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14. ABSTRACT

Neurological injury [brain and cord] is always accompanied by tissue deprivation of glucose and oxygen (ischemia/hypoxia). Most of the damage seems to be mediated by mechanisms that follow the initial injury (secondary mechanisms). Perfluorocarbons (PFCs) are one of the methods by which oxygen delivery to tissue can be achieved after injury. The rationale for PFCs in traumatic brain injury has been well established in animal studies and early phase 2 clinical trials. Currently three perfluorocarbons are available in the United States for testing, but none of these have been FDA approved and only for one of them - Oxycyte has the process of application for FDA approval even been commenced. For the third generation perfluorocarbon (Oxycyte) a possible side effect that has emerged in humans is transient mild thrombocytopenia. It is uncertain at this time whether this side effect will prove to be a limiting factor which may jeopardize the use of these compounds as a class, or just affect Oxycyte in particular following traumatic brain and spinal cord injury. Any agent which might exacerbate thrombocytopenia in intracranial hemorrhage into traumatic contusions is dangerous for obvious reasons. The purpose of this grant therefore is to cross compare the safety and efficacy of three perfluorocarbons namely Oxycyte, Perftec and Oxygent. We assessed these 3 PFC agents in two head injury models (1) new PENETRATING brain injury animal model (human gun-shot wound to head) and (2) closed severe rat TBI -Fluid Percussion Injury (human car crash) with a secondary Hypoxic insult. We measured how the PFCs alter the ability of the injured brain (1) to use glucose, oxygen and (2) lower cell death caused by injury, (3) effect on blood clotting. First, we did not find any evidence of impairment of blood clotting in rats with TBI after treatment with PFCs unlike in humans. Secondly the PFCs modestly improved use of oxygen, surprisingly even glucose; however these improvements did not translate into fewer dead cells. Using novel techniques we found that there is persistent reduction in blood flow to brain after injury. For the first time we also showed by electron microscopy that PFCs appear to improve membrane integrity. Although we could not find them beneficial in this model, PFCs can be improved.

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(2) Section I

- A brief introduction covering the purpose and scope of the research effort.

Introduction and grant rationale

Perfluorocarbons are one of the methods by which oxygen delivery to tissue can be achieved after injury. Neurological injury [brain and cord] is always accompanied by tissue ischemia/hypoxia and much of the damage seems to be mediated by this secondary mechanism. The rationale for perfluorocarbons in traumatic brain injury has been well established in animal studies and early phase 2 clinical trials. Currently three perfluorocarbons are available in the United States for testing, but none of these have been FDA approved and only for one of them – Oxycyte has the process of application for FDA approval even been commenced. For the third generation perfluorocarbon (Oxycyte) a possible side effect that has emerged in humans is transient mild thrombocytopenia. It is uncertain at this time whether this side effect will prove to be a limiting factor which may jeopardize the use of these compounds as a class, or just affect Oxycyte in particular following traumatic brain and spinal cord injury. Any agent which might exacerbate thrombocytopenia in intracranial hemorrhage into traumatic contusions is dangerous for obvious reasons. The purpose of this grant therefore is to cross compare the safety and efficacy of three perfluorocarbons namely Oxycyte, Perftec and Oxygent. We assessed these 3 PFC agents in a new **PENETRATING brain injury animal model**, devised at WRAIR (the Tortella PTBI model) and in closed severe rat TBI -Fluid **Percussion Injury...FPI**—with and without a secondary Hypoxic insult, for the first time, with such agents.

The 4 specific aims are stated below:

Aim 1: PFC will be effective in mitigating penetrating TBI, as tested in the WRAIR/Tortella model of penetrating ballistic-like brain injury (PBBI), with acute brain histology, at 24 and 72 hours after injury, in the rat.

