

**60th Medical Group (AMC), Travis AFB, CA**

**INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)**

**FINAL REPORT SUMMARY**

**(Please type all information. Use additional pages if necessary.)**

**PROTOCOL #:** FDG20180017A

**DATE:** 24 Feb 2020

**PROTOCOL TITLE:** Study to Compare High Versus Low Volume Fluid Resuscitation Strategies in a Porcine Model (*Sus scrofa*) of Burn and Traumatic Brain Injury.

**PRINCIPAL INVESTIGATOR (PI):** Dr. Ian E. Brown

**DEPARTMENT:** UC Davis

**PHONE #:** 916-734-2011

**INITIAL APPROVAL DATE:** 24 May 2018

**LAST TRIENNIAL REVISION DATE:** N/A

**FUNDING SOURCE:** CDMRP

**1. RECORD OF ANIMAL USAGE:**

| <b>Animal Species:</b> | <b>Total # Approved</b> | <b># Used this FY</b> | <b>Total # Used to Date</b> |
|------------------------|-------------------------|-----------------------|-----------------------------|
| Sus scrofa             | 42                      | 10                    | 34                          |
|                        |                         |                       |                             |
|                        |                         |                       |                             |

**2. PROTOCOL TYPE / CHARACTERISTICS: (Check all applicable terms in EACH column)**

- |  |  |  |
|--|--|--|
| <input type="checkbox"/> Training: Live Animal                     | <input type="checkbox"/> Medical Readiness             | <input type="checkbox"/> Prolonged Restraint       |
| <input type="checkbox"/> Training: non-Live Animal                 | <input type="checkbox"/> Health Promotion              | <input type="checkbox"/> Multiple Survival Surgery |
| <input type="checkbox"/> Research: Survival (chronic)              | <input type="checkbox"/> Prevention                    | <input type="checkbox"/> Behavioral Study          |
| <input checked="" type="checkbox"/> Research: non-Survival (acute) | <input type="checkbox"/> Utilization Mgt.              | <input type="checkbox"/> Adjuvant Use              |
| <input type="checkbox"/> Other ( )                                 | <input checked="" type="checkbox"/> Other (Treatment ) | <input type="checkbox"/> Biohazard                 |

**3. PROTOCOL PAIN CATEGORY (USDA): (Check applicable)  C  D  E**

**4. PROTOCOL STATUS:**

**\*Request Protocol Closure:**

- Inactive, protocol never initiated
- Inactive, protocol initiated but has not/will not be completed
- Completed, all approved procedures/animal uses have been completed

**5. Previous Amendments:**

List all amendments made to the protocol. **IF none occurred, state NONE. Do not use N/A.**

**For the Entire Study Chronologically**

| <b>Amendment Number</b> | <b>Date of Approval</b> | <b>Summary of the Change</b> |
|-------------------------|-------------------------|------------------------------|
| 1                       |                         | Personnel changes            |
| 2                       |                         | Personnel changes            |

6. **FUNDING STATUS:** Funding allocated: \$ 290,955.00

Funds remaining: \$ 0.00

7. **PROTOCOL PERSONNEL CHANGES:**

Have there been any personnel/staffing changes (PI/CI/AI/TC/Instructor) since the last IACUC approval of protocol, or annual review?  Yes  No

If yes, complete the following sections (Additions/Deletions). For additions, indicate whether or not the IACUC has approved this addition.

**ADDITIONS:** (Include Name, Protocol function - PI/CI/AI/TC/Instructor, IACUC approval - Yes/No)

| <u>NAME</u> | <u>PROTOCOL FUNCTION</u> | <u>IACUC APPROVAL</u> |
|-------------|--------------------------|-----------------------|
|             |                          |                       |
|             |                          |                       |

**DELETIONS:** (Include Name, Protocol function - PI/CI/AI/TC/Instructor, Effective date of deletion)

| <u>NAME</u> | <u>PROTOCOL FUNCTION</u> | <u>DATE OF DELETION</u> |
|-------------|--------------------------|-------------------------|
|             |                          |                         |
|             |                          |                         |

8. **PROBLEMS / ADVERSE EVENTS:** Identify any problems or adverse events that have affected study progress. Itemize adverse events that have led to unanticipated animal illness, distress, injury, or death; and indicate whether or not these events were reported to the IACUC.

None

9. **REDUCTION, REFINEMENT, OR REPLACEMENT OF ANIMAL USE:**

**REPLACEMENT (ALTERNATIVES):** Since the last IACUC approval, have alternatives to animal use become available that could be substituted in this protocol without adversely affecting study or training objectives?

