

# Evidence to support mitochondrial neuroprotection, in severe traumatic brain injury

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Received: 22 July 2014 / Accepted: 13 October 2014 / Published online: 31 October 2014  
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**Abstract** Traumatic brain injury (TBI) is still the leading cause of disability in young adults worldwide. The major mechanisms – diffuse axonal injury, cerebral contusion, ischemic neurological damage, and intracranial hematomas have all been shown to be associated with mitochondrial dysfunction in some form. Mitochondrial dysfunction in TBI patients is an active area of research, and attempts to manipulate neuronal/astrocytic metabolism to improve outcomes have been met with limited translational success. Previously, several preclinical and clinical studies on TBI induced mitochondrial dysfunction have focused on opening of the mitochondrial permeability transition pore (PTP), consequent neurodegeneration and attempts to mitigate this degeneration with cyclosporine A (CsA) or analogous drugs, and have been unsuccessful. Recent insights into normal mitochondrial dynamics and into diseases such as inherited mitochondrial neuropathies, sepsis and organ failure could provide novel opportunities to develop mitochondria-based neuroprotective treatments that could improve severe TBI outcomes. This review summarizes those aspects of mitochondrial dysfunction underlying TBI pathology with special attention to models of penetrating traumatic brain injury, an epidemic in modern American society.

**Keywords** Mitochondrial dynamics · Hypoxia · Cyclosporine A · Mitochondrial dysfunction · Penetrating traumatic brain injury · Secondary brain injury · KTP

## Introduction

In the United States, an estimated 1.4 million people suffer a traumatic brain injury (TBI) each year (Langlois et al. 2005; Binder et al. 2005). About 50,000 people die and at least 5.3 million live with severe disabilities related to TBI (Binder et al. 2005). Social costs, including medical costs and loss of income, are estimated at 48.3 billion dollars a year by the Centers for Disease Control and Prevention. Worldwide, TBI is recognized as the leading cause of mortality and morbidity in young adults (Fu and Tummala 2005; Vink and Nimmo 2009). Although not yet demonstrated in patients, preclinical studies suggest aging increases cortical synaptic mitochondria susceptibility to injury (Gilmer et al. 2010) and changes the nature of innate immune response (Kollmann et al. 2012) which in turn may influence TBI outcomes.

In spite of numerous studies of TBI, patient outcomes remain poor (Langlois et al. 2005). Amongst the different forms of TBI, penetrating traumatic brain injuries (PTBI) are associated with the worst outcomes and highest death rates and especially affect young people (Binder et al. 2005). Severity of the primary brain injury determines the prognosis, but its evolution and magnification by secondary damage heavily influence outcome. There is now much evidence that most secondary brain injury mechanisms such as hypoxia, hypotension, sepsis, hyperthermia, seizures, anemia, and hypoglycemia can be optimally managed in the intensive care unit to favorably influence outcomes. However, despite extensive efforts to develop new neuroprotective therapies, and about 30 phase III clinical trials, no therapies, other than surgery and neurocritical care management, are available for

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PTBI (Fu and Tummala 2005). The consensus in TBI research is that neuroinflammation, free radical formation, and metabolic dysfunction are key determinants of outcomes (Gaetz 2004). All these converge to disrupt metabolism, which has inspired numerous basic and clinical studies on glucose and oxygen metabolism in mitochondria (Soustiel and Svirni 2007; Vespa et al. 2005). Mitochondria have been shown to be a key participant in TBI pathophysiology. Continuous, normal mitochondrial function is vitally important to maintain homeostasis in neural cellular metabolism, therefore, when mitochondrial dysfunction occurs after TBI, patients with profound mitochondrial impairment have the poorest prognosis (Signoretti et al. 2008).

Since the demonstration of mitochondrial dysfunction in TBI (Xiong et al. 1997), several drugs affecting this mechanism have also been studied and few are used in neurocritical care (Clausen and Bullock 2001; Hanafy and Selim 2012). Growing evidence suggests that for neuroprotection after TBI there is an unmet need for mitochondria-targeted therapeutics (Waldmeier et al. 2003).

