Glucose and oxygen metabolism after penetrating ballistic-like brain injury

Shyam Gajavelli, Shimoda Kentaro, Julio Diaz, Shoji Yokobori, Markus Spurlock, Daniel Diaz, Clayton Jackson, Alexandra Wick, Weizhao Zhao, Lai Y Leung, Deborah Shear, Frank Tortella and M Ross Bullock

INTRODUCTION

Despite numerous studies on traumatic brain injury (TBI), patient outcomes remain poor. There are approximately 1.5 million patients and 50,000 deaths from TBI every year and many TBI patients survive with disabilities in the United States. Social costs including medical costs and loss of earning capacity are estimated at 48.3 billion dollars a year according to the Centers for Disease Control and Prevention. Penetrating traumatic brain injuries (PTBI) are associated with the worst outcomes and highest death rates of any form of TBI and predominately affect young adults. No therapies, other than surgery, are available for PTBI. There is an urgent need to explore additional treatment options due to unclear pathophysiology. To gain insights we looked at cerebral metabolism in a PTBI rat model: penetrating ballistic-like brain injury (PBBI). Early after injury, regional cerebral oxygen tension and consumption significantly decreased in the ipsilateral cortex in the PBBI group compared with the control group. At the same time point, glucose uptake was significantly reduced globally in the PBBI group compared with the control group. Examination of Fluorojade B-stained brain sections at 24 hours after PBBI revealed an incomplete overlap of metabolic impairment and neurodegeneration. As expected, the injury core had the most severe metabolic impairment and highest neurodegeneration. However, in the peri-lesional area, despite similar metabolic impairment, there was lesser neurodegeneration. Given our findings, the data suggest the presence of two distinct zones of primary injury, of which only one recovers. We anticipate the peri-lesional area encompassing the PBBI ischemic penumbra, could be salvaged by acute therapies.

Keywords: 2-deoxy glucose; cerebral metabolism; Fluorojade B; glucose; neurodegeneration; oxygen

This work was supported by Department of Defense Grant #PT074521W81XWH-08-1-0419 to RB.

Received 30 September 2014; revised 29 November 2014; accepted 2 December 2014; published online 11 February 2015
In closed TBI, the cellular changes leading to neurodegeneration have been reviewed previously. Briefly, injury-induced glutamate excitotoxicity results in pollution of mitochondria with calcium, activation of calcium-dependent proteases that initiate cytoskeletal destruction, swelling of mitochondria, release of cytochrome c, dissipation of the mitochondrial membrane potential, mitochondrial dysfunction, increased free radical damage, loss of ATP production, and ionic imbalance culminating in necrosis or apoptosis of neurons. Metabolic responses to TBI have given unexpected insights into how to manage TBI. Global brain metabolism usually decreases after TBI. However, paradoxical increases in glucose metabolism have been detected in some regions of the brain proximal to the impact site. This suggests that hypermetabolic changes might produce more ATP to help the brain restore ionic homeostasis after blunt closed TBI. However, cerebral cellular metabolism after PTBI remains unclear. To assess the metabolic consequences of PTBI, we measured brain oxygen tension, oxygen consumption, and glucose uptake during the acute postinjury phase using the PBBI model. In addition, we investigated the extent to which the observed physiologic and metabolic changes were related to injury-induced neurodegeneration to guide the evaluation of potential of future therapies.

MATERIALS AND METHODS

Surgical Procedures and Penetrating Ballistic-Like Brain Injury

All animal procedures were conducted at the University of Miami, Miller School of Medicine. All procedures were conducted in compliance with guidelines established by the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the University of Miami’s Institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats (250 to 330 g) were used in this study and were maintained on a 12-hour/12-hour light/dark cycle and given food ad libitum. Anesthesia was induced with isoflurane (2% to 5%) delivered in a mixture of 70% nitrous oxide and 30% oxygen. Body temperature was maintained at normothermia (37°C ± 1°C) throughout all surgical procedures by means of a homeothermic heating system (Harvard Apparatus, South Natick, MA, USA). Food and water were provided ad libitum postoperatively.

