AWARD NUMBER: W81XWH-13-2-0091

TITLE: Mechanistic Links Between PARP, NAD, and Brain Inflammation After TBI

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**14. ABSTRACT**

This project is a pre-clinical evaluation of the efficacy of veliparib and NAD as agents for suppressing inflammation and improving outcomes after traumatic brain injury. The animal models include pig and rat models of controlled cortical impact and blast injury. Work completed in this year 2 of 3 includes the large majority of the pig CCI and blast studies, and all rat veliparib animal surgeries. The behavioral analyses for these is completed, and the histological analyses are ongoing. Rat blast studies are ongoing. The studies using NAD as an anti-inflammatory agent have also been initiated. Results of the studies completed confirm that veliparib and NAD attenuate microglial activation after both blast and CCI. Veliparib improves outcome on some of the elevated plus maze and several aspects of the 5-choice reaction test when administered within 2 hours of injury. Analysis of long term (2-month) survival is ongoing.

**15. SUBJECT TERMS**

brain injury, blast injury, rat, pig, inflammation, metabolism, microglia
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1. INTRODUCTION
This is a pre-clinical study to establish the effectiveness of two anti-inflammatory approaches in improving recovery after traumatic brain injury. The studies employ rats and pigs, and use blast injury and controlled cortical injury (CCI) models. The underlying rationale for these studies is based on the salutary effects of ketogenic diet, which acts in part by suppressing brain inflammatory responses. Here we aim to suppress inflammation with (a) veliparib (an inhibitor of poly(ADP-ribose) polymerase, which acts by suppressing NF-kB – mediated inflammatory responses; and (b) intranasal NAD, a natural metabolite which we have in prior studies shown to also suppress poly(ADP-ribose) polymerase activity and inflammatory responses. CtBP1/2 knockout mice will be generated to test a specific mechanism by which ketogenic diet could may have anti-inflammatory effects. For all studies, outcome measures include histological indices of inflammation, cell death, and axonal injury, with behavioral indices of motor coordination, cognitive function, and anxiety. Some studies also use electrocorticography measures of brain network activity.

2. KEYWORDS
brain injury, blast injury, mouse, rat, pig, electrocorticography, inflammation, metabolism, microglia, ketogenic diet

3. ACCOMPLISHMENTS
What were the major goals of the project?

From the SOW:
Year 1
a) Establish blast injury models for rats and swine.
   Status:  Completed.

b) Initiate blast injury studies in rats and swine
   Status: Completed

c1) In rats, establish the dose and time window of opportunity’ for treatment with a PARP inhibitor (veliparib).
   Status: Surgery and behavioral outcome studies completed (see Appendix D, E, and F); analysis of histological data is ongoing

c2) In rats, establish the ‘time window of opportunity’ for treatment with intranasal NAD .
   Status: Analysis of histological outcomes at the early time point (15 minutes) is ongoing. Studies at later time points have not yet been initiated.

d) In pigs, establish the time window of opportunity for treatment with a PARP inhibitor.
   Status: Completed (though this aim was truncated to histological analysis at a single time point as described previously.)
Year-2:

a1) In rats, establish the efficacy and ‘time window of opportunity’ for veliparib treatment after blast injury, using histological and behavioral outcome measures
   Status: Behavioral analysis at the early time point is completed (see Appendix E)

a2) In rats, establish the efficacy and ‘time window of opportunity’ for NAD treatment after blast injury, using histological and behavioral outcome measures
   Status: Histological analysis at the earlier time point is ongoing.

b) In pigs, establish the efficacy and ‘time window of opportunity’ for veliparib treatment after blast injury, using histological and behavioral outcome measures.
   Status: Completed (blast injury produced no consistent histological injury)

c) In rats, identify the electrophysiological changes in motor circuit function after CCI during over the acute and recovery periods using cortical and depth electrode arrays.
   Status: Studies are ongoing

d) Using a CtBP1/2 transgenic mouse, test the hypothesis that effects of a ketogenic diet can be replicated by inhibiting CtBP dimerization.
   Status: There have been problems in generating the mouse (see below). We have proof of principle for this idea using a peptide inhibitor.

What opportunities for training and professional development has the project provided?
1. Two members of the research team attended the 2015 California Neurotrauma symposium (Won and Bishop)
2. Three members of the research team attended the Society for Neuroscience Meeting and presented the work described here (Swanson, Won, and Irvine)
3. The P.I. was a participant in the Dept. of Veterans Affairs State of the Art conference on traumatic brain injury.