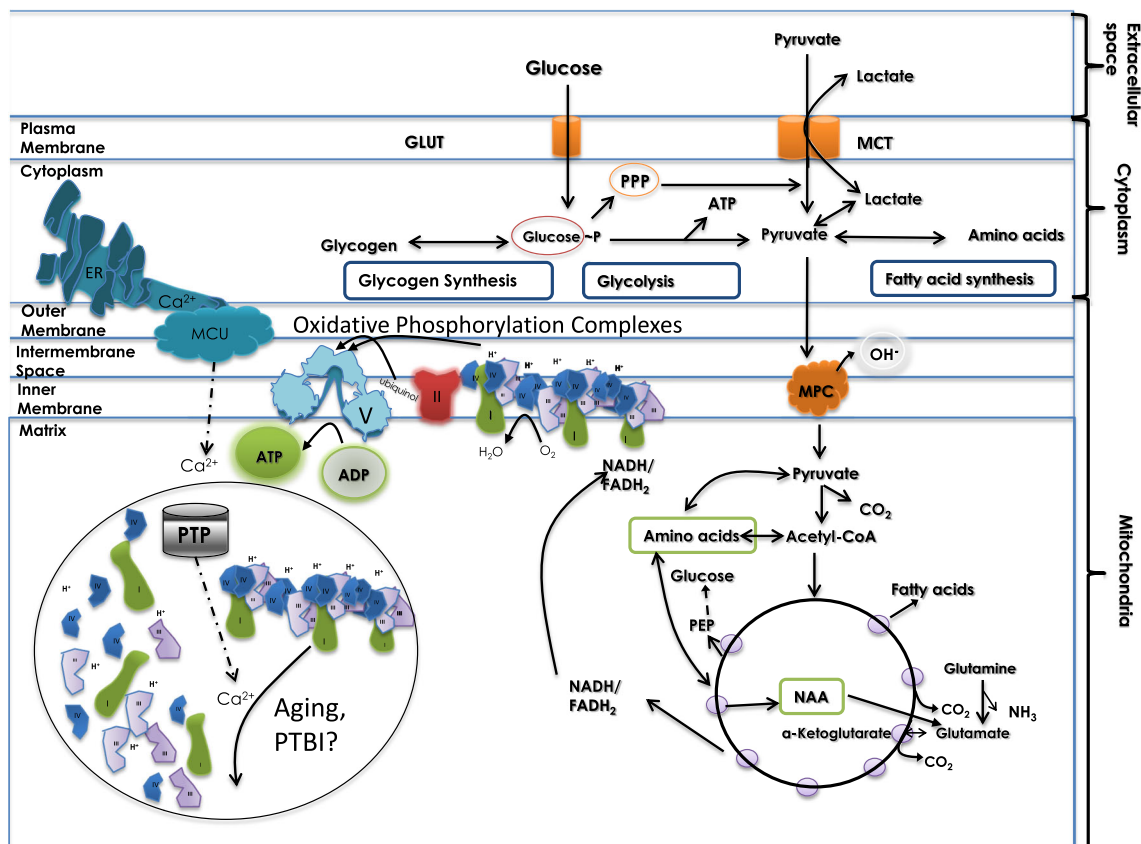
This review will summarize the data on normal mitochondrial function in central nervous system (CNS) cells, outline metabolic impairment following TBI/PTBI, and discuss old and new therapeutic strategies including drug treatment aimed

at cleaning up broken mitochondria and rebuilding metabolic capacity via mitochondrial biogenesis after injury.

## Physiological review

Mitochondria: the metabolic hub of neurons and glia

Core metabolic processes such as glycogen catabolism and glycolysis generate pyruvate, which is a key node in the branching pathways of glucose, fatty acid and amino acid metabolism. Pyruvate can (i) enter mitochondria through the mitochondrial pyruvate carrier (MPC), be subjected to the citric acid cycle/tricarboxylic acid (TCA) and oxidative phosphorylation to produce adenosine triphosphate (ATP), or be carboxylated to form oxaloacetate (ii) accumulate in the cytoplasm, particularly during mitochondrial dysfunction, and is transported into the extracellular space via monocarboxylate transporter (MCT) (DeBerardinis and Thompson 2012; Halestrap 2013; Schell and Rutter 2013) (Fig. 1). The reducing agents produced by the citric acid cycle are used in oxidative phosphorylation on the electron transport chain, which is comprised of four high conductance multimeric enzyme supercomplexes (CI–CIV) to generate an



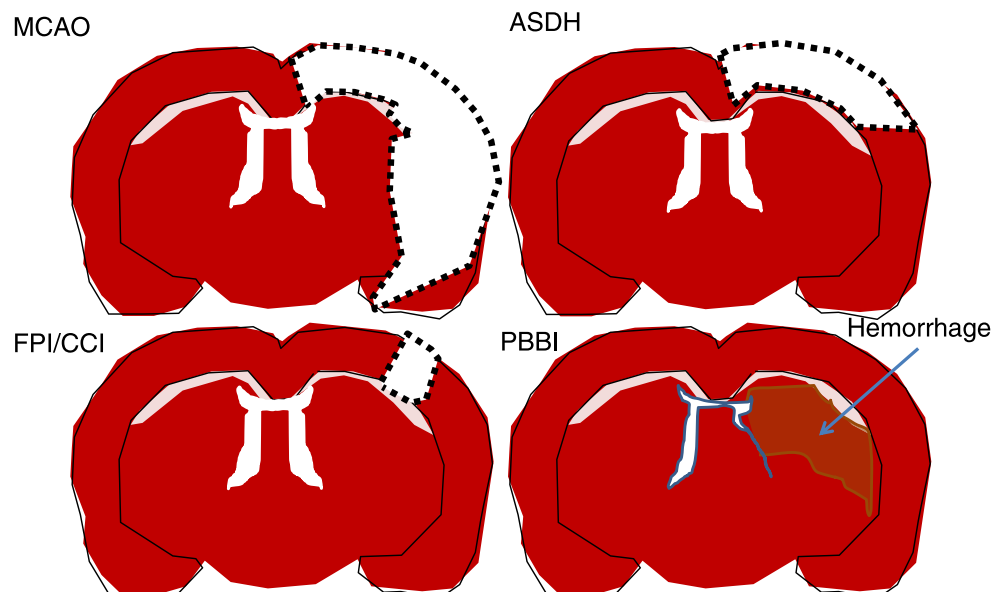
**Fig. 1** Schematic showing mitochondria at the hub of metabolic pathways

