%

Specific Aim 2: Compare the beneficial effects of DRP-RF on long-term recovery and neurologic outcomes compared to crystalloid and colloid fluid treatments for up to 4 weeks after TBI/HS.

Major Tasks 1-4: – 20-36 months – 0%

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.

For the reported period, we were focused on Specific Aim 1 – Elucidation of the major mechanisms and beneficial effects of DRP resuscitation fluid (DRP-RF) infused in animals at the "pre-hospital" acute phase up to 6 hours after traumatic brain injury with hemorrhagic shock (TBI/HS) compared to crystalloid or colloid fluids in controls and contrasted to HS or TBI only using sham as a control.

This included four Major Tasks evaluated: Improvement of microvascular cerebral blood flow and preventing blood brain barrier degradation (1); Improving oxygen delivery to brain tissue (2); the effect of DRP-RF on oxidative stress and survival of neurons (3); and physiological monitoring (4).

On Fig. 1 is presented general experimental protocol which, with variations, was used for the reporting period. TBI was induced after baseline in-vivo 2-photon laser scanning microscopy (2PLSM) and followed by a 1-h hemorrhagic phase (Battlefield), where blood was slowly withdrawn through the femoral vein to reduce mean arterial pressure (MAP) to 40 mmHg. In the following 1-h pre-hospital care phase (Transportation, PFC), resuscitation fluids (LR-RF or DRP-RF) were slowly infused i.v. to raise MAP to ~55 mmHg and CBF to ~65% of baseline. In a subsequent 3-h definitive hospital care phase, shed blood was re-infused to a MAP of 70 mmHg and CBF of ~75% of baseline. *In vivo* 2PLSM or LSCM were done throughout the study over the parietal cortex of the rat brain. Brain and rectal temperatures were monitored and maintained at 38 ± 0.5°C. Arterial blood gases, electrolytes, hematocrit and pH were measured hourly (epoc Blood Analysis System, Alere Inc., Waltham, MA, USA).

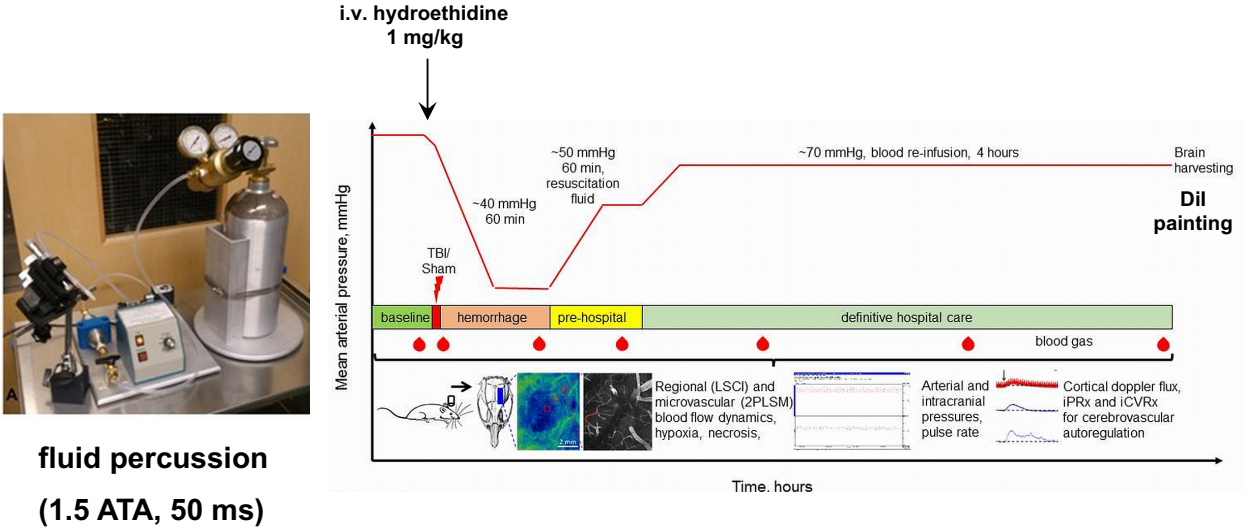


Fig. 1 Experimental Protocol

Experiments on rats with crystalloid RF vs crystalloid RF+DRP and colloid RF vs colloid RF+DRP were performed. Laser speckle contrast imaging and two-photon microscopy showed that addition of DRP to RF significantly improves cerebral regional and microvascular circulation and tissue oxygenation, reduced by TBI/HS (Fig. 2). The effect persisted during the whole monitored period (6 hours). The results are presented in the published manuscript attached. Colloid-based DRP-RF was more effective than crystalloid, and hypertonic—based DRP-RF.

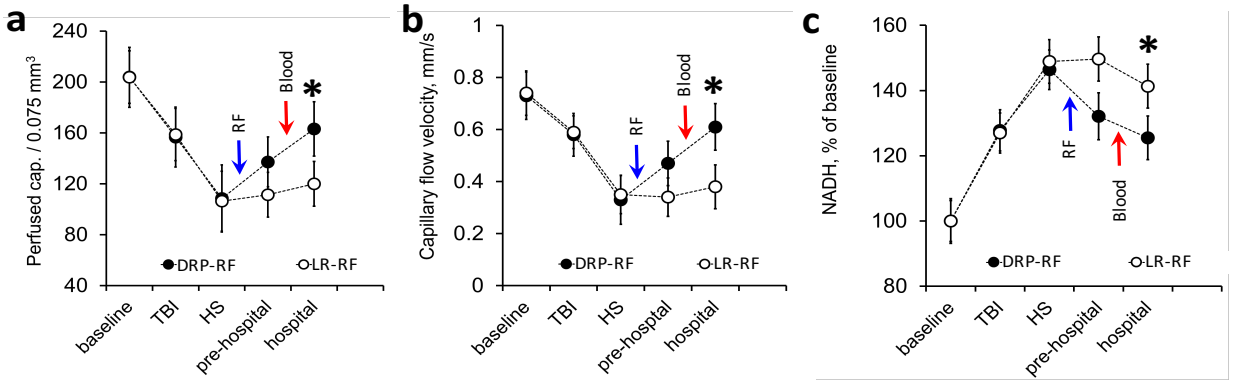


Fig. 2 Resuscitation with crystalloid based DRP-RF improves cerebral microvascular perfusion and tissue oxygenation impaired by TBI/HS, as shown by a) increased number of perfused capillaries; b) increased capillary flow velocity; c) increased tissue oxygenation (NADH decrease). Mean ± SEM, N=10 rats per group, *P < 0.05 from the LR-RF group.

We have evaluated TBI/HS-induced metabolic stress of mitochondria that leads to excessive oxidative phosphorylation and the increased production of reactive oxygen species (ROS) such as superoxide. Using 2PLSM imaging of i.v. injected hydroethidine to visualize superoxide, we have shown that TBI/HS lead to oxidative stress which was less in a group resuscitated with DRP added fluid (Fig. 3). The results are presented in the accepted manuscript attached.

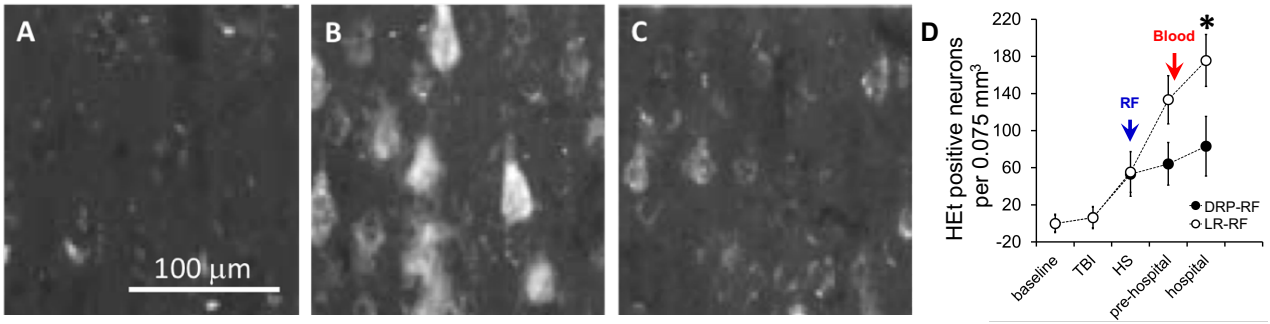


Fig. 3 Resuscitation with DRP-RF reduces superoxide production in cortical neurons after TBI with HS: a) Representative image of a rat cortex at baseline without ET positive neurons; b) Neurons with diffuse cytosolic ET fluorescence in a rat cortex from LR-RF group by the end of experiment; c) and from DRP-RF group; The dynamics of the increase in ET positive cortical neurons. Mean ± SEM, N=10 rats per group, *P < 0.05 from the LR-RF group.

Using DiI vascular painting technique, we have evaluated microvascular changes in extracted brain and found massive microthrombosis in both, contralateral and ipsilateral to trauma hemispheres, that was less in DRP-RF groups. In the injured hemisphere in DRP-RF, microvascular density was higher than in LR-RF (% vessel/total area*100 was 4.9 ± 0.4 vs. 3.1 ± 0.3, respectively, $p<0.05$) as oppose 6.8 ± 0.4 to in Sham rat. In contralateral to the injury hemisphere, microvascular density was also reduced (% vessel/total area*100 was 6.1 ± 0.5 vs 5.2 ± 0.5, in DRP-RF, vs. LR-RF, respectively, $p<0.09$) (Fig. 4). The results are presented in the accepted manuscript attached.