The sample size calculations, criteria for exclusion/inclusion of injured animals in experiments and randomization of animals into different groups were performed as described earlier by Williams et al.13 Anesthetized rats were positioned in a stereotaxic device (Kopf, Tujunga, CA, USA) and unilateral PBBI was induced. Briefly, a specially designed stainless steel probe (Popper & Sons Inc., New Hyde Park, NY, USA) with fixed perforations sealed by airtight elastic tubing on one end was mounted to a stereotaxic arm at an angle of 50° from vertical axis and 25° counter-clockwise from anterior-posterior axis. The other end of the probe was connected to a simulated ballistic injury device (Mitre Corp., McLean, VA, USA). The probe was manually inserted through the right frontal cortex of the anesthetized rat via a cranial window (+4.5 mm A-P, +2 mm M-L from bregma) to a distance of 12 mm (from dura). The ballistic component of the injury was induced by a rapid (∼40 ms) water pressure pulse delivered by the injury device, forming an elliptical-shaped balloon injury while restrained in a lower body cast to secure the cannulas, as described in Sokoloff et al. Blood samples were collected at 20 seconds, 45 seconds, and 85 seconds; and 2 minutes, 5 minutes, 7 minutes, 12 minutes, 20 minutes, 30 minutes, and 45 minutes after injection to assess serum glucose levels and incorporation of radioactivity. After 45 minutes, the animals were euthanized with 1 mL pentobarbital (60 mg/ mL per rat); and the brain was removed as quickly as possible and frozen in isopentane and dry ice. The blood samples were processed and counted with a scintillation counter. The frozen brains were trimmed to −6.3 millimeters embedded in a rat brain matrix, and cut on a dedicated cryostat into 20-μm-thick coronal sections. Three consecutive sections of 300 μm were collected generating a total of 19 series. These serial sections were dried on glass coverslips, glued on poster board, and exposed to [14C]-sensitive PhosphorImaging screens for 36 hours along with [14C] standards of known radioactivity concentration (Amersham Biosciences, Piscataway, NJ, USA). The screens were read and digitized by a PhosphorImaging system (Cyclone Storage Phosphor System; PerkinElmer Life Sciences, Waltham, MA, USA). Individual calibration curves were calculated based on absolute gray levels of the [14C] standards on each film.

| Table 1. Experimental groups, sample size, and figures |
|---------------------------------|
| **# Measurement** | **Experimental groups** | **n/group** | **Results** |
| 1 | PtO2-continuous brain O2 measurement by Licox | Sham | 6 | Figure 1A |
| 2 | Microrespirometry VO2 measurements | PBBI | 8 | Figure 1A |
| 3 | 2-Deoxy glucose (2-DG) mapping of glycolysis | PBBI | 10 | Figure 1B |
| 4 | Fluorojade B cell counts | Sham | 4 | Figure 2 |
| 5 | Hypoperfusion lectin staining | Sham | 3 | Figure 4 |

Abbreviations: PBBI, penetrating ballistic-like brain injury. *Excludes ~ 10% attrition rate for PBBI surgery.

2-Deoxy Glucose Uptake

Two hours after PBBI, a femoral vein was cannulated in the anesthetized rat. Then, immediately before anesthesia was terminated, 50 μCi of [14C]-2-deoxy-D-glucose ([14C]DG) was administered using a 23-gauge blunt needle via the femoral vein over 30 seconds, followed by a 0.2-mL bolus of saline. The anesthesia was then terminated and the animal was allowed to wake up while restrained in a lower body cast to secure the cannulas, as described in Sokoloff et al.20 Blood samples were collected at 20 seconds, 45 seconds, and 85 seconds; and 2 minutes, 3 minutes, 5 minutes, 7 minutes, 12 minutes, 20 minutes, 30 minutes, and 45 minutes after injection to assess serum glucose levels and incorporation of radioactivity. After 45 minutes, the animals were euthanized with 1 mL pentobarbital (60 mg/ mL per rat); and the brain was removed as quickly as possible and flash-frozen in isopentane and dry ice. The blood samples were processed and counted with a scintillation counter. The frozen brains were trimmed to −6.3 millimeters embedded in a rat brain matrix, and cut on a dedicated cryostat into 20-μm-thick coronal sections. Three consecutive sections of 300 μm were collected generating a total of 19 series. These serial sections were dried on glass coverslips, glued on poster board, and exposed to [14C]-sensitive PhosphorImaging screens for 36 hours along with [14C] standards of known radioactivity concentration (Amersham Biosciences, Piscataway, NJ, USA). The screens were read and digitized by a PhosphorImaging system (Cyclone Storage Phosphor System; PerkinElmer Life Sciences, Waltham, MA, USA). Individual calibration curves were calculated based on absolute gray levels of the [14C] standards on each film.
Subsequent densitometric measurements were performed by conversion to radioactivity units of nanoCuries per gram (nCi/g). The average metabolic activities in ipsilateral versus contralateral brain were then calculated, using the equations of Sokoloff et al. To generate averaged 2-deoxy glucose (2-DG) distribution maps by using our established image processing procedure, histologic sections were digitized at 8 standards. Degradation from +4.0 mm to −7.0 mm anterior-posterior to bregma. Serial sections (16 mm thick) between bregma levels −4.8 mm and −5.8 mm were stained for Fluorojade B (FJB) (Millipore, Billerica, MA, USA) to identify degenerate neurons. For unbiased stereology, the whole brain and each hemisphere were contoured at × 5 magnification. The physical fractionator method following the work of stereology to measure the cerebral rate of oxygen consumption (CMRO$_2$) perfectly overlapped with the histologic descriptions of the PBBI (Figure 2A). The mean glucose utilization over the entire brain volume between the bregma levels noted above. To enable examination of the relationship between extent of glucose uptake impairment and incidence of neurodegeneration, the data from identical levels were used. To normalize/control between extent of glucose uptake impairment and incidence of neurodegeneration, the numbers of FJB+ cells in a section at a given distance from bregma was divided by the value of glucose uptake for injured groups (Figure 1B) (*P < 0.05). Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