electrochemical proton gradient ( $\Delta\psi_m$ ) (Fig. 1) (Yokobori et al. 2013a). The mammalian CI supercomplex is referred to as the “respirasome” or “respiratory string.” (Gomez et al. 2009). Chemical staining for this complex serves as an indicator of mitochondrial function in TBI research (Fig. 2), and new molecules with such properties may help image human TBI in the near future (Tsukada et al. 2014). Apart from ATP synthesis, membrane potential ( $\Delta\psi_m$ ) is also used to electrophoretically import nuclear proteins. In addition, the citric acid cycle is the major metabolic hub of the cell, as it provides intermediates such as  $\alpha$ -ketoglutarate and oxaloacetate for amino acid biosynthesis (Owen et al. 2002). In the brain, the citric acid cycle intermediate  $\alpha$ -ketoglutarate is lost through synaptic release of its derivatives glutamate and GABA from neurons and through export of glutamine from glia (Hertz 2006). Glutamate is the most abundant brain metabolite and is used as an indicator of a functional citric acid cycle in TBI patients. In addition to glutamate, N-Acetyl-aspartate (NAA) is used as “a neuronal marker” in the clinical setting, primarily by nuclear magnetic resonance (NMR) practitioners. NAA is synthesized in neuronal mitochondria from aspartic acid and acetyl-coenzyme A. NAA signals are lost following traumatic brain injury. However, to date there is no generally accepted physiological (primary) metabolic role for NAA (Simmons et al. 1991; Clark et al. 2006). Genetic mutation to the aspartoacylase (ASPA) gene leads to acetate deficiency and is associated with Canavan disease (CD), a fatal neurodegenerative disorder (Traeger and Rapin 1998). In addition, it is hypothesized that NAA can be converted to glutamate via the citric acid cycle, and hence could act as a glutamate reservoir (Clark et al. 2006).

## Mitohormesis and mitochondrial dynamics

Resident mitochondrial proteins in combination with proteins drawn across mitochondrial membranes maintain the dynamic network of mitochondria that is continuously remodeled by fusion and fission (Harbauer et al. 2014). Cells do not generate mitochondria de novo, but instead identify and dispose of defective mitochondria while stimulating healthy mitochondria to proliferate through mitochondrial biogenesis (Yun and Finkel 2014). This process called mitochondrial dynamics has become an exciting new frontier in TBI mitochondrial biology (Perez-Pinzon et al. 2012). Mitochondrial dynamics is comprised of four broad categories: organelle biogenesis, movement, fusion-fission, and mitophagy which are all critical to maintain function (Chan et al. 2011). Mitochondrial fusion involves merging the inner and outer mitochondrial membranes (Palmer et al. 2011). Fusion of the outer membrane is governed primarily by the GTPases mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2) (Perez-Pinzon et al. 2012). Fusion of the inner mitochondrial membrane is dictated by the optic atrophy 1 (Opa-1) protein. Cleavage of Opa-1 dissipates mitochondrial membrane potential ( $\Delta\psi_m$ ) (Ehse et al. 2009) leading to greater fission (Palmer et al. 2011). In healthy cells, the PTEN Induced Kinase 1 (PINK1) is partially imported into mitochondria in a membrane potential ( $\Delta\psi_m$ )-dependent manner and processed by the inner membrane rhomboid protease, presenilin-associated rhomboid-like (PARL), which cleaves within the transmembrane segment and generates a destabilizing N-terminus, followed by retro-translocation of cleaved PINK1 into the cytosol and degradation by the ubiquitin-proteasome system. Dissipation of  $\Delta\psi_m$  in damaged mitochondria leads to an accumulation of unprocessed PINK1 and recruitment of the ubiquitin ligase Parkin to