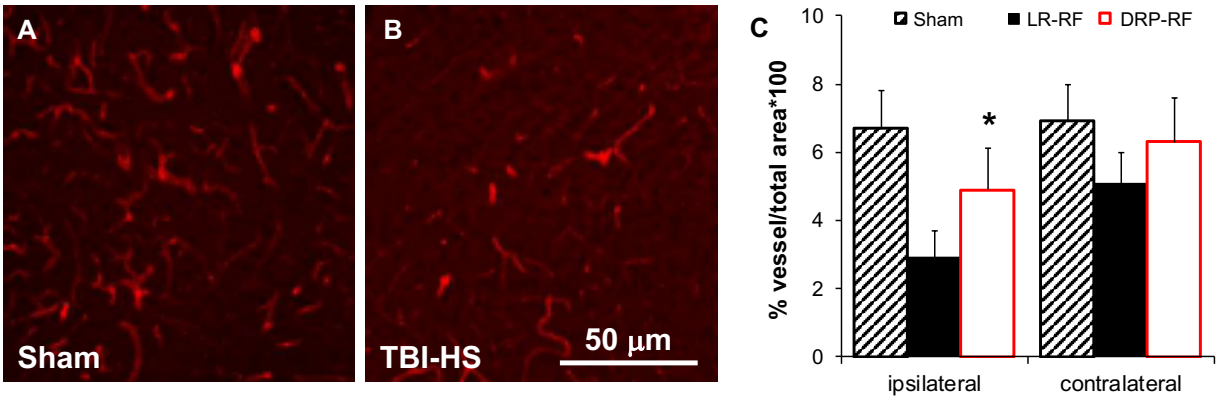


Fig. 4 Resuscitation with DRP-RF reduces microthrombosis in both hemispheres after TBI with HS as shown by post-mortem DiI vascular painting. a) Cortical microvascular network in Sham mouse brain; and b) after TBI with HS; c) Graph showing reduced cortical microvasculature in LR-RF group and better-preserved microvasculature in DRP-RF group in both, traumatized and contralateral hemispheres. Mean ± SEM, N=10 rats per group, *P < 0.05 from the LR-RF group.

To evaluate *neuronal survival*, 200 µL of a propidium iodide (PI)/saline, which labels only necrotized cells with damaged membrane, was injected intravenously during surgical preparation. CBF and tissue oxygenation reduction after TBI/HS caused progressive necrosis of neurons. DRP-RF reduced progression of necrosis of neurons while standard RF (LR) did not decrease dynamic of necrosis. Fig. 5). The results are presented in the published manuscript attached.

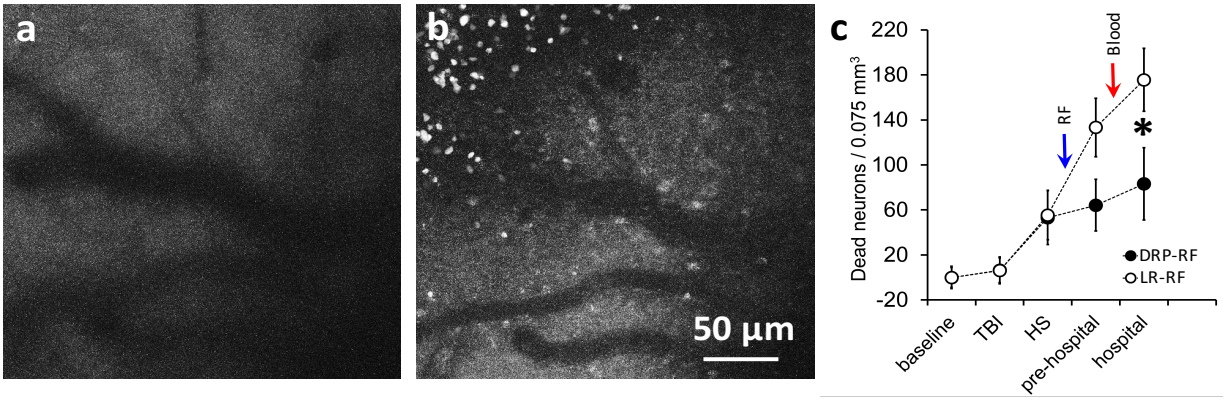


Fig. 5 Resuscitation with DRP-RF is neuroprotective: a) 2PLSM image of a rat cortex at baseline without dead neurons; b) Propidium Iodide stains neurons with damaged membranes reflecting necrosis of neurons after TBI/HS; c) DRP-RF protects neurons from necrosis (*=P<0.05). Mean ± SEM, N=10 rats per group, *P < 0.05 from the LR-RF group.

The anticipated parts of Major Tasks 1-4 of the Specific Aim 1, and thus, this period goals, were completed

Sub-Contractor performed experiments on (Sub-Aim 1a, Major Task 1). Optimization of the DRP-RF preparation process for combat casualty use.
The goal of is to develop a strong protocol for polyethylene oxide (DRP-RF) preparation and storage which can be used without delay and degradation of molecules in solutions.

Here is a short description of the properties and quality of the polyethylene oxide (PEO) which was selected by our research group as a candidate for preclinical and clinical use.

Very low concentrations of the high molecular weight (4000 – 4500 kDa) PEO of linear structure and water-soluble polymer is able to reduce the turbulent frictional drag of the water by as much as 80%. The flexibility of ether linkages combined with the extremely high molecular weight of water-soluble PEO produces solutions with elastic behavior. At high concentrations, with good lubricating, binding and film forming properties, PEO (POLYOX^{TM}) retards the release rate of drug/s and hence is widely used in pharmaceutical formulations like controlled release dosage forms, hot-melt technology and mucoadhesive dosage forms [; Ma L, Deng L, Chen J. Applications of poly(ethylene oxide) in controlled release tablet systems: a review. Drug Development and Industrial Pharmacy. 40(7):845-851; 2013]. PEO water-soluble molecules are nontoxic and have received FDA approvals for a number of food and drug applications. Aqueous solutions of PEO are environmentally degradable due to oxidation and aerobic biodegradation. Water-soluble PEO are nontoxic and have received FDA approvals for a number of applications [Shah AP and Bhandary SR. POLYOX (polyethylene oxide) - applications in pharma industry. Pharmaceutical Reviews 8(3) 2010].

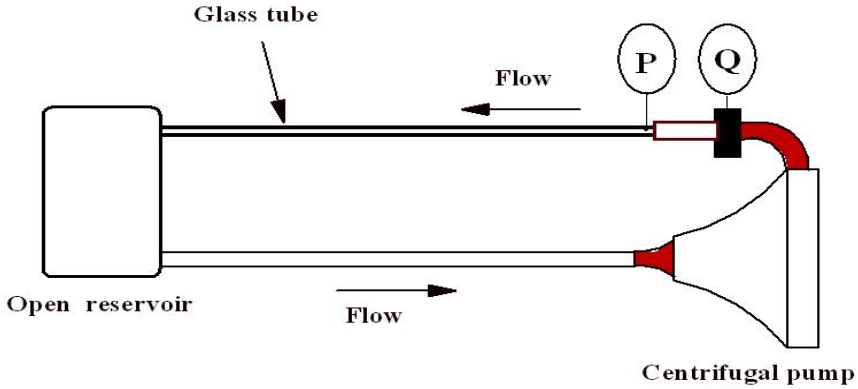
The major problem of producing DRP solutions in advance is their mechanical stress- and storage time-related degradation, which currently makes it necessary to prepare the injectable solution from commercial DRP powder prior to each animal experiment. In this Sub-Aim we planned to develop and optimize a novel process of preparation, sterilization, and storage conditions for creation of the concentrated DRP-RF (PEO) solutions which would be **Figure 1** few minutes which will be needed for quick defrosting and dilution of the stored frozen concentrated solution when it is needed for treatment. The following parameters will be optimized to create DRP solutions which will have little or no degradation after dissolving, dialysis, sterilization, and frizzing storage: optimal DRP concentrations in solutions exposed to sterilization via filtration, variation of freezing temperatures and storage time, exposure to rapid freezing and thawing processes, and quick dilution to nanomolar concentrations for IV injections or as an additive to the resuscitation fluid.

These solutions are tested in the turbulent flow system to confirm the polymer drag reducing properties (the most important physical properties of the DRP solutions), and in the measurement of polymer viscoelastic properties over a large range of shear rates relevant to those in vascular system.

We tested sterilization procedure on PEO to confirm the optimal concentration and filtration conditions to preserve drag reducing and viscoelastic properties of these polymers. Filtration was performed at -200 and -600 mmHg using the Millex Flip-Cup 0.22 um filters with a PEO concentration of 1500 ppm. Following the filtration experiments, we tested filtered solutions using a viscoelastometer (Vilastic) and compared results to the unfiltered polymer solutions. We found that filtration of PEO (linear molecular structure) solution had little or no effect on the drag-reducing ability and viscoelastic properties of the PEO after a short filtration process (about10 min)

Preparation of PEO solutions for optimizations and stability toward it storage: Since DRPs have a tendency to mechanical degradation over the time and due to exposure to high shear stress conditions, special care should be taken to prevent polymer degradation due to handling. The DRP-RF used in most of our in vivo and in vitro experiments, PEO with MW ~ 4000 kDa (Sigma-Aldrich, Saint Louis, MO). The powder of PEO was dissolved in sterile phosphate buffered saline (PBS) at concentrations of 4000 ppm and 1000 ppm. The solutions were tested in the turbulent flow system to verify drag-reducing efficiency.

Drag reduction test circuit: Mechanical degradation of drag reducing polymer molecules dissolved in fluid diminish their drag reducing ability. At the beginning experiments in this program, our lab has used pumps commonly used for clinical circulatory support (Centrifugal pump Medtronic BioMedicus) to induce shear mediated degradation of polymer solutions in a turbulent flow system. However, the shear profile within these pumps and the total amount of accumulated shear stress delivered to the polymer solution ultimately which leads to polymer degradation and reduced drag reduction is not well characterized. Prior several month experiments comparing storage conditions at 4 ºC vs. -80 ºC and sample storage under Argon gas vs. room air yielded inconclusive results which we hypothesized to be due quick PEO degradation generated by the pump which prohibited recording accurate data collection on storage degradation.