In this study, PBBI induced statistically significant acute transient hypoxia consistent with Murakami et al. Figure 1A) (*P < 0.05, n = 6 to 8, Multiple t-tests followed by Holm–Sidak method). To examine the consequences of PBBI-induced hypoxia, we used microspheres to measure the cerebral rate of oxygen consumption (CMRO$_2$) in cortical tissue cores. In the ipsilateral cerebral cortex between 2.5 and 3.0 hours after PBBI, oxygen consumption was significantly reduced compared with samples from uninjured animals (Figure 1B) (*P < 0.05, n = 10, Mann–Whitney test).

In the brain, oxygen availability influences glucose metabolism, therefore we analyzed global and regional cerebral glucose metabolism. A total of 130 regions of interest from 19 sections, spanning +2.7 mm to −6.3 mm from bregma were examined by $^{13}$C autoradiography and rendered as a heat map (see Materials and methods). Color-coded maps of local cerebral metabolic rate of glucose (LCMRglu) perfectly overlapped with the histologic descriptions of the PBBI (Figure 1A). The mean glucose utilization over the entire brain (sum of all 19 sections) for the sham, probe (no inflation), and PBBI treatment groups was 3,075.95, 3,098.73, and 1,794.29, µmol/100 g per minute, respectively. Total cerebral glucose uptake was significantly reduced by the injury (PBBI) compared with sham groups (*P < 0.05) (F = 19.33) and probe-only groups (no-inflation control). Additionally, there was no statistically significant difference between sham groups and probe-only groups (n=4 to 5/group, one-way ANOVA followed by Bartlett’s test) (Figure 2B). The core of the injury could be clearly visualized as an oval cavity with the lowest 2-DG.

![Figure 1. Cerebral oxygen metabolism after penetrating ballistic-like brain injury (PBBI). (A) Time course of brain hypoxia. There were significant differences in partial pressure of brain tissue oxygen (PbO$_2$) percent change from baseline between sham and PBBI groups from 105 to 125 minutes after injury (asterisk, *P < 0.05). (B) Brain tissue oxygen consumption in ipsilateral cortex. There was a significant difference in VO$_2$ between probe and PBBI groups 2.5 hours after injury (*P < 0.05).](image-url)
After insertion of the probe alone (with no balloon inflation) reductions in 2-DG uptake were limited to anterior brain regions, corresponding to the entrance route of the probe (Probe-only group; Figure 2A). Although the injury was unilateral, it resulted in bilateral hypoglycolysis: Sham Ipsi versus PBBI Ipsi (with mean difference of 39.92), and Sham Ipsi versus PBBI Contra (with mean difference of 26.75), Sham Contra versus PBBI Contra (with mean difference of 28.06). Interestingly, a trend for hypoglycolysis was also limited to the anterior and posterior length of the injury tract (Figure 2C).