Aim 2:

- A. Two doses of PFC, given 24 hours apart will be safe and effective in mitigating secondary ischemic damage, superimposed upon severe closed TBI in the rat.
- B. TEG will be performed in collaboration with the Wallace Coulter Platelet Function laboratory at the University of Miami, in both FPI rat models, and in Human blood In Vitro..

Aim 3. PFC's will improve both

- A. Oxygen consumption (CMRO₂) and
- **B.** Glucose use, in the rat brain, after PTBI.

Aim 4. PFC will improve cell survival, in an in vitro model of mild TBI, when applied in the supernatant culture medium.

Our letter of award was made in August 2011 and in this <u>*report*</u> we outline progress that has been made in the **2 year period (Sept 2011-Sept 2013)**

(3) Section II - A brief description of overall progress to date plus a separate description for each task or other logical segment of work on which effort was expended during the report period. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. If this award includes the recruitment of human subjects for clinical research or a clinical trial, report progress on subject recruitment (i.e., number of subjects enrolled versus total number proposed).

The **<u>SCHEDULE OF WORK</u>** from the grant application is attached below, and the status of each task is reported in the following tables.

SCHEDULE OF WORK----PROJECT TASKS

TITLE: The Role of Perfluorocarbons in Mitigating Traumatic Brain Injury. PI: M. Ross Bullock, MD, PhD, University of Miami Miller School of Medicine, Department of Neurological Surgery, 1095 NW 14th Terrace LPLC 3-18, Miami, Florida 33136

- 1. TASK 1: Initial Preparation/Logistics (Months 1-2)
 - a. Hire and assemble a research team, purchase equipment, and reagents; prepare the logistics for experiments over the following 2 years
 - b. With guidance from USAMRMC ACURO we will write, review protocols for animal studies and obtain approval by both DOD and the University of Miami Animal care and use committee.
 - c. Order reagents, surgical supplies, hire a post-doctoral fellow, technologist, and train staff 1-3 months
 - d. Ordering of the Animals, as needed throughout the 2-year project, see Grant chart, below.
 - e. Obtain the PBBI instrument, with help from Tortella lab postdoc, Dr Leung conduct PBBI.
- 2. TASK 2: Aim 1. PFC will be effective in mitigating Penetrating TBI, as tested in the WRAIR/Tortella model, with acute brain histology, at 24 and 72 hours after injury, in the rat. (Months 2-10).
 - a. Start the experiment with reproducible PBBI and the establish treatments of PFCs
 - b. We will begin the histopathological and immunocytochemical staining and analyses during this time frame and should be completed with the majority of the analysis completed for specific Aim 1 by month 12, Task 2 50 male Sprague Dawley rats
- 3. TASK 3: Aim 2. Two doses of PFC, given 24 hours apart will be safe and effective in mitigating secondary ischemic damage, superimposed upon severe closed TBI in the rat. (Months 10-16)
 - a. Model is fluid percussion injury (FPI TBI) +hypoxia treatment with different PFCs.
 - b. The 'run in' group will be 2-3 animals per task to address any technical difficulties.
 - a. –Training of personnel. Month 6, Animal surgeries…months 7-9, Task 3 60 male Sprague Dawley rats

- b. Histopathology....months 8—14, Data analysis and final report –months 12— 16.
- 4. TASK 4: Aim 3. PFC's will improve both oxygen consumption (CMRO₂) and glucose use, in the rat brain, after TBI.
 - a. Model is FPI TBI with PFC to assess oxygen consumption (CMRO₂) and 2-DG uptake.
 - **b.** –**Training of personnel. Month 10. Animal surgeries...months 10—17** Task 4 160 male Sprague Dawley rats
 - c. Histopathology....months 14—20, Data analysis and final report –months 19-22.
- 5. TASK 5: PFC will improve cell survival, in an *in vitro* model of <u>MILD TBI</u>, when applied in the supernatant culture medium.
 - a. *In vitro* experiment to explore if PFC mediated neuroprotection is via membrane stabilizing effect.—month 10, Task 5 20 female time pregnant Sprague Dawley rats.