New gravity driven turbulent flow system: This system is gravity-driven (ΔP =~200 mmHg) and consists of a tube with 0.25-inch inner diameter that produces turbulent flow with Reynolds numbers greater than 12,500 (development turbulence). Pressure and flow parameters are recorded for each run of the PEO solution, and drag reduction, as well as shear stress and shear rates, are calculated. The effect of the shear stress on the drag reducing ability is quantified and compared across solutions stored for different lengths of time. The reproducibility of this new method is also tested by comparing the effect the system has on PEO degradation each test day, keeping all other parameters equal.

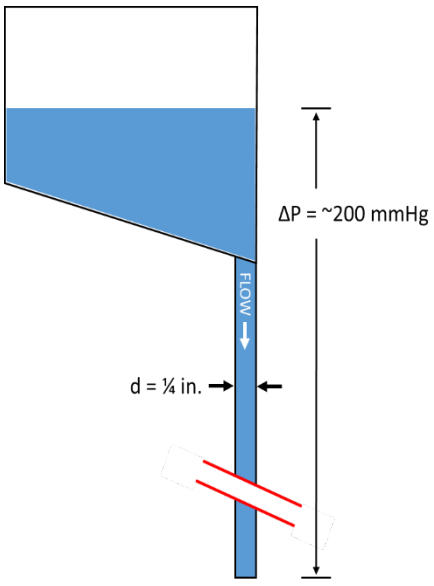


Figure 2

Each tested PEO solution was prepared by dilution of 4000 ppm and 1000 ppm to 10 ppm before it started to run through the turbulent system. Then, viscosity and elasticity of the original PEO solutions were measured using a Vilastic-3 viscoelasticity analyzer (Vilastic Inc, Austin, TX). Our current results indicate that there is no significant decrease of the PEO solution effectiveness as storage time increases. This indicates that refrigerating PEO solutions at either concentration may be viable long-term storage options. Each sample was run through the system 15 times and the time the solution took to reach the beaker was measured. Drag reduction was calculated for each run using the following equation:

$$\boldsymbol{drag\ reduction\ (DR)\%} = \frac{\boldsymbol{time_{H2O} - time_{trial}}}{\boldsymbol{time_{H2O}}}$$

Degradation of PEO caused by turbulent flow is demonstrated in Tables 1 and 2 and in graphs presented the effect of number of runs on increase of flow time due to degradation of the PEO by exposure to turbulent flow.

Table 1: Drag reduction calculated for the first and last run of the 1000 ppm PEO

	Drag Reduction%				
Run #	Week 2	Week 3	Week 4	Week 5	Week 6
1	33.9	35.1	37.7	37.6	38.8
15	12.0	16.0	17.3	17.4	16.0

Table 2: Drag reduction calculated for the first and last run of the 4000 ppm PEO

	Drag Reduction%				
Run #	Week 2	Week 3	Week 4	Week 5	Week 6
1	34.9	32.0	37.6	37.0	38.1
15	12.8	16.0	14.8	16.4	19.3

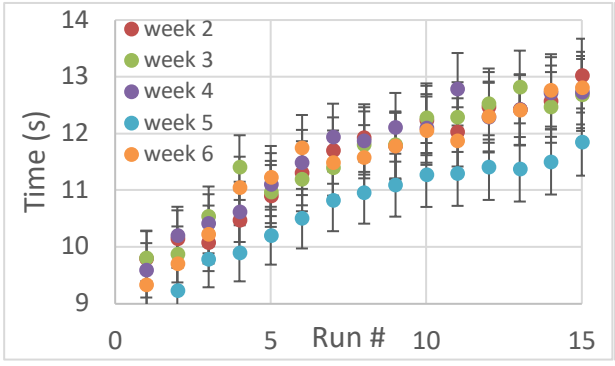


Figure 3: Effect of number of runs on time length of run the samples prepared from 4000 ppm PEO solution

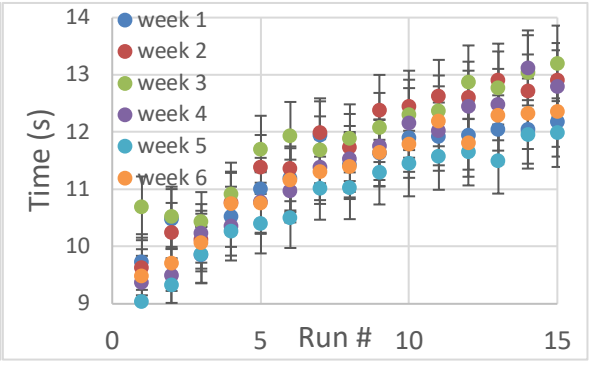


Figure 4: Effect of number of runs on time length of run the samples prepared from 1000 ppm PEO solution

PEO solutions characterization and storage: Polyethylene oxide solutions (4M MW, Sigma Aldrich, USA) at concentrations of 1000 ppm and 4000 ppm were prepared and their viscosity and elasticity were measured using the Vilastic-3 viscoelasticity analyzer (Vilastic Scientific, Inc., Austin, TX, USA) over shear rates range from 1 to 500 s^{-1} (presented in the graphs below). Higher repeatability using the Vilastic-3 instead of a Brookfield rheometer, with which prior storage tests were conducted will better depict changes in DRP due to storage degradation. Solutions of prepared mixtures were separated into aliquots and stored at 4ºC for preparation of our now ongoing storage degradation study using our new flow system. Samples will be measured weekly for DRP viscosity and elasticity as well as drag reducing ability (see below) to ensure sample viability.

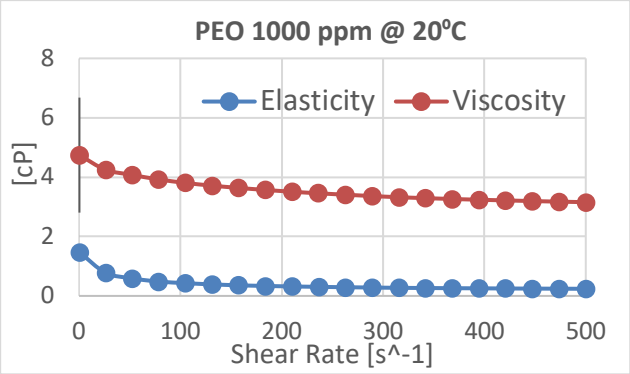


Figure 5: Viscosity and elasticity of the PEO-1000 solution recorded by Vilastic-3 viscoelasticity analyzer

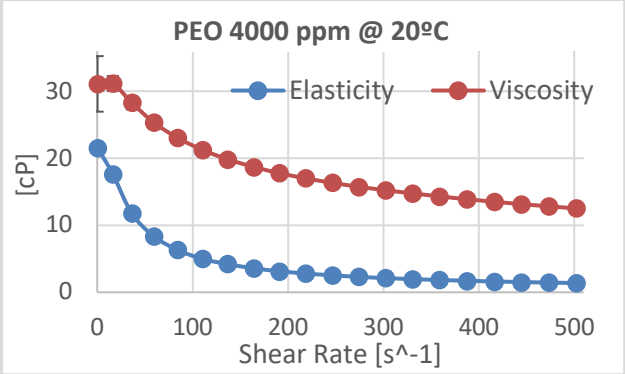
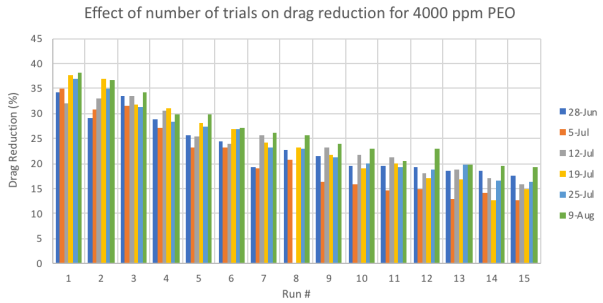
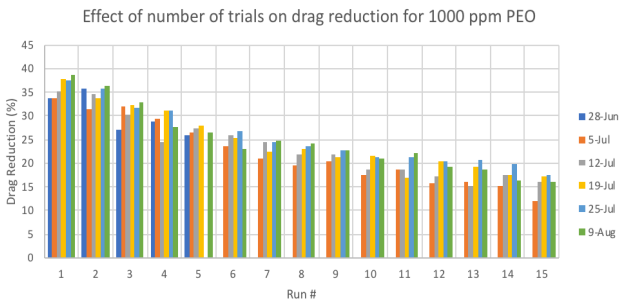


Figure 6: Viscosity and elasticity of the PEO-4000 solution recorded by Vilastic-3 viscoelasticity analyzer

The two pictures below demonstrate the diminished drag reduction during 15 runs of the samples obtained from PEO-4000 and PEO-1000 solutions which were stored over 5 weeks in refrigerator and were tested every week.





Conclusions: These experiments demonstrated that while PEO solutions of high concentration were not very sensitive to storage in refrigerator. The variation of a drag reduction at the first run is mostly related to the procedure steps from worming the sample to a room temperature and dilution for injection. To develop a strong protocol for producing the primary concentrated PEO solution and its storage, from which DRP-RF will be prepared and used without delay and with no degradation of the polymer molecules in solutions, we will continue to vary thickness/concentration of the original solutions to be frozen, and to optimize volume of the remedy samples.

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.

Local Public and Scientific Community:

1. Poster presentation at UNM HSC Neuroscience Day, Albuquerque, NM, 03/16/2018 – "Novel Advanced Resuscitation Fluid for TBI with HS"

National and International Scientific Community:

1. Oral presentation at the Meeting of the International Society on Oxygen Transport to Tissue (ISOTT), Seoul, S. Korea, July 1-5, 2018 – "Resuscitation with drag reducing polymer after traumatic brain injury with hemorrhagic shock reduces microthrombosis and oxidative stress"
2. Poster presentation at the Joint Symposium of the International and National Neurotrauma Societies and AANS/CNS Section on Neurotrauma and Critical Care, Toronto, Canada, August 11-16, 2018: – "Resuscitation with drag reducing polymer reduces microthrombosis and oxidative stress after traumatic brain injury with hemorrhagic shock"
3. Oral Presentation at the Military Health System Research Symposium, Kissimmee, FL, August 20-23, 2018 – "A novel advanced resuscitation fluid with drag reducing polymer enhances cerebral microcirculation and tissue oxygenation after traumatic brain injury complicated by hemorrhagic shock"

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

To continue working on specified tasks according to SOW. I plan to finish Major tasks 1-4 of the Specific Aim 1 by the end of the next period, and to start working on tasks of the Specific Aim 2.