Multiple t-tests were used to compare the 2-DG uptake in sections between groups. Glucose metabolism after PBBI was reduced bilaterally in a number of brain regions including the cingulate cortex, frontal cortex, parietal cortex, ventrolateral orbital cortex, nucleus accumbens, caudate putamen, dorsal agranular insular cortex, intermediate and lateral septal nuclei, limb cortical areas, globus pallidus, posterior agranular cingular cortex, retrosplenial cortex, hippocampus, striatum, and occipital cortex, temporal cortex.
central gray, and dentate gyrus. The levels of glucose in peripheral circulation were unaltered between groups (data not shown).

Neurodegenerative cell counts 24 hours after PBBI were compared between PBBI rats and uninjured rats. Increased evidence of neurodegeneration was detected in the injury core and in the peri-lesional zone of the injured hemisphere (Figure 3A). Verification of neurodegeneration by Fluorojade staining is shown in Figure 3B. In the ipsilateral hemisphere, the number of FJB+ cells was significantly greater than that in the contralateral hemisphere (P = 0.0152, n = 6, Mann–Whitney test GraphPad Prism 6.0, GraphPad Software, Inc., La Jolla, CA, USA; Figure 3D). To assess status of microvasculature after PBBI, we combined fluorescent lectin perfusion with light sheet microscopy. A composite image of the each hemisphere shows the extent of lectin labeling (hence perfusion) (Figures 4A and 4B). Compared with the contralateral side, hypoperfusion was evident on the ipsilateral hemisphere (values for lectin labeling volume 6.4E10 μm³ versus 4.6E10 μm³, respectively (Figure 4A). Surface reconstruction using Imaris software clearly shows the absence of perfusion of a major blood vessels ipsilateral to the injury in contrast to the contralateral side (Figure 4A). This region of hypoperfusion also shows least uptake of 2-DG and corresponds to the center of the injury tract. This is rostro-caudally flanked by regions with higher 2-DG uptake (Figure 4C). The cortical neurodegeneration (FJB staining) data across five evenly spaced brain sections spanning (−0.3 mm to −4.3 mm from bregma, n = 6/group) were compared with the 2-DG uptake data to explore the possible existence of a penumbra (Figure 4D). The 2-DG uptake was least in the cortex at −0.8 mm and −2.8 mm from bregma (Figure 4C). In contrast, the FJB+ cell counts peaked at −0.3 mm but decreased exponentially caudally at −4.3 mm from bregma. The FJB/LCMRglc ratio across the sections revealed the numbers of FJB+ cells at the injury core decreased from 100% at −0.3 mm to 8% at −4.3 mm from bregma. In the brain sections (−0.3 mm to −1.3 mm) recovery may be hampered due to severe ischemia as evidenced by lack of a capillary network (Figures 4A and 4B) < 31% of maximal LCMRglc thus constitute the irrecoverable injury core (** in Figure 4C). While in the flanking regions (−2.3 mm to −4.3 mm from bregma) there is robust recovery from ischemia as evidence by lower FJB counts (Figure 4D). Taken together, this region could constitute the penumbra (* in Figure 4C).

**DISCUSSION**

The passage of a high velocity projectile (e.g., bullet or shrapnel) through the brain produces intense local shearing forces,
especially adjacent to the compression/cavitation zone and which progress along the entire course of the injury tract. When the projectile is of a sufficient mass and velocity, the entire brain is affected, and death is usually rapid, as seen in high velocity rifle wounds. However, when the mass of the projectile or its velocity is low enough to restrict the compression/cavitation damage zone to a smaller tissue zone in the brain, as seen in handgun victims and those injured through shrapnel, etc., the PBBI rat model depicts the neuropathologic consequences of these survivable PTBI injuries in humans, with a high degree of accuracy.13

The delayed nature of cell death, over weeks or months in humans, and over days in rats, is well known.24,25 However, no therapies have been shown to influence this process. As part of a Department of Defense-funded effort to gain insights into mechanisms underlying PTBI, we have determined the oxygen tension (PbO2), oxygen consumption (CMRO2), glucose uptake (2-DG method), and their relationship to regional cell death in the PBBI rat model.