No

**REFINEMENT:** Since the last IACUC approval, have any study refinements been implemented to reduce the degree of pain or distress experienced by study animals, or have animals of lower phylogenetic status or sentience been identified as potential study/training models in this protocol?

No

**REDUCTION:** Since the last IACUC approval, have any methods been identified to reduce the number of live animals used in this protocol?

No

10. **PUBLICATIONS / PRESENTATIONS:** (List any scientific publications and/or presentations that have resulted from this protocol. Include pending/scheduled publications or presentations).

None yet. Article is in preparation.

11. **PROTOCOL OBJECTIVES:** (Were the protocol objectives met, and how will the outcome or training benefit the DoD/USAF?)

The protocol objectives were met and demonstrated expected responses to the treatments delivered. Military members with combined burn/brain injuries could be resuscitated using the aggressive fluids protocol.

12. **PROTOCOL OUTCOME SUMMARY:** (Please provide, in "ABSTRACT" format, a summary of the protocol objectives, materials and methods, results - include tables/figures, and conclusions/applications.)

**Objectives:** We developed a porcine model of combined burn and traumatic brain injury and compared different fluid resuscitation strategies.

**Methods:** Swine were anesthetized and instrumented. Baseline data were collected prior to any injuries and again at a time point either 7 or 8 hours from injury depending upon the variable. Forty percent body surface area burns were created with steel plates heated to 275° C. Traumatic brain injuries were generated using a controlled cortical impactor to deliver a reproducible force to the brain through a burr hole. Swine were randomly assigned to receive either aggressive (Parkland formula) or restrictive (modified Brooke formula) resuscitation with lactated Ringer's solution and hypertonic saline solution. Following 8 hours of critical care, swine were euthanized and brain slices were stained with 2,3,5-triphenyl tetrazolium chloride (TTC). Following fixation, the volume of each brain injury was measured. Tissue samples from brain, skin, liver, kidney, and lung were routinely processed and stained with hematoxylin and eosin. Immunohistochemistry of selected brain sections with antibodies against  $\beta$ -amyloid precursor protein was conducted in a conventional manner. All tissue sections were evaluated and scored by a blinded observer.

**Results:** Treatment groups did not differ in any respect at baseline except for starting body weight (Table 1). Pigs receiving aggressive resuscitation had significantly higher intraoperative weight gain, more urine output, longer time at goal mean arterial pressure, longer time at goal sodium concentration and lower blood urea nitrogen (BUN) concentrations compared with pigs receiving restrictive treatment (Table 2). Relative to baseline values, end of study hematocrit, BUN, aspartate transaminase, and potassium concentrations were significantly higher in both groups but not substantially different from each other. Grossly, there were no significant differences in brain lesion sizes between groups and no significant differences in histological features. Lung and liver pathology did not differ significantly between groups, while the restrictive group had slightly more kidney lesions compared to the aggressive resuscitation group. Histologic features in these tissues reflected congestion and edema.

**Discussion/Conclusion:** Both the treatment groups appeared to be adequately resuscitated during the 8 hours of critical care. Elevated BUN and AST concentrations are commonly reported in burn patients and likely don't reflect kidney or liver pathology, as creatinine and liver-specific alanine aminotransferase concentrations were not elevated. Aggressive fluid therapy for burns did not appear to worsen concurrent brain injuries.



(PI / TC Signature)



(Date)

**Attachments:**

Attachment 1: Defense Technical Information Center (DTIC) Abstract Submission (**Mandatory**)

**Attachment 1****Defense Technical Information Center (DTIC) Abstract Submission**

**This abstract requires a brief (no more than 200 words) factual summary of the most significant information in the following format: Objectives, Methods, Results, and Conclusion.**

**Objectives:** We developed a porcine model of combined burn and traumatic brain injury and compared different fluid resuscitation strategies.

**Methods:** Burns and traumatic brain injuries were created. Swine received either aggressive or restrictive fluid resuscitation. Following 8 hours of critical care, swine were euthanized. Tissue samples were collected, processed and reviewed by a blinded observer.

**Results:** Pigs receiving aggressive resuscitation had significantly higher intraoperative weight gain, more urine output, longer time at goal mean arterial pressure, longer time at goal sodium concentration and lower blood urea nitrogen (BUN) concentrations compared with pigs receiving restrictive treatment. Relative to baseline, end of study hematocrit, BUN, aspartate transaminase, and potassium concentrations were significantly higher in both groups but not substantially different from each other. Grossly, there were no significant differences in brain lesions sizes between groups. Lung and liver pathology did not differ significantly between groups. The kidneys did. Histologic features in these tissues reflected congestion and edema.