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).

During this year we obtained principal evidences of neuroprotective efficiency of our DRP-enhanced resuscitation fluid. We have also developed, for the first time, in-vivo two-photon imaging of superoxide (hydroethidine) in a rat cortex. Previous studies we performed on post-mortem sections. Further study will lead to the development to the new, neuroprotective strategy of field resuscitation in patients with TBI and HS due to polytrauma.

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.*

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.*

By presenting our results on local, national and international conferences we improved public knowledge about novel resuscitation strategy we are developing in in lay and scientific, as well as civilian and military communities

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.

No significant changes anticipated

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.

No problems encountered

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.

We had a several changes during reporting period that affected expenditures resulting in less cost than anticipated. Among them are delays in hiring technical staff and 2 months outage of the two-photon microscope

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.

No changes

Significant changes in use of biohazards and/or select agents

No changes

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).*

Bragin DE, Lara DA, Bragina OA, Kameneva MV, Nemoto EM. Resuscitation Fluid with Drag Reducing Polymer Enhances Cerebral Microcirculation and Tissue Oxygenation After Traumatic Brain Injury Complicated by Hemorrhagic Shock. Adv Exp Med Biol. 2018; 1072:39-43. doi: 10.1007/978-3-319-91287-5_7. PubMed PMID: 30178321. D.E. Bragin, D.A. Lara, O.A. Bragina, M.V. Kameneva and E.M. Nemoto. Resuscitation with Drag Reducing Polymer after Traumatic Brain Injury with Hemorrhagic Shock Reduces Microthrombosis and Oxidative Stress, *Adv Exp Med Biol.* Accepted, August 2018

The manuscript, describing the beneficial effects of advanced resuscitation fluid on cerebral microcirculation and metabolism in TBI with HS is in preparation.

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.*

Abstracts

1. D. Bragin, D, Lara, O. Bragina, M. Kameneva, E. Nemoto. Novel Advanced Resuscitation Fluid for TBI with HS, Abstract Book for UNM Neuroscience Day, Albuquerque, NM, 03/16/2018, P. 31.

2. D. Bragin, O. Bragina, L. Berliba, M. Kameneva, E. Nemoto. Resuscitation with drag reducing polymer after traumatic brain injury with hemorrhagic shock reduces microthrombosis and oxidative stress, Abstract Book for the International Society on Oxygen Transport to Tissue, 2018, P. 49.
3. D. Bragin, O, Bragina, M. Kameneva, E. Nemoto. Resuscitation with drag reducing polymer reduces microthrombosis and oxidative stress after traumatic brain injury with hemorrhagic shock, J. Neurotrauma, 35, A-136. (Abstracts for National Neurotrauma Symposium 2018).

Conference Presentations:

1. UNM HSC Neuroscience Day, Albuquerque, NM, 03/16/2018 – "Novel Advanced Resuscitation Fluid for TBI with HS" – Poster presentation

2. International Society on Oxygen Transport to Tissue (ISOTT), Seoul, S. Korea, July 1-5, 2018 – "Resuscitation with drag reducing polymer after traumatic brain injury with hemorrhagic shock reduces microthrombosis and oxidative stress" – Oral presentation

3. Joint Symposium of the International and National Neurotrauma Societies and AANS/CNS Section on Neurotrauma and Critical Care, Toronto, Canada, August 11-16, 2018: – "Resuscitation with drag reducing polymer reduces microthrombosis and oxidative stress after traumatic brain injury with hemorrhagic shock" – Poster presentation

4. Military Health System Research Symposium, Kissimmee, FL, August 20-23, 2018 – "A novel advanced resuscitation fluid with drag reducing polymer enhances cerebral microcirculation and tissue oxygenation after traumatic brain injury complicated by hemorrhagic shock" – Oral Presentation

- **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. In addition to a description of the technologies or techniques, describe how they will be 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. 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.

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;*
  - *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.*

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."

Name:	*Denis Bragin*
Project Role:	*PI*
Nearest person month worked:	*5*
Contribution to Project:	*Dr. Bragin performed optical imaging, data interpretation and manuscripts preparation.*
Funding Support:	*NIH-NIGMS P20 GM109089* *NIH-NINDS 1R01NS082225* *NIH-NINDS 1R21NS091600*

Name:	*Edwin Nemoto*
Project Role:	*Co-investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*0.6*
Contribution to Project:	*Dr. Nemoto has performed data analysis and interpretation.*
Funding Support:	*NIH-NIGMS P20 GM109089* *NIH-NINDS 1R01NS082225* *NIH-NINDS 1R21NS091600* *Neurosurgery Department Fund*

Name:	*Tongsheng Zhang*
Project Role:	*Investigator*
Nearest person month worked:	*1.2*
Contribution to Project:	*Dr. Zhang has performed data analysis.*
Funding Support:	*Albuquerque Magnetic Resonance, Inc*

Name:	*Lucy Berliba*
Project Role:	*Research Specialist*
Nearest person month worked:	*7.2*
Contribution to Project:	*Mrs. Berliba has performed rat surgeries and physiological monitoring.*
Name:	*Olga Bragina*
Project Role:	*Sr. Research Specialist*
Nearest person month worked:	*0.0*
Contribution to Project:	*She has performed histochemistry.*
Funding Support:	*NIH-NIGMS P20 GM109089* *Neurosurgery Department Fund*

Sub-award

Name:	*Marina Kameneva*
Project Role:	*Sub-award PI*
Nearest person month worked:	*1.2*
Contribution to Project:	*Involved in development and testing of a novel process of preparation and storage conditions for DRP solutions.*
Funding Support:	*NIH-NHLBI R01 HL089456* *Commonwealth of PA.*
Name:	*Sarah Tolaymat*
Project Role:	*Undergraduate Student Researcher*
Nearest person month worked:	*6*
Contribution to Project:	*Involved in development and testing of a novel process of preparation and storage conditions for DRP solutions.*

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.

NIH-NIGMS P20 GM109089, NIH-NINDS 1R01NS082225, NIH-NINDS 1R21NS091600 were ended and Dr. Bragin currently has only DOD grant where he has 5-person months effort. It would be helpful to increase his effort on this grant for boosting the study.

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

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

A Novel Advanced Resuscitation Fluid for Traumatic Brain Injury with Hemorrhagic Shock

Log Number: DM160142

Award Number: W81XWH-17-2-0053

PI: Bragin, D.E. **Org:** Dept. of Neurosurgery, University of New Mexico School of Medicine/ McGowan Inst. for Regenerative Medicine, University of Pittsburgh (Sub-contractor)

Award Amount: $1,416,397.00

Study/Product Aim(s)

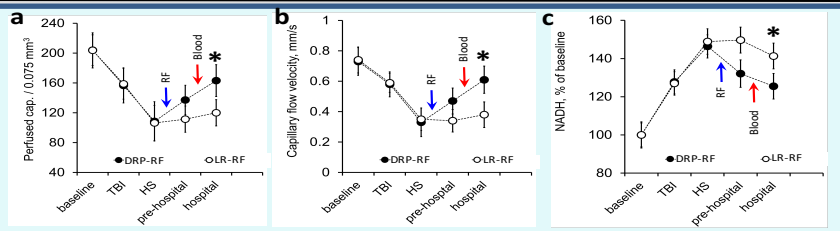
Current resuscitation fluids for traumatic brain injury with hemorrhagic shock (TBI/HS) do not ameliorate impaired cerebral microvascular flow leading to hypoxia, neuronal death, increased mortality and poor neurological outcome. Nanomolar concentrations of blood soluble intravenous drag reducing polymers (DRP) improve cerebral microcirculation and tissue oxygenation. The proposed research aims to ameliorate impaired cerebral and systemic microcirculation by restoring capillary perfusion after TBI/HS using novel, advanced by DRP addition, resuscitation fluid.

Hypothesis: Addition of DRP to resuscitation fluid (DRP-RF) for TBI/HS will attenuate the severity of injury, increase survival, improve neurologic recovery and reduce the volume of fluid required to prevent the transition of HS to the irreversible stage or functional impairment of the brain.

- Specific Aim 1: Demonstrate the major mechanisms and the acute, beneficial effects of DRP-RF for up to 8 hours after TBI/HS compared to crystalloid or colloid fluids on both the brain and systemic microcirculation, metabolism and pathology.
- Specific Aim 2: Prove the beneficial effects of DRP-RF on long-term recovery and neurologic outcome comparing with crystalloid and colloid fluids for up to 4 weeks after TBI/HS.

Approach

- Using rat fluid percussion injury for TBI in rats, we will evaluate the beneficial effects of DRP-RF brain circulation, metabolism and neuronal survival in acute phase of TBI/HS (up to 8 hrs.) by in-vivo Laser Speckle Contrast Imaging and Two-photon Laser Scanning Microscopy.
- Long term anatomical and neurological outcome will be evaluated for up to 4 wks. after TBI/HS by magnetic resonance imaging, behavioral tests and histochemistry.



Resuscitation with DRP-RF improves cerebral microvascular perfusion and tissue oxygenation impaired by TBI/HS, as shown by a) increased number of perfused capillaries; b) increased capillary flow velocity; c) increased tissue oxygenation (NADH decrease). Mean ± SEM, N=10 rats per group, *P < 0.05 from the LR-RF group.

During reported period we showed that colloid and hypertonic-based DRP-RF vs. colloid significantly improves cerebral regional and microvascular circulation and tissue oxygenation impaired by TBI/HS. Effect lasts at least 6 hours. Colloid-based DRP-RF was more effective than crystalloid and hypertonic—based DRP-RF tested earlier. We have also done evaluation of TBI/HS-induced metabolic stress of mitochondria, neuronal survival and microthrombosis and beneficial effects of DRP-RF-vs. RF.