Hypoxia is also associated with deficits in CMRO2 both in TBI patients and in experimental TBI. The results of the current study provide the first demonstration of acute impaired CMRO2 in the PBBI model (Figure 1). This apparent hypoxia occurs in limited patients and in experimental TBI. The results of the current study suggest 'mitochondrial dysfunction' similar to that seen in PTBI patients.19,26 Acute hypoxia and lowered CMRO2 after PBBI are consistent with PTBI patient data and previous work in PBBI.7,9,18 However, there is no precedent to targeting CMRO2 after PTBI to improve outcomes. On the basis of the PBBI literature and findings in this study, it can be expected that PTBI-induced mitochondrial dysfunction may limit benefits from manipulation of CMRO2. Yao et al27 compared tissue viability after PBBI and middle cerebral artery occlusion using TTC (2,3,5-triphenyltetrazolium chloride) staining and found that, unlike middle cerebral artery occlusion, TTC staining after PBBI was preserved at 24 hours after injury.27 The TTC staining is a measure of mitochondrial oxidative phosphorylation complex I activity and the absence of staining reflects tissue ischemia.28 Therefore, PBBI appears to induce a metabolic defect that is between the oxidative phosphorylation complex I (based on intact TTC) and complex IV (based on reduced CMRO2). The persistence of oxidative phosphorylation complex I activity in the absence of oxygen consumption may increase production of reactive oxygen species contributing to PBBI-induced neurodegeneration. In contrast to stroke and closed TBI where there was an initial increase followed by a decrease in CMRO2 in PBBI there is only a decrease. On the basis of these data, it may be speculated that damage to the mitochondria in the PBBI compression/cavitation zone triggers the mechanical release of well-characterized factors such as cytochrome c, or novel factors such as inflammasomes that increase apoptosis and necrosis in the neighboring tissues and spreading mitochondrial dysfunction to areas beyond the primary injury.29,30

Importantly, astrocyte-mediated transcellular mitophagy has recently been implicated as a mechanism that helps maintain neuronal metabolism.31 However, the degree to which increased intracranial pressure and reactive gliosis may impair intra/transcellular mitophagy after PBBI is not yet known. Before
manipulating oxygen levels, targeted mitochondrial stability/biogenesis may be necessary. Preclinical work has shown that mitochondrial uncoupling after injury may be beneficial by reducing reactive oxygen species production, albeit the downside of such intervention (i.e., hyperthermia) needs to be mitigated. Alternatively, increased clearance of dysfunctional mitochondria by transcellular mitophagy may serve as a better antioxidant than any extraneous agents with antioxidant activity. Given our findings, there is no evidence to suggest that manipulation of brain oxygen tension alone can improve PTBI outcomes.

In the current study, significant decreases in glucose uptake were detected both globally and in a region-specific manner after PBBI in rats. To our knowledge, these findings are the first reports to show profound and global decrease in glucose uptake after PBBI. Previous studies using brain injury models reported an acute period of increase glucose metabolism on the order of minutes. In our study, the metabolic measurements were made hours after the insult. Therefore, the period of hyperglycolysis might have been missed hence cannot be ruled out. Another caveat that needs consideration is that compared with TBI, PBBI injury may have relatively greater compromise of blood–brain barrier. In TBI patients and the fluid percussion injury model of closed head injury, less severe regional decreases in cerebral metabolic rate of glucose were seen using 13C nuclear magnetic resonance spectroscopy and 18F-fluorodeoxyglucose positron emission tomography, and using the 2-DG method, in animal models, such as fluid percussion injury. Similarly, in a stroke model (reversible middle cerebral artery occlusion) glucose utilization is decreased regionally, and a core lesion of depressed 2-DG uptake is surrounded by a zone of increased 2-DG uptake. In contrast, in this study at the time pointed tested we could not observe any regions of hyperglycolysis, after PBBI.

Under normal physiologic conditions oxygen helps degrade hypoxia inducible factor 1α while hypoxia stabilizes it. Hypoxia inducible factor 1α associates with a basic helix–loop–helix transcription factor to upregulate genes encoding enzymes involved in cellular metabolism, including the glycolytic enzymes. Despite acute hypoxia, the hypoxia inducible factor 1α protein is not detectable ipsilateral to the injury after PBBI (unpublished data and Cartagena et al35). The absence of hyperglycolysis in PBBI is consistent with lack of hypoxia inducible factor 1α. Thus, the use of glucose or lactate to supplement metabolism may rescue the ‘PBBI penumbra’ tissue surrounding the injury core. Global reduction of glucose utilization could be due to the much larger lesion volume in PBBI than that in closed TBI, fluid percussion injury, and stroke models in the rat.