How were the results disseminated to communities of interest?
1.) Poster presentation at the 2015 Society for Neuroscience Meeting (see appendix)

What do you plan to do during the next reporting period to accomplish the goals?
Studies will proceed as described in the award proposal/SOW. In particular, we will proceed to studies involving behavioral outcome measures and the electrocorticography measures, and studies with the CtBP mouse.
4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
Nothing to Report

What was the impact on other disciplines?
Nothing to Report

What was the impact on technology transfer?
Nothing to Report

What was the impact on society beyond science and technology?
Nothing to Report

5. CHANGES/PROBLEMS

Changes in approach and reasons for change.

1. Pig behavioral studies – showed no behavioral deficits after either blast or CCI. Our evaluations included a novel object recognition task, a hurdle crossing tasks, and a video analysis of gait. Discussion of this issue with other researchers in the field identifies this as a major limitation with use of pigs in general, stemming from their proclivities as herd animals, lack of digits, and small numbers available for any given experiment. We have discontinued the pig behavioral experiments and will use only histological outcome measures from the pigs.

2. Rat blast model - showed little blast-induced injury when the head is fully immobilized. There was a reproducible signal on some of the behavioral studies, which is interesting in itself, but as a criteria for evaluation of NAD and veliparib this is insufficient. We have revised the blast studies to allow reproducible head movement in one direction. This is thus a more complex model – involving both blast and closed head trauma – but it is on the other hand much more realistic that a “pure” blast exposure.

3. Constructs for generating conditional CtBP2-/- mice are were sequenced and found to be correct, but 2 attempts at generating ES cells from these constructs failed. We have therefore proceeded with the new TALENS system for generating the mice.

Changes that had a significant impact on expenditures. None

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents. None

Significant changes in use or care of human subjects. N/A

Significant changes in use or care of vertebrate animals. None

Significant changes in use of biohazards and/or select agents. N/A
6. PRODUCTS

**Journal & book publications.** None

**Other publications, conference papers, and presentations.**
See Appendix C. Presentation at 2015 Society for Neuroscience meeting

**Website(s) or other Internet site(s).** None.

**Technologies or techniques.** None.

**Inventions, patent applications, and/or licenses.** None

**Other Products.** None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

<table>
<thead>
<tr>
<th>Name:</th>
<th>Raymond A. Swanson MD</th>
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<tbody>
<tr>
<td>Project Role:</td>
<td>PI</td>
</tr>
<tr>
<td>Researcher Identifier</td>
<td>ORCID 0000-0002-3664-5359</td>
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<tr>
<td>Nearest person month worked</td>
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<tr>
<td>Contribution to project</td>
<td>Study design, personnel recruitment, compliance, data analysis</td>
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<td>Funding support</td>
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<tr>
<th>Name:</th>
<th>S. Scott Panter PhD</th>
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<td>Faculty</td>
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<td>Supervision of all studies done with pigs</td>
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<tr>
<th>Name:</th>
<th>Karunesh Ganguly, MD, PhD</th>
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<tr>
<th>Name:</th>
<th>Valerie Coppes</th>
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<td>Large animal surgery technician</td>
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<td>David Kapfhamer, PhD</td>
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<td>Katherine Hamel</td>
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<td>Seok Joon Won, Robin Bishop, PhD</td>
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<td>Karen-Amanda Irvine, PhD</td>
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<td>Robin Bishop, MS</td>
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<td>Contribution to project</td>
<td>Purchasing, stocking, coordinates studies, assists in behavioral assessments, conducts rat blast injury experiments.</td>
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**Change in active other support of the PD/Pi's or senior/key personnel**
Dr. Panter has retired due to health reasons and as of May 2015 is no longer receiving support through this grant. Support to Valerie Coppes, Katherine Hamel, has also ended, and support to David Kapfhamer will end 11/15/15.

**What other organizations were involved as partners?**
None

8. **SPECIAL REPORTING REQUIREMENTS**
Not applicable

9. **APPENDICES**
   - Appendix A.  Updated Quad Chart
   - Appendix B.  Presentation at University of California Traumatic Brain Injury conference
   - Appendix C.  Presentation at 2015 Society for Neuroscience meeting
   - Appendix D.  Rat behavioral data summaries
   - Appendix E.  Dose response veliparib summary
   - Appendix F.  Gene expression changes after rat CCI
APPENDIX A

Mechanistic Links between PARP, NAD, and Brain Inflammation after TBI

Log Number 13306001
Award Number W81XWH-13-2-0091
PI: Raymond A. Swanson, M.D.  Org: Northern California Institute for Research and Education  Award Amount: $1,979,662

Study/Product Aim(s)

Objective: Establish a validated treatment approach for TBI, targeting brain inflammation, that can be implemented hours-to-days after injury.