Sub-Contractor performed experiments on DRP characterization and storage and drag reduction test circuit development

Timeline and Cost

Activities CY	17	18	19	20
Specific Aim 1: Demonstrate the major mechanisms and the acute, beneficial effects of DRP-RF for up to 8 hours after TBI/HS compared to crystalloid or colloid fluids on both the brain, systemic microcirculation, metabolism and pathology.				
Specific Aim 2: Prove the beneficial effects of DRP-RF on long-term recovery and neurologic outcome comparing with crystalloid and colloid fluids for up to 4 weeks after TBI/HS.				
Estimated Budget ($1,416K)	**$195K**	**$455K**	**$457K**	**$309K**

Updated: 03/30/2018

Goals/Milestones

CY17 Goal– Specific Aim 1: Acute effects of DRP-RF (up to 8 hrs.). ☑ Brain microvascular circulation and tissue oxygenation (DRP vs. crystalloids

CY18 Goals – Specific Aim 2: Long term neurological and anatomical recovery (up to 4 weeks). ☑ Continuation of Specific Aim 1.

CY19 Goal – Completion of Specific Aim 1, Continuation of Specific Aim 2, data interpretation and transition to translational phase of research (pre-clinical and clinical studies)

CY20 Goal – Completion of Specific Aim 2, data interpretation and transition to translational phase of research (pre-clinical and clinical studies)

Comments/Challenges/Issues/Concerns

- No changes/No concerns

Budget Expenditure to Date

Projected Expenditure: $1,416K

Actual Expenditure: $380K

9. APPENDICES:

Resuscitation Fluid with Drag Reducing Polymer Enhances Cerebral Microcirculation and Tissue Oxygenation After Traumatic Brain Injury Complicated by Hemorrhagic Shock

D. E. Bragin, D. A. Lara, O. A. Bragina, M. V. Kameneva, and E. M. Nemoto

Abstract

Traumatic brain injury (TBI) is frequently accompanied by hemorrhagic shock (HS) which significantly worsens morbidity and mortality. Existing resuscitation fluids (RF) for volume expansion inadequately mitigate impaired microvascular cerebral blood flow (mvCBF) and hypoxia after TBI/HS. We hypothesized that nanomolar quantities of drag reducing polymers in resuscitation fluid (DRP-RF), would improve mvCBF by rheological modulation of hemodynamics. Methods: TBI was induced in rats by fluid percussion (1.5 atm, 50 ms) followed by controlled hemorrhage to a mean arterial pressure (MAP) = 40 mmHg. DRP-RF or lactated Ringer (LR-RF) was infused to MAP of 60 mmHg for 1 h (pre-hospital), followed by blood re-infusion to a MAP = 70 mmHg (hospital). Temperature, MAP, blood gases and electrolytes were monitored. In vivo 2-photon laser scanning microscopy was used to monitor microvascular blood flow, hypoxia (NADH) and necrosis (i.v. propidium iodide) for 5 h after TBI/HS followed by MRI for CBF and lesion volume. Results: TBI/HS compromised brain microvascular flow leading to capillary microthrombosis, tissue hypoxia and neuronal necrosis. DRP-RF compared to LR-RF reduced microthrombosis, restored collapsed capillary flow and improved mvCBF (82 ± 9.7% vs. 62 ± 9.7%, respectively, $p < 0.05$, $n = 10$). DRP-RF vs LR-RF decreased tissue hypoxia (77 ± 8.2% vs. 60 ± 10.5%, $p < 0.05$), and neuronal necrosis (21 ± 7.2% vs. 36 ± 7.3%, respectively, $p < 0.05$). MRI showed reduced lesion volumes with DRP-RF. Conclusions: DRP-RF effectively restores mvCBF, reduces hypoxia and protects neurons compared to conventional volume expansion with LR-RF after TBI/HS.

D. E. Bragin (✉) · D. A. Lara · O. A. Bragina
E. M. Nemoto
Department of Neurosurgery, University of New Mexico School of Medicine, Albuquerque, NM, USA
e-mail: dbragin@salud.unm.edu

M. V. Kameneva
McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, USA

1 Introduction

Traumatic brain injury (TBI) is frequently accompanied by arterial hypotension resulting from hemorrhagic shock (HS) which significantly

O. Thews et al. (eds.), *Oxygen Transport to Tissue XL*, Advances in Experimental Medicine and Biology 1072, https://doi.org/10.1007/978-3-319-91287-5_7

worsens neurologic outcome, and increases mortality [1]. The main reason for this is increased severity of reduced cerebral blood flow (CBF) leading to capillary microthrombosis, hypoxia, neuronal death and twofold increase in contusion volume [2]. In TBI/HS, arterial pressure reductions, normally well-tolerated, causes severe reductions in CBF and increased ischemia even after mild TBI [2]. We previously showed that even in a healthy rat brain, decreasing arterial pressure to 40 mmHg for 1 h compromised cerebral microcirculation and CBF autoregulation leading to tissue hypoxia [3, 4].

Current resuscitation fluids (RF) for volume expansion after TBI/HS do not adequately ameliorate impaired microvascular cerebral blood flow (mvCBF). In our previous studies in a rat TBI model, we have shown that nanomolar concentrations of intravascular blood soluble drag reducing polymers (DRP) significantly enhanced microvascular perfusion and tissue oxygenation in peri-contusional areas and protected neurons [5]. It has been also demonstrated that DRP improved hemodynamics, blood chemistry, and survival in various animal models of HS [6]. Hence, we hypothesized that the addition of nanomolar quantities of DRP to resuscitation fluids would improve mvCBF and tissue oxygenation by rheological modulation of hemodynamics. We compared the effects of lactated Ringer resuscitation fluid with DRP (DRP-RF) and without (LR-RF) on mvCBF, tissue oxygenation and neuronal survival in the rat brain after TBI and HS.

2 Methods

Most of the procedures used in these studies we described previously [5]. Protocol #200640 was approved by the Institutional Animal Care and Use Committee of the University of New Mexico and the studies were conducted according the NIH Guide for the Care and Use of Laboratory Animals.

Surgical preparation Laboratory-acclimated male Sprague-Dawley rats (250–300 g) were mechanically ventilated on isoflurane (2%), nitrous oxide (69%) oxygen anesthesia (29%). Femoral vein and artery catheters were inserted. For imaging and TBI, a 5-mm craniotomy over the left parietal cortex was filled with 2% agarose in saline and sealed by cover glass. The fluid percussion was used as a model of TBI and was induced by 1.5 atm 50 ms pulse from a custom-built Pneumatic Impactor connected to the brain through a pressure transducer filled with artificial cerebrospinal fluid. HS was performed in a way similar to that described by Robertson et al. [2].

Overall design of the study TBI was induced after baseline in-vivo 2-photon laser scanning microscopy (2PLSM) and followed by a 1-h hemorrhagic phase, where blood was slowly withdrawn through the femoral vein to reduce mean arterial pressure (MAP) to 40 mmHg. In the following 1-h pre-hospital care phase, resuscitation fluids (LR-RF or DRP-RF) were slowly infused i.v. to raise MAP to ~55 mmHg and CBF to ~65% of baseline. In a subsequent 3-h definitive hospital care phase, shed blood was re-infused to a MAP of 70 mmHg and CBF of ~75% of baseline. In vivo 2PLSM was done throughout the study over the peri-contusion area of the parietal cortex of the rat brain. Monitored variables included: cerebral microvascular blood flow velocity, number of perfused capillaries, tissue oxygenation (NADH) and neuronal necrosis (i.v. Propidium Iodide). The laser Doppler flux was measured via a lateral temporal window using a 0.9 mm diameter probe (DRT4, Moor Inst., Axminster, UK) in the same region of the brain studied by 2PLSM. Brain and rectal temperatures were monitored and maintained at 38 ± 0.5 °C. Arterial blood gases, electrolytes, hematocrit and pH were measured hourly (epoc Blood Analysis System, Alere Inc., Waltham, MA, USA).

DRP preparation Polyethylene oxide (PEO, MW ~4000 kDa) was dissolved in saline to 0.1% (1000 ppm), dialyzed against saline using a 50 kD cutoff membrane, diluted in saline to

50 ppm, slow rocked for ~2 h and then sterilized using a 0.22 μm filter [6]. DRP-RF was prepared before infusion by adding DRP to Lactated Ringer to reach final DRP concentration of 0.0005% (5 ppm).

Two-Photon Laser Scanning Microscopy Fluorescent serum (i.v. fluorescein isothiocyanate (FITS) dextran, 150 kDa in physiological saline, 5% wt/vol) was visualized using an Olympus BX 51WI upright microscope and water-immersion LUMPlan FL/IR 20X/0.50 W objective. Excitation was provided by a PrairieView Ultima multiphoton microscopy laser scan unit powered by a Millennia Prime 10 W diode laser source pumping a Tsunami Ti: Sapphire laser (Spectra-Physics, Mountain View, CA, USA) tuned to 750 nm centre wavelength. Band-pass-filtered epifluorescence (510–550 nm for FITS and 425–475 nm for NADH) was collected by photomultiplier tubes of the Prairie View Ultima system. Images (512 × 512 pixels, 0.15 um/pixel in the x- and y-axes) or line scans were acquired using Prairie View software. Red blood cell flow velocity was measured in microvessels ranging from 3 to 50 μm diameter up to 500 μm below the surface of the parietal cortex, as described previously [5]. Tissue hypoxia was assessed by measurement of NADH autofluorescence. In offline analyses using NIH ImageJ software, three-dimensional anatomy of the vasculature in areas of interest were reconstructed from two-dimensional (planar) scans of the fluorescence intensity obtained at successive focal depths in the cortex (XYZ stack). To evaluate neuronal survival, 200 μL of a propidium iodide (PI)/saline, which labels only necrotized cells with damaged membrane, was injected intravenously during surgical preparation. Propidium iodide fluorescence was band-pass filtered at 600–620 nm (as described previously) to visualize damaged neurons [7].