The reasons for decreased glucose uptake after PBBI could be similar to those seen in TBI such as poor perfusion, glucose transport across the blood–brain barrier, diminished hexokinase activity, and severe loss of cerebral perfusion pressure after PBBI. To test whether poor perfusion underlies the phenomenon, we assessed the density of the microvasculature using a tomato-lectin loop and found no detectable ischemia-like state, as seen after vessel occlusion, in stroke. One limitation of extending these findings to PTBI is that the PBBI mathematical models the intracranial cerebral temporary cavitation, tissue tearing and shearing accurately capturing the secondary events. However, the model does not model the rapid entry of projectile and associated metabolic consequences. The injury is proportional to the balloon expansion and consequent secondary mechanisims.

Experimental studies using stroke models to document the ischemic flow thresholds of brain tissue have shown the existence of two critical levels of decreased perfusion: first, a level representing the flow threshold for reversible functional failure (functional threshold); second, a lower threshold below which irreversible membrane failure and morphologic damage occur. In PBBI, demonstrating the presence of an ischemic penumbra region, and its severity, could help improve outcomes. For example, reduced glycolysis may suggest that lactate or ketone administration may be beneficial. For this reason, we assessed the relationship between depression of 2-DG uptake and extent of cortical neurodegeneration at multiple bregma levels. Neurodegeneration as assessed using FJB has been found to peak from 24 to 72 hours after PBBI. This result is similar to previous report and indicates that neuronal cell death occurs primarily ipsilateral after PBBI. Due to technical limitations, 2-DG uptake and labeling for neurodegeneration (FJB) were performed in separate groups of animals. However, anatomically homologous sections from bregma were matched using hematoxylin and eosin stained sections. The hypoperfusion, hypoxia, decreased glucose uptake, acute impaired cerebral oxygen consumption (2.5 hours after injury) culminate to produce neurodegeneration at the PBBI injury core (at 24 hours after injury). Despite the aforementioned pathophysiology contributing factors, rostrocaudally from the injury epicenter there is lower neurodegeneration suggesting similarities to stroke penumbra (Figure 4D), where studies in ischemic stroke models suggest that such a region is characterized by low perfusion, electrical silence, and damaged but viable neurons that can be putatively rescued by early thrombolytic therapy. Consistent with our PBBI CMRO2 and LCMRglc results, a positron emission tomography study of closed TBI patients has shown a decrease in CBF, oxygen extraction fraction, CMRO2, and LCMRglc with an aerobic metabolism in the hypodense gray matter at the pericontusional area.

To better guide future therapeutic strategies for PTBI, we have focused on elucidating the pathophysiology. Our data show significant reductions in glucose utilization in the whole brain hemisphere occurring concomitantly with significant decreases of cerebral oxygen consumption and consequent neurodegeneration localized to the ‘core lesion’ (albeit using different animals). The metabolic impairments appear to be tied to impaired perfusion and spontaneous recovery from the early PBBI metabolic impairments is apparent at 24 to 72 hours after injury. Brain regions that show evidence of injury-induced mitochondrial dysfunction transition into neurodegeneration, whereas regions absent of mitochondrial impairment remain free of neurodegeneration. These data suggest that, unlike in closed TBI, use of normobaric hyperoxia or hyperbaric oxygen as a clinical intervention may produce mixed results in PTBI. However, as reported earlier for both PBBI and acute subdural hematoma, hypothermia may provide to be a better option. Gradual metabolic restoration via slow rewarming giving injured tissue time to carry out repairs (such as mitochondrial biogenesis) and reverse metabolic derangements is theoretically attractive extrapolation of our data.

**DISCLOSURE/CONFLICT OF INTEREST**

Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official, or as reflecting true views of Department of the Army or Department of Defense.

**ACKNOWLEDGMENTS**

Contributions of Professor Helen M. Bramlett and Jessie Truettner to the 2-DG work are acknowledged.
REFERENCES

1. Coronado VG, Xu L, Basavaraju SV, McGuire LC, Wald MM, Faul MD et al. Centers for Disease Control and Prevention (CDC). Surveillance for traumatic brain injury-related deaths—United States, 1997-2007. MMWR Surveill Summ 2011; 60: 1–32.