**Fig. 2** Schematics of TTC stained sections from different TBI models shows the nature of mitochondrial dysfunction specifically that of complex I (white area) (Baskaya et al. 2000; Perri et al. 1997; Yao et al. 2009; Yokobori et al. 2013a)



mitochondria. Parkin mediates ubiquitination of mitochondrial outer membrane proteins (including mitofusins), leads to a degradation of damaged mitochondria by mitophagy (Chan et al. 2011; Jin and Youle 2012; Matsuda et al. 2010). Defects in mitochondrial dynamics that result in respiratory chain impairment underlie Parkinson's disease (Kim et al. 2007; Twig et al. 2008; Westermann 2010; Amo et al. 2011). Mitophagy selectively removes a single deleterious mitochondrion (which is discussed later) (Westermann 2010), separating damaged from healthy mitochondria (Twig et al. 2008). Damaged/depolarized/reactive oxygen species (ROS) producing mitochondria are marked by externalized cardiolipin and surface ubiquitination, recognized by PINK1-Parkin and removed by mitophagy (Nunnari and Suomalainen 2012; Ji et al. 2012). Until recently, each cell was thought to degrade its own broken mitochondria. However, recent work by Davis et al., uncovered a novel mechanism of acidified mitochondrial degradation called transmitophagy. In retinal ganglion cell axons of mice, mitochondria are shed at the optic nerve head (ONH), and these mitochondria are internalized and degraded by adjacent astrocytes (Davis et al. 2014; Nguyen et al. 2011). This new phenomenon remains to be explored in the context of TBI. We can postulate this role for the astrocytes could be impeded by edema and perhaps explain how improved intracranial pressure (ICP) management impacts outcomes. The need for multiple mitophagy mechanisms arises from the highly deleterious effects of broken mitochondria. Mitochondrial components such as formyl peptides and mitochondrial DNA due to their bacterial origin can activate innate immunity leading to cardiomyopathy or neurodegeneration (Oka et al. 2012; Adamczak et al. 2014; Zhang et al. 2010). Mitochondria have been compared to “Pandora's box” (Zamzami and Kroemer 2001), recalling the Greek legend of a gift from Zeus to Pandora which should have never been opened, and once opened, spread all the troubles the world had ever experienced. Unlike the consequences of the “Pandora's box” opening, genetic engineering has been successfully used to decrease the burden of sick mitochondria and promote healing in inherited mitochondrial models (Moraes 2014).

Aside from its role in mitochondrial fusion, PINK 1 regulated Mfn2 has also been noted to play a critical role in the axonal transport of mitochondria (Liu et al. 2012; Misko et al. 2010; Russo et al. 2009). Mutations that disrupt mitochondrial import, dynamics, and transport result in neurodegenerative disorders (Duncan and Goldstein 2006; De Vos et al. 2008; Wang et al. 2014). Mitochondrial fission and fusion are altered by injury induced hypoxia and reactive oxygen species (Suliman and Piantadosi 2014). Recently insulin has been shown to regulate these processes (Parra et al. 2014). Further, mitochondrial biogenesis is altered by stimuli as diverse, as nutrient availability, hormones, growth factors and temperature fluctuations. Several signaling pathways

regulate mitochondrial biogenesis. Among these transcription factors, nuclear respiratory factors (NRF1 and NRF2), estrogen-related receptors (ERR- $\alpha$ , ERR- $\beta$ , ERR- $\gamma$ ) and the peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1 $\alpha$ ) are major modulators of mitochondrial proliferation (Palikaras and Tavernarakis 2014). Post-transcriptionally cytosolic kinases such as casein kinase 1 and 2 and protein kinase A also affect mitochondrial biogenesis (Palikaras and Tavernarakis 2014). A delicate balance of mitochondrial dynamics and biogenesis confers the normal cell an ability to respond to external stimuli. Theoretically, the regulation of the balance of fusion-fission, biogenesis could be used as a strategy for neuroprotection.

### Measuring mitochondrial function

The standard methods employed to assess mitochondrial function include measurement of ATP levels, production in living tissue, or oxygen consumption (Lanza and Nair 2010). These methods are surrogates of the status of the mitochondria, especially of oxidative phosphorylation. This information can be obtained by indirect methods such as chemical staining of brain sections for activity of complexes or, by directly measuring oxygen consumption by tissues either by microrespirometry, or by isolated mitochondria in polarographic respiratory complex rate experiments. The polarographic methodology has been reviewed elsewhere (Barrientos et al. 2009). Changes in mitochondrial respiration are measured in term of respirator control ratio (RCR), an index of how coupled respiration is to ATP production. A RCR of 5 indicates well-coupled mitochondria, a value which significantly drops with increasing injury severity. Alterations in mitochondrial respiration were evident as early as 1 h post-injury and persisted for up to 3 h in mitochondria isolated from rat cortical tissue after cortical contusion injury (Gilmer et al. 2009). A significant decrease in RCR was also observed in ipsilateral cortical mitochondria as early as 30 min after injury in experimental mouse models of controlled cortical impact, suggesting a decreased mitochondrial ATP production capacity. This study was the first to demonstrate that the earliest consequences of TBI included alterations in mitochondrial bioenergetics. The mitochondrial dysfunction was proportional to injury severity and preceded many of the TBI injury cascades, including oxidative damage. The study indicated that therapeutic interventions need to be aimed at assisting mitochondria and initiated early for possible neuroprotection (Gilmer et al. 2009). It started the search for the elusive mito-/neuroprotectant including cyclosporine A (CsA) and its analogues (Sullivan et al. 1999). More recently aminopropyl carbazole agents that preserve mitochondrial function have been shown to be neuroprotective after TBI (Blaya et al. 2014).