Statistical analyses were done using GraphPad Prism software 6.0 (La Jolla, CA, USA) by Student's t-test or Kolmogorov-Smirnov test where appropriate. Differences between groups were determined using two-way analysis of variance (ANOVA) for multiple comparisons and post-hoc testing using the Mann-Whitney U-test.

3 Results

TBI did not cause notable changes in MAP, while cortical CBF, measured by surface laser Doppler probe in the peri-contusional area, fell to 87.5 ± 6.2% from baseline ($P < 0.05$). In the peri-contusion area after TBI, microvascular CBF, measured by in-vivo 2PLSM showed a reduction in capillary flow velocity to 0.59 ± 0.03 mm/s from 0.77 ± 0.05 mm/s and about ~25% reduction in the number of functioning capillaries due to microthrombosis leading to tissue hypoxia, reflected by a ~25% increase in NADH autofluorescence (Fig. 1; $P < 0.05$ from baseline) which agrees with our previous observations [5].

Subsequent hemorrhagic shock reduced MAP to 40.2 ± 3.7 mmHg and cortical CBF to 45 ± 7.1%, which caused a further twofold reduction of mvCBF and tissue oxygenation leading to neuronal necrosis with 59.6 ± 6.1 dead neurons per 0.075 mm^3 at the end of the HS phase (Figs. 1 and 2; $P < 0.05$ from TBI).

In the pre-hospital phase, conventional LR-RF, slowly infused in the amount of 5.4 ± 2.1 ml, restored MAP to 55 ± 5.4 mmHg and cortical CBF to 62.8 ± 7.2% ($P < 0.05$ from the HS phase). However, no notable changes were observed in mvCBF and tissue oxygenation which remained at the same level as in the HS phase (Fig. 1), while the number of dead neurons increased to 138.3 ± 7.6 per 0.075 mm^3 of brain parenchyma (Fig. 2, $P < 0.05$ from the HS phase).

DRP-RF, infused during the pre-hospital phase in the smaller amount of 2.1 ± 0.3 ml, increased MAP to 47.1 ± 4.6 mmHg while cortical CBF increased to 65.5 ± 6.9% ($P < 0.05$ from the HS phase). In contrast to LR-RF, addition of DRP-RF improved mvCBF and tissue oxygenation, i.e., the number of perfused capillaries increased to 141.2 ± 6.8 per 0.075 mm^3, and capillary flow velocity increased to 0.55 ± 0.04 leading to NADH autofluorescence reduction to 129.1 ± 8.4% (Fig. 1, $P < 0.05$ from the HS

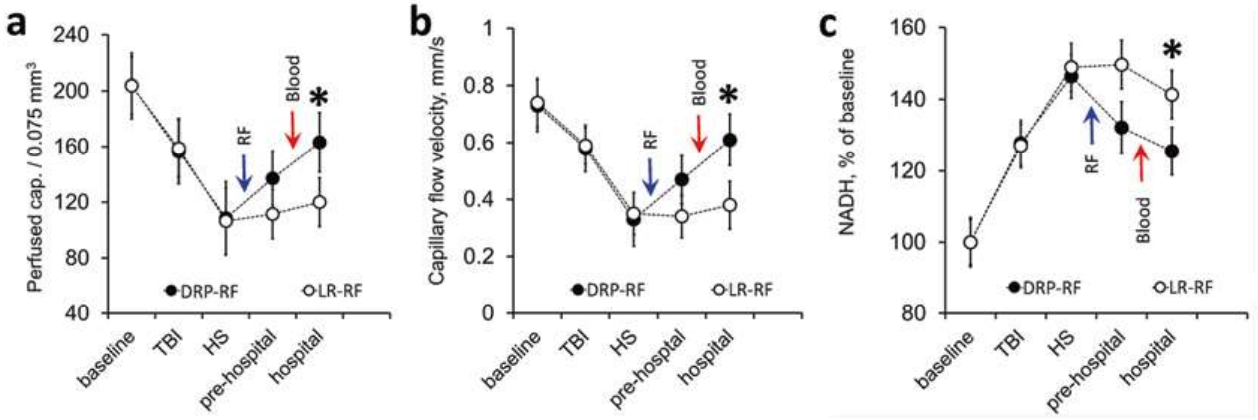


Fig. 1 Resuscitation with DRP-RF improves cerebral microvascular perfusion and tissue oxygenation impaired by TBI/HS, as shown by: (**a**) increased number of perfused capillaries; (**b**) increased capillary flow velocity; (**c**) increased tissue oxygenation (NADH decrease). Mean ± SEM, N = 10 rats per group, *P < 0.05 from the LR-RF group

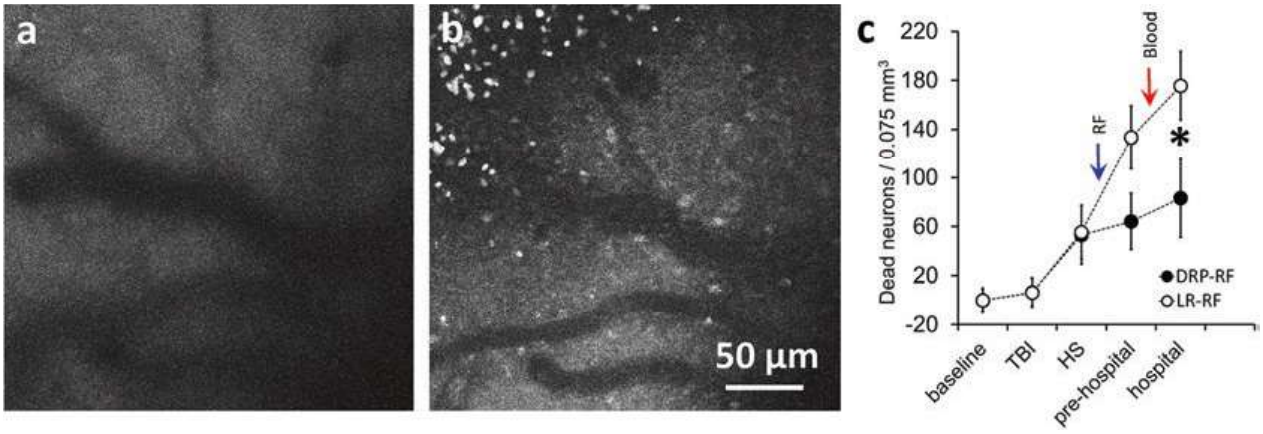


Fig. 2 Resuscitation with DRP-RF is neuroprotective: (**a**) 2PLSM image of a rat cortex at baseline without dead neurons; (**b**) Propidium Iodide stains neurons with damaged membranes reflecting necrosis of neurons after TBI/HS; (**c**) DRP-RF protects neurons from necrosis (*=P < 0.05). Mean ± SEM, N = 10 rats per group, *P < 0.05 from the LR-RF group

phase). Improved oxygen transport to tissue protected neurons from necrosis, as the number of dead neurons showed no significant increase: 62.3 ± 3.1 per 0.075 mm^3 (Fig. 2).

During the hospital phase in the LR-RF group, re-infusion of blood increased MAP to 70.2 ± 6.7 mmHg and cortical CBF to 76.8 ± 8.4%. However, as in the pre-hospital phase, mvCBF and tissue oxygenation in the peri-contusion regions did not change significantly; however, there was a trend toward improvement (Fig. 1). The number of dead neurons increased to 177.3 ± 9.2 per 0.075 mm^3 of tissue by the end of this phase (Fig. 2). In the DRP-RF group, re-infusion of blood during the hospital phase increased MAP to 69.7 ± 5.9 mmHg and cortical CBF to 78.5 ± 7.3%. mvCBF and tissue oxygenation further improved: the number of perfused capillaries increased to 162.3 ± 9.2 per 0.075 mm^3, capillary flow velocity increased to 0.64 ± 0.05, and NADH autofluorescence reduced to 121.8 ± 9.1% (Fig. 1, P < 0.05 from the LR-RF group). Improved oxygen transport to tissue protected neurons from necrosis, as the number of dead neurons was significantly less than in the LR-RF group: 78.2 ± 9.3 vs. 181.5 ± 10.2 per 0.075 mm^3 (Fig. 2c, P < 0.05). Anatomical T2 MRI at the end of the study revealed reduced lesion volumes in the DRP-RF compared to LR-RF (P < 0.05).

4 Discussion

Based on our data and previous studies [5, 8], the mechanisms of restoring mvCBF by DRP-RF include increasing the arteriolar blood volume flow via the increase of flow velocity by reduction of flow separations and vortices at vessel bifurcations and decreasing pressure loss across the arterial network due to the viscoelastic properties of DRP. This leads to a rise in the precapillary blood pressure thus enhancing capillary perfusion, countering capillary stasis, increasing the density of functioning capillaries and the number of RBC passing through capillaries to improve tissue oxygenation. The decrease of cerebral hypoxia due to increased oxygen transport to tissue by restored mvCBF explains the neuroprotective effect of DRP. Another beneficial property of DRP-RF is that it significantly reduced the amount of the fluid required to increase tissue perfusion, which is particularly essential for the traumatized brain. One of the main problems of current resuscitation fluids is the need for infusion of large volumes that can exacerbate brain edema [9] and cause hemodilution, thereby reducing blood oxygen carrying capacity that can further compromise oxygenation of the injured brain [10]. Adequate capillary perfusion, which can be restored by DRP-RF, is essential to maintain homeostasis, to remove metabolic waste products which, if allowed to accumulate, exert toxic effects, and to overcome deficits in oxygen delivery.