2. Cavaleri R, Cavenago L, Siccardi D, Viale GL. Gunshot wounds of the brain in civilians. Acta Neurochir (Wien) 1988; 94: 133–136.

3. Kalesan B, French C, Fagan JA, Fowler DL, Galea S. Flame-related hospitalizations and in-hospital mortality in the United States, 2000-2010. Am J Epidemiol 2014; 179: 303–312.

4. Fu ES, Tummala RP. Neuroprotection in brain and spinal cord trauma. Curr Opin Anaesthesiol 2005; 18: 181–187.

5. Kazim SF, Shamim MS, Tahir MZ, Enam SA, Waheed S. Management of penetrating brain injury. J Emerg Trauma Shock 2011; 4: 395–402.

6. Joseph B, Aziz H, Pandit Y, Kulvatunyoo N, O’Keeffe T, Wynne J et al. Improving survival rates after civilian gunshot wounds to the brain. J Am Coll Surg 2014; 218: 58–65.

7. De Fazio M, Rammro R, O’Phelan K, Bullock MR. Alterations in cerebral oxidative metabolism following traumatic brain injury. Neurocrit Care 2011; 14: 91–96.

8. Glenn TC, Kelly DF, Boscardin WL, McArthur DL, Vespa P, Oertel M et al. Energy dysfunction as a predictor of outcome after moderate or severe head injury: indices of oxygen, glucose, and lactate metabolism. J Cereb Blood Flow Metab 2003; 23: 1239–1250.

9. Henry B, Emile C, Bertrand P, Erwan D. Cerebral microdialysis and PiO2 to decide unilateral decompressive craniectomy after brain gunshot wound. J Emerg Trauma Shock 2012; 5: 103–105.

10. Williams AJ, Wei HH, Dave JR, Tortella FC. Acute and delayed neuroinflammatory response following experimental penetrating ballistic brain injury in the rat. J Neuroinflammation 2007; 4: 17.

11. Williams AJ, Ling GS, Tortella FC. Severity level and injury track determine outcome following a penetrating ballistic-like brain injury in the rat. Neurosci Lett 2006; 408: 183–188.

12. Williams AJ, Hartings JA, Lu XC, Rolli ML, Tortella FC. Penetrating ballistic-like brain injury in the rat: differential time courses of hemorrhage, cell death, inflammation, and remote degeneration. J Neurotrauma 2006; 23: 1828–1846.

13. Williams AJ, Hartings JA, Lu XC, Rolli ML, Dave JR, Tortella FC. Characterization of a new rat model of penetrating ballistic brain injury. J Neurotrauma 2005; 22: 313–331.

14. Shear DA, Lu XC, Bombard MC, Pedersen R, Chen Z, Davis A et al. Longitudinal characterization of motor and cognitive deficits in a model of penetrating ballistic-like brain injury. J Neurotrauma 2010; 27: 1911–1923.

15. Wei G, Lu XC, Yang X, Tortella FC. Intracranial pressure following penetrating ballistic-like brain injury in rats. J Neurotrauma 2010; 27: 1635–1641.

16. Yokobori S, Gajavelli S, Mondello S, Mo-Seaeny J, Bramlett HM, Dietrich WD et al. Neuroprotective effect of preoperatively induced mild hypothermia as determined by biomarkers and histopathological estimation in a rat subdural hematoma decompression model. J Neurosurg 2013; 118: 370–380.

17. Prins M, Greco T, Alexander D, Giza CC. The pathophysiology of traumatic brain injury at a glance. Dis Model Mech 2013; 6: 1307–1315.

18. Murakami Y, Wei G, Yang X, Lu XC, Leung LY, Shear DA et al. Brain oxygen tension monitoring following penetrating ballistic-like brain injury in rats. J Neurosci Methods 2012; 203: 115–121.

19. Levasseur JE, Alessandri B, Reinert M, Bullock R, Kontos HA. Fluid percussion injury transiently increases then decreases brain oxygen consumption in the rat. J Neurotrauma 2000; 17: 101–112.

20. Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD et al. The [14C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem 1977; 28: 897–916.

21. Zhao W, Ginsberg MD, Smith DW. Three-dimensional quantitative autoradiography by disparity analysis: theory and application to image averaging of local cerebral glucose utilization. J Cereb Blood Flow Metab 1995; 15: 552–565.