b. experiments...months 11-12, data analysis and reports...months 13-15

- 6. TASK 6: Aim 5: PFC mitigating TBI induced cognitive deficits, as tested by Morris Water maze
 - a. Identify the most effective PFC in previous Aims, and compare with Oxycyte in a FPI TBI model with cognitive component
 - b. experiments...months 22—24, data analysis and reports...months 23—24 Task
 6 30 male Sprague Dawley rats.
- 7. TASK 7; Interim Analysis
 - a. Interim statistical analysis of the data obtained from different aims of the study
 - **b.** Quarterly progress reports (every 3 months) and annual reports to be written for DOD reviewers.

8. FINAL DELIVERABLES

- a. Final report to DOD CDMRP and initial manuscripts as available,
- b. Detailed manual of operations for surgery, behavior and histopathology
- c. Manuscripts to journals, detailing results of each specific aim.

Grant Chart								
TASK	Jan-June	2012 July-Dec	Jan-June	2013 July-Dec				
Aim 1				Final Report				
Aim 2				Final Report				
Aim 3				Final Report				
Aim 4				Final Report				

Table 1 animal use gant chart

We have completed all experiments involving animal use, according to the Schedule of Work (SOW), except for ...

1) As outlined in the Grant Chart in SOW, we have completed experiments for Aim 2, however the delay in availability of the third PFC resulted in delayed execution of the surgical procedures, and thus subsequently histopathological analysis of these brains, in Aim 2—each brain requires about 8 hours, for cell counts, and analysis. These counts, and analyses, will be completed, over the next few months, **during the No cost extension period, through March 2014.**

2) For Aim 4, microphotography has been completed, but further analysis of the data, is in progress. This will be competed in the next 2 months.

3) Several manuscripts, arising from these studies are in preparation, (see appendix) and these will be completed, in the no cost extension (NCE) period (September 2013 to March 2014) as shown the adjacent chart.





Figure 2 -The processes interrogated by the Aims of this proposal are shown in this schematic. Glucose and oxygen are transported into the brain by vasculature. PFC facilitates this. Glucose is taken up by the cells (Aim 3A) and oxidized via consumption of oxygen (Aim 3B); these metabolic processes keep cells alive. Following injury interruption of these processes could lead to neurodegeneration (Aims 1 and 2).

OVERALL EXECUTIVE SUMMARY, of results to date. No beneficial neuroprotective effect, of any of the 3 PFC tested, was seen, as judged by FJ positive cell counts. Fig 3, 4, 5.





Figure 4 - FJB stained brain section to illustrate green-stained neurons(right panel) which were stereology, by a blinded investigator. (high power view, right, lower panel). This data suggest that PBBI-induced neurodegeneration could not be reduced by use of intravenous PFCs at 2.5h, and 24 hrs post injury. 2. These studies have completed the most detailed histopathological analysis done to date of the effect of the PTBI model (i) upon neuronal degeneration, and (ii) upon the VASCULATURE. We have observed a progressive increase in the severity of the degree of vascular impairment, after PTBI, over the first week, using the tomato red lectin labeling of vasculature in combination with tissue clearing and a newly developed Ultramicroscopy method(Erturk *et al.*, 2012). We have not demonstrated vasospasm as a possible explanation for the progressively worsening vascular impairment, seen clearly in fig 5 below. We speculates that microvascular occlusion, at the capillary and regulatory penetrating arteriolar levels, is responsible. More studies are needed, possibly with ultrastructure to resolve this mechanism and further qualitative vessel counting studies to make a quantitative analysis. Our data suggests a central role for progressive microvascular impairment, as a major cause of the neurodegeneration, after PTBI and could underlie the tissue loss observed by Williams et al 2005(Williams et al., 2005). Figures, 5, 6—lectin vascular labeling. Further analyses will assess the relationship between vascular occlusion, and cell death in this PBBI model.