5 Conclusions

Rheological modulation of blood flow using advanced resuscitation fluid with DRP in nanomolar amounts effectively restores cerebral microcirculation, reduces hypoxia and protects neurons after TBI complicated by hemorrhagic shock compared to conventional volume expansion with lactated Ringer. In addition, DRP-RF requires infusion of a smaller volume to improve tissue perfusion and oxygen utilization which reduces brain edema formation due to hypervolemia, which often occurs with a standard fluid resuscitation.

Acknowledgments Supported by DOD DM160142, R21NS091600 and RMSE № 17.1223.2017/AP.

References

1. Manley G, Knudson MM, Morabito D et al (2001) Hypotension, hypoxia, and head injury: frequency, duration, and consequences. Arch Surg 136(10):1118–1123
2. Navarro JC, Pillai S, Cherian L et al (2012) Histopathological and behavioral effects of immediate and delayed hemorrhagic shock after mild traumatic brain injury in rats. J Neurotrauma 29(2):322–334
3. Bragin DE, Statom GL, Yonas H et al (2014) Critical cerebral perfusion pressure at high intracranial pressure measured by induced cerebrovascular and intracranial pressure reactivity. Crit Care Med 42(12):2582–2590
4. Bragin DE, Bush RC, Muller WS et al (2011) High intracranial pressure effects on cerebral cortical microvascular flow in rats. J Neurotrauma 28(5):775–785
5. Bragin DE, Kameneva MV, Bragina OA et al (2017) Rheological effects of drag-reducing polymers improve cerebral blood flow and oxygenation after traumatic brain injury in rats. J Cereb Blood Flow Metab 37(3):762–775
6. Kameneva MV, Wu ZJ, Uraysh A et al (2004) Blood soluble drag-reducing polymers prevent lethality from hemorrhagic shock in acute animal experiments. Biorheology 41(1):53–64
7. Fumagalli S, Coles JA, Ejlerskov P et al (2011) In vivo real-time multiphoton imaging of T lymphocytes in the mouse brain after experimental stroke. Stroke 42(5):1429–1436
8. Kameneva MV (2012) Microrheological effects of drag-reducing polymers in vitro and in vivo. Int J Eng Sci 59:168–183
9. Falk JL (1995) Fluid resuscitation in brain-injured patients. Crit Care Med 23(1):4–6
10. Lee EJ, Hung YC, Lee MY (1999) Anemic hypoxia in moderate intracerebral hemorrhage: the alterations of cerebral hemodynamics and brain metabolism. J Neurol Sci 164(2):117–123

Resuscitation with Drag Reducing Polymer after Traumatic Brain Injury with Hemorrhagic Shock Reduces Microthrombosis and Oxidative Stress

Denis E. Bragin[1], Olga A. Bragina[1], Marina V. Kameneva[2] and Edwin M. Nemoto[1]

[1]Dept of Neurosurgery, University of New Mexico School of Medicine, Albuquerque, USA
E-mail: dbragin@salud.unm.edu

[2]McGowan Institute for Regenerative Medicine, University of Pittsburgh, USA

Abstract: Outcome after traumatic brain injury (TBI) is worsened by hemorrhagic shock (HS), but existing volume expansion approach with resuscitation fluids (RF) is controversial as do not adequately alleviates impaired microvascular cerebral blood flow (mCBF). We previously reported that resuscitation fluid with drag reducing polymers (DRP-RF) improves CBF by rheological modulation of hemodynamics. Here, we evaluate the efficacy of DRP-RF, compared to lactated Ringers resuscitation fluid (LR-RF), in reducing cerebral microthrombosis and reperfusion mitochondrial oxidative stress after TBI complicated by HS. *Method* Fluid percussion TBI (1.5 ATA, 50 ms) was induced in rats and followed by controlled HS to a mean arterial pressure (MAP) of 40 mmHg. DRP-RF or LR-RF was infused to restore MAP to 60 mmHg for one hour (pre-hospital period), followed by blood reinfusion to a MAP=70 mmHg (hospital period). In vivo 2-photon laser scanning microscopy over the parietal cortex was used to monitor microvascular blood flow, NADH (hypoxia) and mitochondrial oxidative stress (superoxide by i.v. hydroethidine [HEt], 1 mg/kg) for 4 hours after TBI/HS, followed by DiI vascular painting during perfusion-fixation. *Results* TBI/HS decreased mCBF resulting in capillary microthrombosis and tissue hypoxia. Microvascular CBF and tissue oxygenation were significantly improved in the DRP-RF compared to the LR-RF treated group ($p<0.05$). Reperfusion-induced oxidative stress, reflected by HEt fluorescence, was 32 ± 6% higher in LR-RF vs. DRP-RF ($p<0.05$). Post-mortem whole-brain visualization of DiI painted vessels revealed multiple microthromboses in both hemispheres that were 29 ± 3% less in DRP-RF vs. LR-RF group ($p<0.05$). *Conclusions* Resuscitation after TBI/HS using DRP-RF effectively restores mCBF, reduces hypoxia, microthrombosis formation, and mitochondrial oxidative stress compared to conventional volume expansion with LR-RF.

1 Introduction

Outcome after traumatic brain injury (TBI) is significantly worsened by hemorrhagic shock (HS) due to increased severity of reduced cerebral blood flow (CBF) leading to capillary microthrombosis, hypoxia, reactive oxygen species formation due to mitochondrial dysfunction, neuronal death and a two-fold increase in contusion volume [1, 2]. Existing volume expansion approach with resuscitation fluids (RF) is controversial as do not adequately alleviates impaired microvascular cerebral blood flow (mCBF) and thus not neuroprotective. We previously reported that resuscitation fluid with drag reducing polymers (DRP-RF) improves CBF and reduces cerebral hypoxia and neuronal necrosis by rheological modulation of hemodynamics. Here, we evaluate the efficacy of DRP-RF, compared to lactated Ringers resuscitation fluid (LR-RF), in reducing cerebral microthrombosis and reperfusion mitochondrial oxidative stress after TBI complicated by HS.

2 Methods

Most of the procedures used in these studies we described previously [3]. Protocol #200640 was approved by the Institutional Animal Care and Use Committee of the University of New Mexico and the studies were conducted according to the NIH Guide for the Care and Use of Laboratory Animals.

Surgical preparation. Laboratory-acclimated male Sprague-Dawley rats (250–300 g) were mechanically ventilated on isoflurane (2%), nitrous oxide (69%) oxygen anesthesia (29%). Femoral vein and artery catheters were inserted. For imaging and TBI, a 5-mm craniotomy over the left parietal cortex was filled with 2% agarose in saline and sealed by a cover glass. The fluid percussion was used as a model of TBI and was induced by 1.5 atm 50 ms pulse from a custom-built Pneumatic Impactor connected to the brain through a pressure transducer filled with artificial cerebrospinal fluid. HS was performed in a way similar to that described by Robertson et al. [2].

Overall design of the study. TBI was induced after baseline in-vivo 2-photon laser scanning microscopy (2PLSM) and followed by a 1-h hemorrhagic phase, where blood was slowly withdrawn through the femoral vein to reduce mean arterial pressure (MAP) to 40 mmHg. In the following 1-h pre-hospital care phase, resuscitation fluids (LR-RF or DRP-RF) were slowly infused i.v. to raise MAP to ~55 mmHg and CBF to ~65% of baseline. In a subsequent 3-h definitive hospital care phase, shed blood was re-infused to a MAP of 70 mmHg and CBF of ~75% of baseline. *In vivo* 2PLSM was done throughout the study over the peri-contusion area of the parietal cortex of the rat brain. Monitored variables included: cerebral microvascular blood flow velocity, number of perfused capillaries, tissue oxygenation (NADH)

and superoxide production (i.v. Hydroethidine). The laser Doppler flux was measured via a lateral temporal window using a 0.9 mm diameter probe (DRT4, Moor Inst., Axminster, UK) in the same region of the brain studied by 2PLSM. Brain and rectal temperatures were monitored and maintained at 38 ± 0.5°C. Arterial blood gases, electrolytes, haematocrit and pH were measured hourly (epoc Blood Analysis System, Alere Inc., Waltham, MA, USA). At the end of experiments animals were subjected to perfusion with vessel painting.

Superoxide production evaluation. 0.5 mg of HEt in a concentration of 1 mg/ml in 0.1 M phosphate buffer solution (PBS) containing 20% DMSO, was injected intravenously during surgical preparation [4]. Hydroethidine (HEt) is a fluorescent dye that is oxidized to ethidium (ET) by superoxide. ET fluoresces at a different wavelength (Em=595 nm) than HEt (Em=415 nm) and thus may be used to visualize superoxide production.

Animal perfusion and Vessel Painting. Vessel painting was done during cardiac perfusion according to Hughes with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) which binds directly and preferentially to endothelial cells [5]. The Vessel Painting included the following sequential order for delivery of perfusion solutions: (a) PBS (150 ml), (b) 50 ml of DiI (13 μg/ml) and (c) paraformaldehyde (4%, 200 ml). After fixation and perfusion, the brain was extracted from the cranium and all were meninges removed with care. Imaging was done with 2PLSM in a custom-made fixed tissue imaging chamber

DRP preparation. Polyethylene oxide (PEO, MW ~4000 kDa) was dissolved in saline to 0.1% (1000 ppm), dialyzed against saline using a 50 kD cutoff membrane, diluted in saline to 50 ppm, slow rocked for ~2 hours and then sterilized using a 0.22 μm filter [6]. DRP-RF was prepared before infusion by adding DRP to Lactated Ringer to reach final DRP concentration of 0.0005% (5 ppm).