In our studies with a rat model of penetrating brain trauma, penetrating ballistic-like brain injury (PBBI), we employed microrespirometry as a method to measure oxygen consumption in cores of brain tissue isolated from defined regions and incubated in glucose phosphate buffer. Details of application of the methods in various TBI studies have been reviewed previously (Alessandri et al. 2009).

## TBI pathophysiology

### Cerebral oxygen metabolism after PTBI

Continuous monitoring of brain oxygen tension revealed that about one-third of severe head injured patients (including penetrating traumatic brain injury (PTBI)) reduced brain oxygen tension ( $<25$  mmHg  $O_2$ ) for the first 6 to 12 h and had significantly worse outcomes (Henry et al. 2012; Valadka et al. 1998; van den Brink et al. 1998). The cause for reduced cerebral oxygen tension in patients who do poorly is unknown.

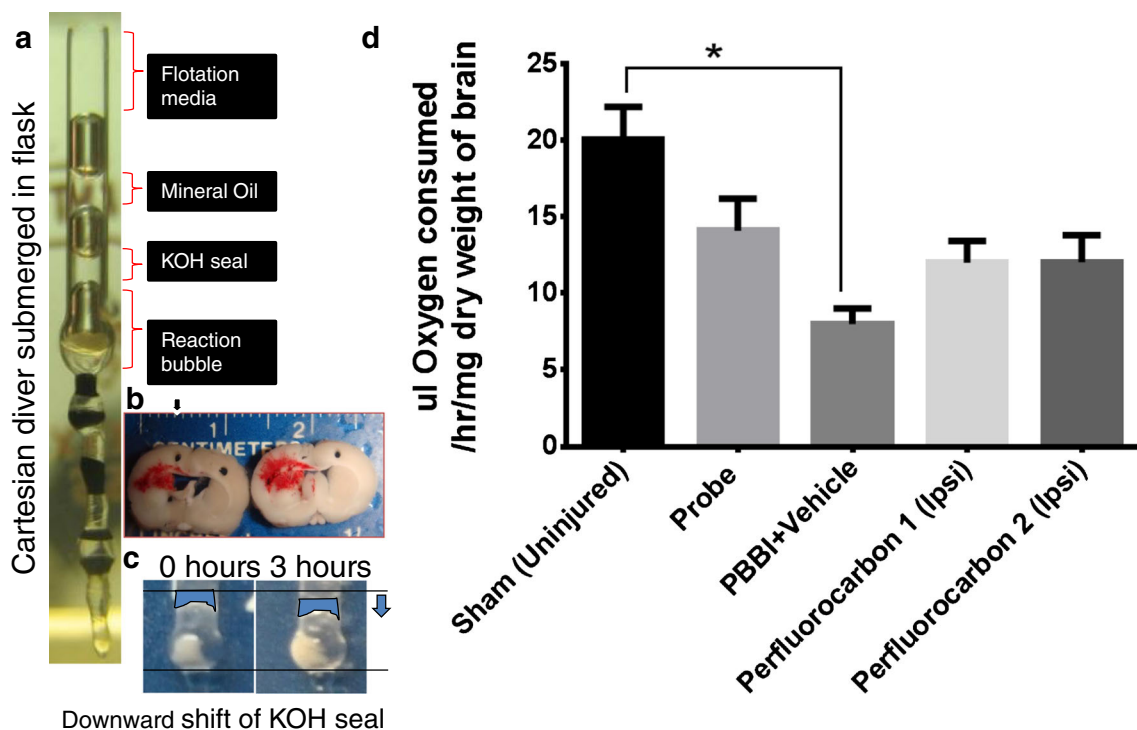
Reduced cerebral oxygenation may arise from at least four mechanisms:

1. Reduced oxygen delivery due to impaired cerebral blood flow.
2. Reduced oxygen delivery by diminished hemoglobin content or hemoglobin function (e.g., carbon monoxide poisoning or anemia)
3. Reduced oxygen uptake from the lungs (e.g., Acute Respiratory Distress Syndrome (ARDS), severe lung disease, or pulmonary contusions).
4. Reduced oxygen unloading into the tissue (e.g., if mitochondrial damage incapacitates aerobic metabolism).