22. Back T, Zhao W, Ginsberg MD. Three-dimensional image analysis of brain glucose metabolism-blood flow uncoupling and its electrophysiological correlates in the acute ischemic penumbra following middle cerebral artery occlusion. J Cereb Blood Flow Metab 1995; 15: 566–577.

23. Jahrling N, Becker K, Dott HU. 3D-reconstruction of blood vessels by ultra-microscopy. Organonews 2009; 5: 227–230.

24. Maxwell WL, Mackinnon MA, Stewart JE, Graham DL. Stereology of cerebral cortex after traumatic brain injury matched to the Glasgow outcome score. Brain 2010; 133: 139–160.

25. Bramlett HM, Dietrich WD. Quantitative structural changes in white and gray matter 1 year following traumatic brain injury in rats. Acta Neuroallpathol 2002; 103: 607–614.

26. Venweij BH, Muijzelar JP, Vinas FC, Peterson PL, Xiong Y, Lee CP. Impaired cerebral mitochondrial function after traumatic brain injury in humans. J Neurosurg 2000; 93: 815–820.

27. Yao C, Williams AJ, Ottens AK, Lu XC, Liu MC, Hayes RL et al. P43/pro-EMP1: a potential biomarker for discriminating traumatic versus ischemic brain injury. J Neurotrauma 2009; 26: 1295–1305.

28. Tsukada H, Ohba H, Kishiyama S, Kanazawa M, Kikuchi T, Harada N. PET imaging of ischemia-induced impairment of mitochondrial complex I function in monkey brain. J Cereb Blood Flow Metab 2014; 34: 708–714.

29. Sullivan PG, Keller JN, Bussen WL, Scheff SW. Cytochrome c release and caspase activation after traumatic brain injury. Brain Res 2002; 949: 88–96.

30. de Rivero Vaccari JP, Dietrich WD, Keane RW. Activation and regulation of cellular inflammatory: gaps in our knowledge for central nervous system injury. J Cereb Blood Flow Metab 2014; 34: 369–375.

31. Davis CH, Kim KY, Bushong EA, Mills EA, Boassa D, Shih T et al. Transcerebral degradation of axonal mitochondria. Proc Natl Acad Sci USA 2014; 111: 9633–9638.

32. Pandya JD, Pauly JR, Sullivan PG. The optimal dosage and window of opportunity to maintain mitochondrial homeostasis following traumatic brain injury using the uncoupler FCCP. Exp Neurol 2009; 218: 381–389.

33. Kamawata T, Katayama Y, Hovda DA, Yoshino A, Becker DP. Administration of excitatory amino acid antagonists via microdialysis attenuates the increase in glucose utilization seen following concussive brain injury. J Cereb Blood Flow Metab 1992; 12: 12–24.

34. Semenza GL. Hypoxia-inducible factor 1: regulator of mitochondrial metabolism and mediator of ischemic preconditioning. Biochim Biophys Acta 2011; 1813: 1263–1268.

35. Cartagena CM, Phillips KL, Tortella FC, Dave JR, Schmid KE. Temporal alterations in aquaporin and transcription factor HIF1alpha expression following penetrating ballistic-like brain injury (PBI). Mol Cell Neurosci 2014; 60: 81–87.

36. Zoltewicz JS, Mondello S, Yang B, Newsom KJ, Kobeissy F, Yao C et al. Biomarkers track damage after graded injury severity in a rat model of penetrating brain injury. J Neurotrauma 2013; 30: 1161–1169.

37. Astrup J, Siesjo BK, Symon L. Thresholds in cerebral ischemia - the ischemic penumbra. Stroke 1981; 12: 723–725.

38. Heiss WD. The ischemic penumbra: correlates in imaging and implications for treatment of ischemic stroke. The Johann Jacob Wepfer award 2011. Cerebrovasc Dis 2011; 32: 307–320.

39. Wu HM, Huang SC, Vespa P, Hovda DA, Bergsneider M. Redefining the peri-contusional penumbra following traumatic brain injury: evidence of deteriorating metabolic derangements based on positron emission tomography. J Neurotrauma 2013; 30: 352–360.

40. Yao C, Wei G, Lu XC, Yang W, Tortella FC, Dave JR. Selective brain cooling in rats ameliorates intracerebral hemorrhage and edema caused by penetrating brain injury: possible involvement of heme oxygenase-1 expression. J Neurotrauma 2011; 28: 1237–1245.

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-sa/3.0/