**Discussion/Conclusion:** Both groups appeared to be adequately resuscitated during the 8 hours of critical care. Elevated BUN and AST concentrations are commonly reported in burn patients and likely don't reflect kidney or liver pathology, as creatinine and liver-specific alanine aminotransferase concentrations were not elevated.

**TABLE 1. Characteristics at Baseline (mean  $\pm$  SD or median [IQR] depending on distribution).**

| Variable or Analyte                           | Restrictive (n=14) | Aggressive (n=14) | P    |
|---|--------------------|-------------------|------|
| Weight (kg)                                   | 68.4 $\pm$ 7.8     | 76.0 $\pm$ 9.2    | 0.03 |
| Core Temperature ( $^{\circ}$ C) <sup>a</sup> | 35.7 $\pm$ 3.8     | 34.5 $\pm$ 5.9    | 0.55 |
| Percent BSAB <sup>b</sup> (%)                 | 40.0 $\pm$ 0.6     | 40.0 $\pm$ 0.6    | 0.98 |
| Heart Rate (Beats per Minute) <sup>a</sup>    | 100 $\pm$ 12       | 89 $\pm$ 13       | 0.04 |
| Mean Arterial Pressure (mm Hg) <sup>a</sup>   | 65 [56 – 73]       | 60 [56 – 70]      | 0.43 |
| Central Venous Pressure (mm Hg) <sup>a</sup>  | 8 [5 – 12]         | 11 [9 – 13]       | 0.08 |
| Intracranial Pressure (mm Hg) <sup>a</sup>    | 17 [13 – 30]       | 17 [4 – 47]       | 0.69 |
| SPO <sub>2</sub> (%) <sup>a</sup>             | 97 [96 – 98]       | 98 [94 – 99]      | 0.84 |
| Preinjury Blood Loss (mL/kg)                  | 0.9 $\pm$ 0.4      | 0.8 $\pm$ 0.5     | 0.43 |
| White Blood Cell Count (x10 <sup>9</sup> /L)  | 15.0 $\pm$ 4.7     | 13.7 $\pm$ 3.1    | 0.42 |
| Hematocrit (%)                                | 27.0 $\pm$ 3.3     | 25.3 $\pm$ 3.3    | 0.18 |
| Platelet Count (x10 <sup>9</sup> /L)          | 273 [247 – 364]    | 287 [268 – 334]   | 0.82 |
| Fibrinogen (mg/dL)                            | 403 $\pm$ 65       | 401 $\pm$ 62      | 0.95 |
| Alkaline Phosphatase (U/L)                    | 106 [90 – 138]     | 92 [84 – 108]     | 0.09 |
| Alanine Transaminase (U/L)                    | 39 $\pm$ 13        | 39 $\pm$ 11       | 0.90 |
| Aspartate Transaminase (U/L)                  | 11 [10 – 15]       | 13 [10 – 18]      | 0.60 |
| Albumin (g/dL)                                | 2.5 [2.4 – 2.7]    | 2.6 [2.4 – 2.6]   | 0.53 |
| Blood Urea Nitrogen (mg/dL)                   | 8.4 $\pm$ 1.7      | 8.0 $\pm$ 2.0     | 0.55 |
| Creatinine (mg/dL)                            | 1.2 $\pm$ 0.1      | 1.3 $\pm$ 0.2     | 0.16 |
| Total Bilirubin (mg/dL)                       | 0.3 $\pm$ 0.1      | 0.4 $\pm$ 0.1     | 0.06 |
| pH.   | 7.47 $\pm$ 0.05    | 7.44 $\pm$ 0.04   | 0.08 |
| pO <sub>2</sub> /FiO <sub>2</sub> (mm Hg/%)   | 470 $\pm$ 63       | 448 $\pm$ 109     | 0.52 |
| K <sup>+</sup> (mmol/L)                       | 3.2 [3.1 – 3.5]    | 3.4 [3.1 – 3.7]   | 0.50 |
| Glucose (mg/dL)                               | 94 $\pm$ 24        | 98 $\pm$ 19       | 0.68 |
| Lactate (mmol/L)                              | 2.8 [2.6 – 3.0]    | 3.0 [2.6 – 3.4]   | 0.34 |

<sup>a</sup>At T30, otherwise at T0. <sup>b</sup>BSAB=Body Surface Area Burned.