Lowered brain oxygen tension/hypoxia, as seen in PTBI patients has been recapitulated in PBBI (Murakami et al. 2012). Candidate mechanisms include microvascular compromise due to astrocytic foot process swelling or reduced cerebral blood flow (CBF). This has been shown in brain microvessels of patients with traumatic cerebral contusions (Bullock et al. 1991; Vaz et al. 1997). Attempts to raise CBF by use of pressors and increasing cerebral perfusion pressure have not proven successful in a large NIH funded trial (Narayan et al. 2002). Restoration of cerebral tissue oxygenation in non-human primates within 3 h lead to preservation of neuronal integrity, resolution of brain swelling, and slow restoration of function during the recovery period (Morawetz et al. 1978). Previously, we have shown that augmenting tissue oxygen tension, by use of hyperbaric oxygen (HBO), normobaric hyperoxia (NBH) 100 % fraction of inspired oxygen ( $FiO_2$ ) and perfluorocarbons (PFCs), resulted in better

functional outcome, brain oxygen consumption ( $VO_2$ ) and less neuronal death, after severe TBI, in 2 rat models (Morawetz et al. 1978; Daugherty et al. 2004; Kwon et al. 2005). A recent report of a Phase II of trial of HBO and NBH that excluded high velocity penetrating TBI patients concluded that combined HBO/NBH treatments significantly improved markers of oxidative metabolism relative to uninjured brain as well as in pericontusional tissue, with reduced intracranial hypertension, and demonstrated improvement in microdialysis markers of cerebral integrity. There was significant reduction in mortality and improved favorable outcome as measured by GOS (Glasgow Outcome Scale), in the acute HBO group (Rockswold et al. 2013). However, HBO is not available in most trauma centers. On the other hand, PFCs were also highly effective, in restoring partial brain oxygen tension ( $ptiO_2$ ) after TBI, and they were especially attractive in this role. PFCs can transport oxygen without the need for erythrocytes, perfuse and oxygenate “peri-contusional” brain tissue and have been found to be safe in laboratory animals and humans (Faithfull 1992; Zhou et al. 2008). PFCs remain in the circulation for  $\sim 20$  h after a single 30-min rapid infusion. Unfortunately, PFCs are only slowly eliminated from the body due to their inertness and remain in macrophages in liver and spleen up to 10 days, limiting multiple dosing.

To test if PTBI pathophysiology could be ameliorated with PFCs, we exposed PBBI rats to PFCs acutely after injury. Brain oxygen tension but not oxygen consumption was modestly improved by multiple PFCs when injected within 30 min of injury. Brain oxygen tension was monitored up to 2.5 h post injury and oxygen consumption was assayed at 3 h post injury (Fig. 3a). Based on these results it is apparent that an inability to use supplied oxygen exists after PTBI. We speculate that this inability is mostly likely due to mitochondrial dysfunction. Compared to other TBI models, PBBI has a unique feature: an active complex I which can be seen by the presence of 2,3,5-triphenyltetrazolium chloride (TTC) reduction that is present up to 24 h post injury (Fig. 2) (Yao et al. 2011; Baskaya et al. 2000; Perri et al. 1997; Yao et al. 2009; Yokobori et al. 2013a). Based on lowered oxygen consumption, inability to use supplied oxygen and ability to reduce TTC, we propose that the mitochondrial defect after PBBI lies in the complex IV, similar to mitochondrial neurodegenerative disorders such as Parkinson’s disease (PD) (Kirkinetzos and Moraes 2001). In a recent case, we observed that PTBI patient metabolism does not respond to restored cerebral perfusion pressure (CPP), or lowered ICP suggesting persistent irreversible mitochondrial dysfunction up to 4 days (Fig. 4) (De Fazio et al. 2011). As with TBI patients, in PBBI rats we observed reduced glucose uptake (hypoglycolysis; unpublished data). In PTBI, the shearing forces of a travelling projectile mechanically stretch and tear apart the membrane and internal organelles. Apart from physical damage in TBI, hypoxia induces a family of proteins called the hypoxia inducible factors. The