Fig.5 At 1 week post PBBI the core of the PBBI lesion is a ~1.4mm wide ellipsoid of hypoperfused tissue that is lined by lipofucsin positive immune cells (yellow autofluorescence). Ultramicroscopy allows visualization of the entire lesion in an unprecedented quantifiable manner.



Progress with Aim 2

The FPI studies with Oxycyte, Oxygent and Perftec and secondary hypoxia, and controls are completed. Over the next 6 months, the FJB cell counting will be completed, for these animals. To date 35 of the 60 brains have been cut and stained.

<u>Aim 2B –effect of PFC upon platelet function, and coagulation</u> parameters.

This part of the Aim 2 is to investigate the status of platelets after exposure to PFCs in rats with TBI. Below, we show selected data from the TEG results from rats (n=6 per group) undergoing (1) TBIs without PFCs (2) only PTBI, with 3 PFC's, given 30 mins after PTBI, and blood was sampled 2.5 hrs, after injury.



The CI—Coagulation index data is the most reliable "overview" of coagulation, and NO significant effect, was seen, for any of the PFC's. (See below, Fig16) this suggests no harmful pro-or anti-coagulant effect, of these compounds, in rats, after a severe brain injury. Further consistent with our previous report that platelets were not aggregated in liver, spleen or lungs, Oxygen Biotherapeutics presented at Military Health System Research Symposium (MHSRS) 2013 Ft. Lauderdale, that radio labeled platelets mature into radiolabeled microparticles and cannot be detected in tissues.



Whereas, these peaks in TBI were at 24h after injury (A,B, and C). *p<0.05 **p<0.01 ***p<0.001

Model	PBBI	FPI	ASDH		
Human equivalent	Gunshot	DAI and small contusion	ASDH with large ischemia		
TEG data on 2.5h after injury (Compared to Control)	R† K† α↓ MA↓G↓CI↓	No severe change	Rtt Ktt all MALL GLL CILL		
Peak / recovery of coagulopathy	7	Peaked on 24h Recovered by 7d	Peaked on 2.5h post injury Recovered by 7d		
Hypotension / shock	None				
Hemoglobin (2.5h)	No significant difference				
Platelet counting (24h)	No Significant difference				
Neurodegeneration measured with FJB (2.5h- <24h post injury)	++	+	*++		
Translation	islation <u>Early (2.5h), mild</u> Enzymatic coagulopathy, Fibrin dysgenesis Platelet-fibrin dysfunction,		Early (2.5h), severe Enzymatic coagulation, Fibrin dysenesis Platelet-fibrin dysfunction,		
Possible primary cause of coagulation disorder	Moderate tissue injury, Acute tissue factor (TF) release?→ Moderate Coagulopathy	Late onset secondary axonotomy?→ Late onset coagulopathy	Severe tissue injury → Higher TF release? → Severe consumptive coagulopathy		

Table 1. Data summary and possible pathomechanisms.



Fig 11. TEG values for R (Initiation phase of enzymatic factor activity) not significantly different with PFCs onboard after PTBI.



Fig 12. TEG values for K (Potentiation phase of enzymatic factors yielding clot strengthening via thrombin cleavage of fibrinogen into fibrin) not significantly different with PFCs onboard after PTBI.



Fig 13. TEG values for α (Rate of clot strengthening through polymerization of available fibrinogen. This also represent the thrombin cleavage of fibrinogen into fibrin) not significantly different with PFCs onboard after PTBL.







Fig 15. TEG values for G (Overall total clot strength resulting from all coagulation interactions, calculated from amplitude (A), G=(5000×A)/(100×A), the process of clot dissolution or fibrinolysis, leads to a decrease in G) not significantly different with PFCs onboard after PTBI.



Fig 16. All TEG values CI (CI is linear combination of R.K.MA and a. CI = -0.1227R+0.0092K+0.1655MA-0.0241a-5.0220) not significantly different with PFCs onboard after PTBI

Progress with Aim 3.