**TABLE 2. Characteristics at End of Study (mean ± SD or median [IQR] depending on distribution).**

| Variable or Analyte                             | Restrictive (n=14)        | Aggressive (n=14)         | P     |
|---|---------------------------|---------------------------|-------|
| Weight Gain (kg)                                | 1.1 ± 1.1                 | 2.3 ± 1.3                 | 0.01  |
| Core Temperature (°C) <sup>a</sup>              | 38.4 [38.0 – 39.3]        | 38.7 [38.4 – 39.4]        | 0.23  |
| Heart Rate (Beats per Minute) <sup>a</sup>      | 127 [112 – 133]           | 113 [107 – 150]           | 0.96  |
| Mean Arterial Pressure (mm Hg) <sup>a</sup>     | 62 [54 – 64]              | 64 [62 – 65]              | 0.11  |
| Central Venous Pressure (mm Hg) <sup>a</sup>    | 8 [6 – 11]                | 10 [7 – 12]               | 0.22  |
| Intracranial Pressure (mm Hg) <sup>a</sup>      | 22 [17 – 42]              | 23 [16 – 72]              | 0.98  |
| SPO <sub>2</sub> (%) <sup>a</sup>               | 98 [97 – 98]              | 97 [96 – 99]              | 0.74  |
| White Blood Cell Count (x10 <sup>9</sup> /L)    | 14.7 ± 2.7                | 12.9 ± 3.7                | 0.16  |
| Hematocrit (%)                                  | 33.4 ± 2.9 <sup>‡</sup>   | 30.6 ± 3.9 <sup>‡</sup>   | 0.04  |
| Platelet Count (x10 <sup>9</sup> /L)            | 314 [248 – 355]           | 309 [267 – 365]           | 0.80  |
| Fibrinogen (mg/dL)                              | 479 ± 89                  | 458 ± 57                  | 0.47  |
| Alkaline Phosphatase (U/L)                      | 134 ± 34 <sup>‡</sup>     | 114 ± 29                  | 0.10  |
| Alanine Transaminase (U/L)                      | 42 ± 12                   | 41 ± 10                   | 0.84  |
| Aspartate Transaminase (U/L)                    | 37 [25 – 66] <sup>†</sup> | 36 [27 – 48] <sup>‡</sup> | 0.85  |
| Albumin (g/dL)                                  | 2.7 ± 0.3                 | 2.5 ± 0.2                 | 0.22  |
| Blood Urea Nitrogen (mg/dL)                     | 15.0 ± 2.0 <sup>‡</sup>   | 12.1 ± 2.5                | <0.01 |
| Creatinine (mg/dL)                              | 1.6 ± 0.2                 | 1.6 ± 0.2                 | 0.36  |
| Total Bilirubin (mg/dL)                         | 1.4 ± 0.4                 | 1.3 ± 0.7 <sup>‡</sup>    | 0.71  |
| pH  | 7.48 ± 0.04               | 7.47 ± 0.02               | 0.58  |
| pO <sub>2</sub> /FiO <sub>2</sub> ≥ 2 (mm Hg/%) | 449 [431 – 506]           | 421 [387 – 480]           | 0.52  |
| K <sup>+</sup> (mmol/L)                         | 4.5 ± 0.6 <sup>†</sup>    | 4.4 ± 0.3 <sup>‡</sup>    | 0.52  |
| Glucose (mg/dL)                                 | 120 ± 23                  | 114 ± 16                  | 0.45  |
| Lactate (mmol/L)                                | 2.6 ± 0.7                 | 2.7 ± 0.8                 | 0.70  |
| Norepinephrine (mcg/kg)                         | 35.8 [21.9 – 73.7]        | 31.9 [25.4 – 70.2]        | 0.92  |
| Lactated Ringer's Solution (mL/kg)              | 42.9 ± 11.6               | 76.1 ± 20.6               | <0.01 |
| 10% Saline Solution (mL/kg)                     | 10.3 ± 1.7                | 11.3 ± 1.8                | 0.12  |
| Urine Output (mL/kg/hr)                         | 5.3 ± 1.8                 | 7.3 ± 1.8                 | <0.01 |
| Brain Injury Area (cm <sup>3</sup> )            | 1.4 ± 0.5                 | 1.6 ± 0.8                 | 0.51  |
| Time at MAP ≥ 65 mm (%)                         | 51.8                      | 58.3                      | <0.01 |
| Time [Na <sup>+</sup> ] at Goal (%)             | 37.3                      | 54.0                      | 0.01  |

<sup>a</sup>At T420, else T480. <sup>†</sup>Significantly greater than baseline, p<0.05. <sup>‡</sup>Significantly greater than baseline, p<0.01.