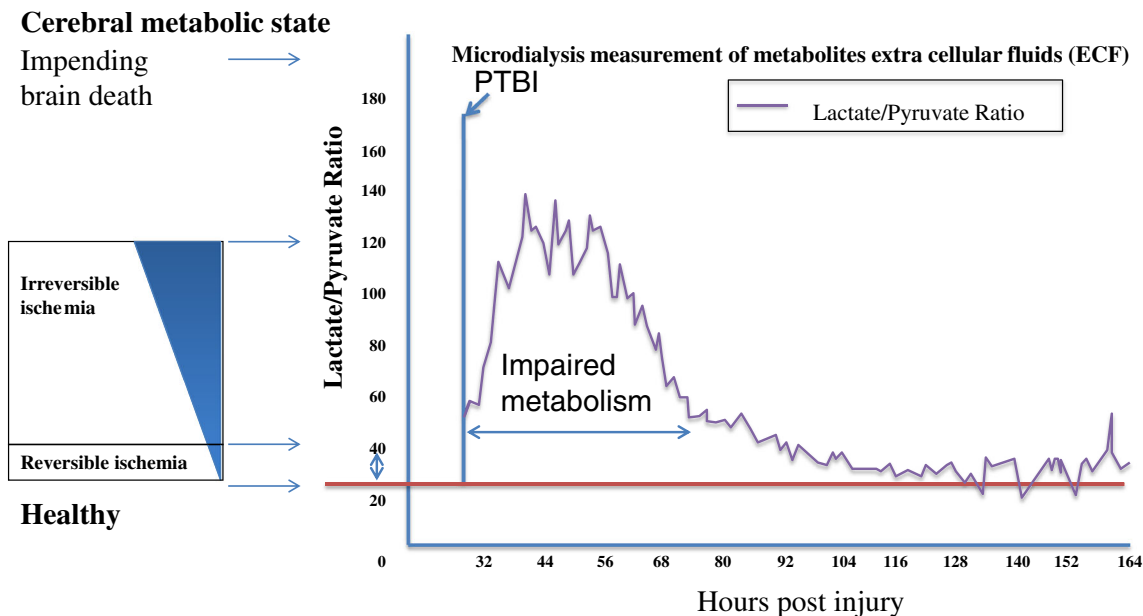


**Fig. 3** Cartesian diver (a) is used to measure oxygen consumption by tissue cores collected from cold saline perfused PBBI cortical slices (arrow in b). Consumption of O<sub>2</sub> results in downward shift of the KOH

seal altering the buoyancy of the diver. Compared to controls PBBI significantly decreased cerebral VO<sub>2</sub> which could partially be ameliorated by perfluorocarbons (d)

hypoxia inducible factor 1 alpha (HIF1α) mediates mitochondrial fission, which could potentially contribute to mitochondrial dysfunction. Despite the upregulation of HIF1α mRNA in PBBI, the protein was not detectable (Cartagena et al. 2014). It remains to be determined whether HIF1α contributes to mitochondrial dysfunction in PTBI/PBBI/in vitro stretch injury models. In addition to hypoxia, inflammation seen in

PTBI has been observed in PBBI (Williams et al. 2007; Oehmichen et al. 2003). Both inflammation and hypoxia induce mitophagy. In an in vitro liver injury model, their respective roles were clarified. Hypoxia was found to be a stronger inducer of mitochondrial dysfunction than inflammation with concomitant induction of neuroprotective hemoxygenase 1 (HO-1) via Nrf2/ nuclear factor kappa-



**Fig. 4** Example of mitochondrial dysfunction in PTBI patient. The ordinate is the lactate/pyruvate ratio and abscissa time (De Fazio et al. 2011)

light-chain-enhancer of activated B cells (NFK $\beta$ ) pathways. In PBBI, there is notable hemorrhage and upregulation of HO-1 (Yao et al. 2011). As with TBI, perhaps PBBI neurodegeneration can be attenuated by activation of neuroprotective HO-1 via Nrf2 (Miller et al. 2013). A variety of diseases have been associated with excessive ROS (Kirkinezos and Moraes 2001). A direct link between ROS mediated oxidative damage to the neuronal ubiquitination/de-ubiquitination machinery and the pathogenesis of sporadic Alzheimer's disease (AD) and PD has been established. Ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) is a neuronal de-ubiquitinating enzyme (Choi et al. 2004). In PBBI, the levels of UCHL1 in serum and CSF increase within minutes of injury and are proportional to the severity of the injury. This indicates that acute mitochondrial dysfunction can occur immediately following PTBI (Ren et al. 2013; Zoltewicz et al. 2013). In a separate but related model of TBI, the acute subdural hematoma (ASDH) model, we have previously shown that the release of injury biomarker UCHL1, TTC staining as well as neurodegeneration can all be blunted during the reperfusion phase with moderate therapeutic hypothermia (Yokobori et al. 2013a). In addition, loss of UCHL1 immunoreactivity precedes neurodegeneration in ASDH model (Gajavelli Shyam et al. 2012). Apart from maintaining ubiquitin pools, UCHL-1 is also responsible for endogenous antioxidant glutathione levels (Coulombe et al. 2014). Increased ROS production, UCHL1 oxidation, and impaired oxidative phosphorylation that manifests as mitochondrial dysfunction could underlie the metabolic dysfunction seen in PTBI patients (De Fazio et al. 2011; Verweij et al. 2000). Although there are multiple mechanisms contributing to mitochondrial dysfunction, several could thus be ameliorated by therapeutic hypothermia and management of intracranial pressure (Piantadosi and Suliman 2012). Clinical use of a TTC positron emission tomography (PET) ligand which precedes UCHL1 release into body fluids could help determine the time window for therapeutic hypothermia.