PTBI significantly reduced both oxygen consumption, and glucose use (based on 1. ¹⁴C 2-deoxy glucose), in the hemisphere of the lesion (ipsi). This study documents, for the first time, the severity and spatial distribution, of these changes in the brain after PTBI, in this rat model (Fig 7, 8, 9). Equipped with data on PBBI global ischemia and neurodegeneration data, we asked of how the spread of global ischemia after PBBI at 2.5h translates into neurodegeneration at 24-72h? The assessment presented in Fig 18-20 shows that the spread of PBBI induced global ischemia is far greater than the neurodegeneration (almost twice). In the 4mm region along the rostro-caudal axis, there is a less than 2-fold difference in percentage of glucose depression between the core (-0.3mm from Bregma) and peri-lesional area (-4.3mm from Bregma) while in contrast there is a 10-fold drop in FJB-positive cells. Thus in this 2mm region -0.3mm to -2.3mm ischemia directly translation into cell death. However, in the next 2 mm (-2.3 to -4.3mm Bregma) there is a dramatic decrease in cell death (34% at -0.3mm Bregma to 3.4% at -4.3mm Bregma) despite almost similar ischemia (34% vs. 20%). Taken together the data suggest that even in PTBI there is a window of opportunity for therapeutic intervention (2.5-72h) and not all tissue subjected to PTBI is destined to perish. However with PFCs did not significantly increase tissue sparing in this study.







Unfortunately, no robust, ameliorative effect of any of the 3 PFC's tested was seen upon VO₂ in the PBBI model (Fig.1) However, significant improvements in glycolysis could be observed, especially with Perftec and Oxycyte after PBBI (Fig. 18-19).

2. Changes in VO_2 in FPI + secondary hypoxia model were different from that of PBBI. No significant differences existed between right and left hemispheres of uninjured animals. The VO₂ levels were depressed in vehicle treated injured group in FPI +secondary hypoxia as seen in **PBBI compared to uninjured group**. Perftec administration improved the VO₂ both on the ipsi and contralateral sides. However, it did not differ significantly from either the uninjured or injured. The counting of the Fluoro jade positive cells for the Aim 2 is still in progress, once those results are available, improvement in VO_2 and its translation into cell survival can be assessed in that model. In summary, the PFCs were associated with slightly improved oxidative brain metabolism, but there was no statistically significant difference between the PFC's in **PBBI or FPI+secondary hypoxia.** (PFC1=Oxycyte PFC 2 = Perftec) Overall, none of the PFC groups differed significantly from the Vehicle on both sides, a disappointing, and surprising finding. Thus in 2 different animal models of TBI, both penetrating and closed, the delivery of more oxygen, by the PFC was NOT robustly associated with better OXIDATIVE METABOLISM. As we had hypothesized.



restores VO₂ after injury to an extent that is not statistically significant from control uninjured or injured samples based on Dunnett's multiple comparison test, *= p < 0.05. Perftec improved more on the contralateral than ipislateral side.

Figure. 21 –Effect of 3 PFC's upon Oxygen metabolism, after FPI+secondary hypoxia



<u>Effect of 3 different PFC upon Glucose use</u>, after both Penetrating TBI, and Fluid percussion injury. (Aim 3B)

Significant and robust improvements in glucose use were seen in multiple brain regions, associated with Perftec administration, when compared to vehicle treated animals (Fig.18-23).



Figure 23. Pixel based "p mapping" method, shows that Perftec amelioration of glucose use is seen near the lesion.