Two-Photon Laser Scanning Microscopy. Fluorescent serum (i.v. fluorescein isothiocyanate (FITS) dextran, 150 kDa in physiological saline, 5% wt/vol) was visualized using an Olympus BX 51WI upright microscope and water-immersion LUMPlan FL/IR 20X/0.50 W objective. Excitation was provided by a PrairieView Ultima multiphoton microscopy laser scan unit powered by a Millennia Prime 10 W diode laser source pumping a Tsunami Ti: Sapphire laser (Spectra-Physics, Mountain View, CA, USA) tuned to 750 nm center wavelength. Band-pass-filtered epifluorescence (510–530 nm for FITS, 445-475 nm for NADH and 565-600 for ET) was collected by photomultiplier tubes of the Prairie View Ultima system. Images (512 x 512 pixels, 0.15 um/pixel in the x- and y-axes) or line scans were acquired using Prairie View software. Red blood cell flow velocity was measured in microvessels ranging from 3-50 μm diameter up to 500 μm below the surface of the parietal cortex, as described previously [5]. Tissue hypoxia was assessed by measurement of NADH autofluorescence. In offline analyses using NIH ImageJ software, a three-dimensional anatomy of the vasculature in areas of interest were reconstructed from two-dimensional (planar) scans of the fluorescence intensity obtained at successive focal depths in the cortex (XYZ stack).

Statistical analyses were done using GraphPad Prism software 6.0 (La Jolla, CA, USA) by Student's t-test or Kolmogorov-Smirnov test where appropriate. Differences between groups were determined using two-way analysis of variance (ANOVA) for multiple comparisons and post-hoc testing using the Mann-Whitney U-test.

3 Results

As in our previous studies, TBI followed by HS decreased mCBF resulting in capillary microthrombosis and tissue hypoxia. Microvascular CBF, number of perfused capillaries and tissue oxygenation in peri contusion areas were significantly better in the DRP-RF compared to the LR-RF treated group.

Post-mortem whole-brain visualization of DiI painted vessels revealed multiple microthromboses and reduction of microvascular density in both hemispheres in rats after TBI with HS (Fig. 1A, B). Wherein, in the injured hemisphere in DRP-RF, microvascular density was higher than in LR-RF (% vessel/total area*100 was 4.9 ± 0.4 vs. 3.1 ± 0.3, respectively, $p<0.05$) as oppose 6.8 ± 0.4 to in Sham rats (Fig. 1C). In contralateral to the injury hemisphere, microvascular density was also reduced (% vessel/total area*100 was 6.1 ± 0.5 vs 5.2 ± 0.5, in DRP-RF, vs. LR-RF, respectively, $p<0.09$) (Fig. 1C).

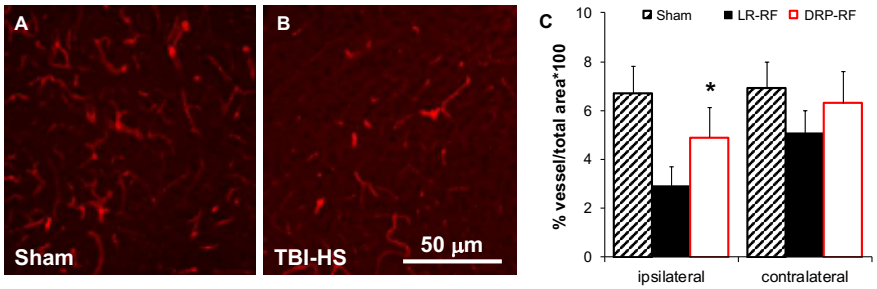


Fig. 1 Resuscitation with DRP-RF reduces microthrombosis in both hemispheres after TBI with HS as shown by post-mortem DiI vascular painting. a) Cortical microvascular network in Sham mouse brain; and b) after TBI with HS; c) Graph showing reduced cortical microvasculature in LR-RF group and better-preserved microvasculature in DRP-RF group in both, traumatized and contralateral hemispheres. Mean ± SEM, N=10 rats per group, *P < 0.05 from the LR-RF group.

Superoxide production was determined by oxidation of HEt to ET. Under normal physiological conditions at the baseline, ET fluorescence appeared as small particles in the cytosol, suggesting mitochondrial generation of superoxide (Fig. 2A). In pathological conditions, the ET fluorescent signal filled the cytosolic space and allowed clear visualization and differentiation of individual soma and dendritic processes. Neurons in which the accumulated fluorescence demonstrated the entire

body of the soma and obscured the nucleus were considered positive for diffuse cytosolic ET fluorescence.

TBI with subsequent hemorrhagic shock caused generation of superoxide, in both groups – the number of Et positive neurons increased to 58.1 ± 5.2 per 0.075 mm^3 (Fig. 2 D). In the *pre-hospital phase*, in the group where conventional LR-RF, slowly infused in the amount of 5.6 ± 2.2 ml, the number of Et positive neurons further increased to 137.9 ± 5.6 per 0.075 mm^3 (Fig. 2D). While in the group where DRP-RF was slowly infused in smaller amount of 2.0 ± 0.3 ml, the number of Et positive neurons increased only to 62.3 ± 4.8 per 0.075 mm^3 (Fig. 2D). *By the end of the hospital phase* with re-infusion of blood, in the LR-RF group, the number of Et positive neurons increased more to 176.5 ± 6.2 per 0.075 mm^3 (Fig. 2B, D), reflecting active reactive oxygen species (superoxide) formation due to, probably, excessive oxidative phosphorylation in metabolically stressed mitochondria. In the DRP-RF group, the number of Et positive neurons increased only to 84.2 ± 5.3 per 0.075 mm^3 (Fig. 2C, D), reflecting lower oxidative stress.

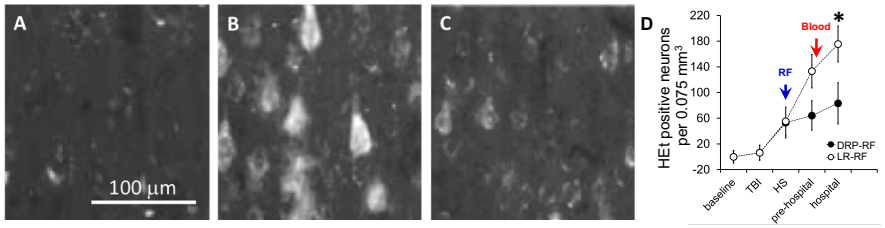


Fig. 2 Resuscitation with DRP-RF reduces superoxide production in cortical neurons after TBI with HS: a) Representative image of a rat cortex at baseline without ET positive neurons; b) Neurons with diffuse cytosolic ET fluorescence in a rat cortex from LR-RF group by the end of experiment; c) and from DRP-RF group; The dynamics of the increase in ET positive cortical neurons. Mean ± SEM, N=10 rats per group, *P < 0.05 from the LR-RF group.

4 Discussion

The most common free radical in TBI, responsible for tissue damage, is superoxide that produced when oxygen molecules gain an electron from other molecules [7, 8]. The major source of superoxide in brain injury is mitochondria [8, 9]. The demonstrated in this work attenuation of oxidative stress by DRP-RF after TBI with hemorrhagic shock is related to mitigation of microthrombosis formation and hypoxia reduction. Hypoxia, induced by TBI and exacerbated by HS, causes mitochondrial impairment leading to excessive and altered oxidative phosphorylation in mitochondria and, as a result, superoxide hyperproduction. Resuscitation re-perfusion exacerbates this process [11]. Attenuation of reduction of cerebral microvascular density by mitigation of microthrombosis formation by DRP-RF does not allow hypoxia to reach the critical level which, possibly, reduces mitochondrial injury and neuronal

excitotoxicity and as a result reactive oxygen species overproduction. The mechanisms of hemorheological modulation by DRP include increasing the arteriolar blood volume flow via the increase of flow velocity by reduction of flow separations and vortices at vessel bifurcations and decreasing pressure loss across the arterial network due to the viscoelastic properties of DRP [11]. This leads to a rise in the pre-capillary blood pressure thus enhancing capillary perfusion, countering capillary stasis, increasing the density of functioning capillaries and the number of RBC passing through capillaries to improve tissue oxygenation [11].

5 Conclusion

Rheological modulation of blood flow using advanced resuscitation fluid with DRP in nanomolar amounts effectively restores microvascular CBF, reduces hypoxia, microthrombosis formation and mitochondrial oxidative stress compared to conventional volume expansion with lactated Ringer.

Acknowledgments Supported by DOD DM160142.

References

1. Manley G, Knudson MM, Morabito D et al (2001) Hypotension, hypoxia, and head injury: frequency, duration, and consequences. Arch Surg 136 (10):1118-1123
2. Navarro JC, Pillai S, Cherian L et al (2012) Histopathological and behavioral effects of immediate and delayed hemorrhagic shock after mild traumatic brain injury in rats. J Neurotrauma 29 (2):322-334
3. Bragin DE, Kameneva MV, Bragina OA et al (2017) Rheological effects of drag-reducing polymers improve cerebral blood flow and oxygenation after traumatic brain injury in rats. J Cereb Blood Flow Metab 37 (3):762-775
4. Hughes, S, Dashkin O, Defazio RA (2014) Vessel painting technique for visualizing the cerebral vascular architecture of the mouse. Methods Mol Biol 1135:127–13
5. Peterson SL, Morrow D, Liu S et al (2002) Hydroethidine detection of superoxide production during the lithium-pilocarpine model of status epilepticus. Epilepsy Res 49(3):226-38
6. Kameneva MV, Wu ZJ, Uraysh A et al (2004) Blood soluble drag-reducing polymers prevent lethality from hemorrhagic shock in acute animal experiments. Biorheology 41 (1):53-64
7. Abdul-Muneer PM, Chandra N, Haorah J (2015) Interactions of oxidative stress and neurovascular inflammation in the pathogenesis of traumatic brain injury. Mol Neurobiol 51(3):966-79
8. Hiebert JB, Shen Q, Thimmesch AR et al (2015) Traumatic brain injury and mitochondrial dysfunction. Am J Med Sci. Aug;350(2):132-8
9. Yonutas H, Vekaria HJ, Sullivan PG (2016) Mitochondrial specific therapeutic targets following brain injury. Brain Research 1640:77–93
10. Fiskum G (2000) Mitochondrial participation in ischemic and traumatic neural cell death. J Neurotrauma. 17(10):843-55
11. Kameneva MV (2012) Microrheological effects of drag-reducing polymers in vitro and in vivo. Int J Eng Sci 59:168-183