Aims:
- Evaluate functional and histological markers of focal injury and diffuse axonal injury.
- Evaluate the effects of a PARP inhibitor (veliparib) on these endpoints.
- Evaluate delayed intranasal administration of NAD on these endpoints.
- Test the hypothesis that the effects of intranasal NAD and ketogenic diet on TBI are each mediated through actions of the NAD-sensitive transcription factor, CtBP, on inflammatory pathways.

Approach

Studies employ two TBI models: blast injury and controlled cortical impact, and 2 species, rat and pig. Animals are treated post-injury with veliparib or NAD, and subsequently assessed by behavioral tests (for anxiety, learning, and motor function) and histological measures (for inflammation, cell death, and axonal injury).

Timeline and Cost

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<th>14</th>
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<td>☑</td>
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<tr>
<td>Evaluate veliparib in these models</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Evaluate NAD in these models</td>
<td></td>
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<td>☑</td>
<td></td>
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<tr>
<td>Identify e-phys correlates of recovery and drug effects</td>
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<td>✓</td>
<td>✓</td>
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<td>Generate CtBP1/2 ko mouse</td>
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Updated: October 24, 2015

Accomplishment: Veliparib 3 mg/kg/d was shown to completely suppress microglial activation after TBI, in both rats and pigs.

Goals/Milestones

CY13 Goal – Equipment acquisition, personnel hires, and approvals ☑ All in place
CY14 Goals – Model characterizations (behavioral and histological)
- Rat CCI
- Rat blast
- Pig CCI
- Pig blast
- Dose / response of veliparib on brain inflammatory response

CY15 Goal – Establish veliparib efficacy at delayed time points after TBI
- Rat CCI
- Rat blast

CY16 Goals – Establish NAD efficacy at delayed time points after TBI
- Dose / response of NAD on brain inflammatory response
- Evaluate e-phys effects of veliparib & NAD
- Evaluate CtBP-/- genotype on TBI outcomes

Comments/Challenges/Issues/Concerns
- CtBP ko mouse still in production
- Behavioral studies in the pig are not feasible

Budget Expenditure to Date
Projected Expenditure: $1,508,000
Actual Expenditure: $1,668,355
APPENDIX B

Oral Presentation to 2014 University of California Brain Trauma meeting

True Blast injury – Fact or Fiction?

DoD-funded project to evaluate effectiveness of using a PARP inhibitor (veliparib) to suppress brain inflammation after TBI in multiple preclinical models:
- CCI and blast injury in rats (Raymond Swanson, Robin Bishop, Seok Joon Won)
- CCI and blast injury in swine (Scott Panter, Valerie Coppes, Katie Hamel, Preeti Mann)

Blast exposure (e.g. land mine, or mortar shell) → multiple mechanisms of brain injury
- Skull penetration
- Brain deformation due to rapid acceleration/deceleration
- Brain vs. skull collision
- Intrinsic effects of a blast wave on axons, capillaries, etc.

Does this mechanism in fact contribute to brain injury?

The technical, experimental issue:
- Experimental blast exposure also causes head movement / skull deformation.

This study:
- Compare histologic outcomes after blast exposure to rats with some head movement vs. “no” head movement.

Blast Tube setup (L-3/Jaycor)

Peak pressure = 220 psi pressure
Positive pressure duration = 2.6 msec

Rotational movement (only)

No head movement
Examples of neuronal injury induced by 3 different grades of blast injury in cortex (left) and hippocampus (right). The highest grade, 15 mil, corresponds to 200 psi overpressure. Dead neurons are stained bright green by fluoro-jade B.

Conclusions: True blast injury is likely minimal relative to the injury caused by head / brain movement in any real-world TBI setting.
APPENDIX C

Figure 2: Imaging of multiple lesion types and associated networks using MRI.

Table 1: Summary of lesion types and associated networks.

Table 2: Summary of lesion types and associated networks.

Figure 3: Imaging of single lesion type and associated networks using MRI.

Figure 4: Imaging of single lesion type and associated networks using MRI.

Figure 5: Imaging of single lesion type and associated networks using MRI.

Figure 6: Imaging of single lesion type and associated networks using MRI.

Figure 7: Imaging of single lesion type and associated networks using MRI.
COMBINING COHORTS 2 AND 4
(VELIPARIB DRUG TRIALS)

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COMBINING COHORTS 1 AND 3
(BLAST TRIALS)
Gene expression in lesioned cortex after CCI in the rat
Effects of veliparib on gene expression at day 3 after CCI