Figure 23. Pixel based "p mapping" method, shows that Perftec amelioration of glucose use is seen near the lesion. The effect of closed head trauma upon Glycolysis, as measured by the 2-Deoxyglucose method, is well known, and the findings in this model accord quite closely with human TBI. However, the effect of Penetrating TBI upon glycolysis has never been studied, in any animal model, nor in humans. We have robust findings , concerning the effect of PTBI upon glucose use, which will be the focus of a future paper. In brief, PTBI was associated with profound reductions in glucose use, both globally, in the whole brain, and focally around the PTBI site, at 2 hours after injury. Surprisingly, no significant hyperglycolysis was seen, in contrast to other animal models . After PTBI, administration of 2 different PFC's was associated with significant amelioration of this depressed glycolysis (Fig 18-23)

This is a counter intuitive, but important finding, which deserves further study, and may open a new mechanism, by which PFC may improve recovery, after PTBI in particular, and TBI in general. This finding was seen in tissues distant from the injury epicenter, but in both hemispheres, after PTBI. This suggests that the rate limiting enzymes for the glycolytic pathway, (chiefly hexokinase) are influenced by PFC, either directly, or via an increase in oxygen tension, in the tissue.

<u>Aim 4. --</u>Mild TBI.

We have a five replicate experiment completed; with each PFC and saline controls, after stretch injury, imaging and counts are ongoing, for that experiment. We have standardized the imaging of the stretch injured cells while still on the thick silastic membrane. This is the first time such experiments have been done to our knowledge. The quantitation of the data revealed that the **Perftec and Oxygent were associated with significantly reduced cell death in these cultures, 24h post stretch injury.** The effect of 2 of the 3 PFC's studied here seems to be independent from its ability to dissolve gases, since these cells were growing in a fully oxygenated supernatant growth medium solution. Numerous membrane-sealing agents are being developed for use in acute TBI; ability to functionalize such molecules with PFCs may increase their utility--more experiments are warranted. (Ingram *et al.*, 1992; Ellis *et al.*, 1995)





PFCs within cytoplasm appear to be sequestered in vesicles (arrows in F, lower right inset shows PFC vesicle at higher magnification) similar to that seen with fluosol, a different PFC (upper left inset) reported by *Ingram et al.*, 1992. Magnification is indicated by the micron bars.

References

- Ellis, E.F., McKinney, J.S., Willoughby, K.A., Liang, S. & Povlishock, J.T. (1995) A new model for rapid stretch-induced injury of cells in culture: characterization of the model using astrocytes. *Journal of neurotrauma*, **12**, 325-339.
- Erturk, A., Becker, K., Jahrling, N., Mauch, C.P., Hojer, C.D., Egen, J.G., Hellal, F., Bradke, F., Sheng, M. & Dodt, H.U. (2012) Three-dimensional imaging of solvent-cleared organs using 3DISCO. *Nature protocols*, 7, 1983-1995.
- Ingram, D.A., Forman, M.B. & Murray, J.J. (1992) Phagocytic activation of human neutrophils by the detergent component of fluosol. *The American journal of pathology*, **140**, 1081-1087.
- Williams, A.J., Hartings, J.A., Lu, X.C., Rolli, M.L., Dave, J.R. & Tortella, F.C. (2005) Characterization of a new rat model of penetrating ballistic brain injury. *Journal* of neurotrauma, 22, 313-331.

(5) Section IV - A description of work to be performed during the next reporting period.

During our "No cost extension," a 6 month period, to march 31 2014, we will complete the volumetric histopathology, for aim 2, (11 animals cut and stained, remaining 20 to be done) and analyze data for this aim. We will also complete further analyses for aim 4. and generate the manuscripts, listed below.

Manuscripts to be prepared, and supported by putatively publishable data

- 1. Effect of PTBI upon Cell death patterns. Fluoro jade staining via unbiased stereology data-never reported.
- 2. Relationship between TEG and severity of brain damage in 3 different animal models, of TBI
- 3. PFC ameliorates anaerobic glycolysis, but not oxygen metabolism, after PTBI, and closed TBI, in rat models.
- 4. Histopathological correlates of spatial and anatomical patterns of alteration of glycolysis, after PTBI.

Respectfully Submitted, R Bullock/Shyam Gajavelli Sept 30 2013.