#### Metabolic substrate augmentation after TBI

Depressed cerebral metabolism following TBI is a consistent finding and associated with poor outcomes (Vespa et al. 2003). Metabolic crisis from mitochondrial dysfunction has been demonstrated by the use of microdialysis, allowing monitoring of neurochemical events as they unfold after severe human TBI and showing lactate surges and glucose decreases after injury (Chen et al. 2000; Vespa et al. 2005). The initial failure of mitochondrial respiratory function in turn compromises mitochondrial integrity. The metabolic crisis persists even after adequate restoration of CPP and substrates of energy metabolism (De Fazio et al. 2011). Clinical investigations in severely head-injured patients have shown increases in brain extracellular lactate levels despite well-preserved, or

restored regional blood flow and tissue oxygen tension (De Fazio et al. 2011). Therefore, mitochondrial dysfunction is a primary focus of metabolic dysfunction following TBI, perpetuating a vicious “cause-and-effect” cycle by which the progression of neurological damage is promoted (De Fazio et al. 2011). Since the “Lund concept”, a protocol aimed at nonsurgical reduction of TBI-induced increase of ICP via manipulation of physiological, pharmacological, and biochemical parameters, clinicians and preclinical researchers alike are tempted to supplement with exogenous energy substrates to restore metabolism (Nordstrom et al. 2013). Such studies appeared to have explored the potential of all metabolic substrates listed in the pathways, from glucose to ATP. Previous results from experimental animal models vary, suggesting that post-TBI hyperglycemia may be harmful (Cherian et al. 1997), neutral (Vink et al. 1997), or beneficial (Gurevich et al. 1997). In a recent study using a rodent model of unilateral controlled cortical impact (CCI) injury, glucose administrations significantly improved cerebral metabolism in approximately half of cortical and subcortical regions assessed (Moro et al. 2013). Several preclinical studies have pointed to the possibility of using lactate and pyruvate supplementation as an alternate energy source to power metabolism after TBI. This concept has been reviewed previously in greater detail (Yokobori et al. 2013b). Recently, two clinical studies using microdialysis have addressed the cerebral metabolic effects of exogenous lactate supplementation on the injured human brain. Both studies concluded that infused lactate was effectively metabolized after TBI (Bouzat et al. 2014; Carpenter et al. 2014). Exogenous lactate was found to dilate cerebral vasculature and increase cerebral blood flow (Gordon et al. 2008) and lactate infusion compared favorably to mannitol in reducing ICP after TBI (Ichai et al. 2009). However, the acceptance of this lactate augmentation intervention strategy is still being debated (Nordstrom and Nielsen 2014). In another study using CCI rats, the effects of the hydrophobic acetate precursor, glyceryltriacetate (GTA), as a method of delivering metabolizable acetate to the injured brain were investigated. GTA administration significantly increased the levels of both NAA and ATP in the injured hemisphere 4 and 6 days after injury, and also resulted in significantly improved motor performance in rats 3 days after injury (Arun et al. 2010). In addition, GTA (4.5 g/kg/day) was well tolerated by infants with the neurodegenerative Canavan disease (Segel et al. 2011). An increase in brain acetyl-CoA levels by acetate supplementation increases brain energy stores (such as phosphocreatine), but has no effect on brain glycogen and neuronal mitochondrial biogenesis (Bhatt et al. 2013). Acute and delayed administration of sodium pyruvate (SP) was also found to be neuroprotective and capable of enhancing memory in CCI rats (Moro et al. 2011; Moro and Sutton 2010).

In addition to hypometabolism of glucose post TBI, decreased cellular energy production (Yoshino et al. 1991) by an

increased flux of glucose through the reparative pentose phosphate pathway (PPP) has been documented in several preclinical studies (Prins et al. 2013). In contrast, in a single clinical study, where the nature of TBI was inadequately described, metabolism of glucose was predominantly via glycolysis, rather than by the PPP, suggesting glycolysis was intact even after TBI (Carpenter et al. 2014). Given that glucose may not be the optimum fuel after TBI, it was hypothesized that decreasing metabolism of glucose in the presence of an alternative substrate would improve cellular metabolism and recovery. The ketogenic diet (KD) has been one of the most common approaches to induce ketosis and increase cerebral metabolism of ketones. The rate of ketosis achieved is dependent on age, with younger animals achieving significant  $\beta$ -hydroxybutyrate levels earlier than adults, which can contribute to the age effect of neuroprotection observed after TBI. In juvenile rats in the weight drop model and adolescent rats in CCI, KD resulted in smaller contusion volumes, better motor and cognitive performances, decreased brain edema, cytochrome c release, expression of molecular markers of apoptosis, markers of oxidative stress, and mitochondrial calcium loading, as well as improved cellular energetics and increased expression of brain-derived neurotrophic factor. Ketosis via fasting or calorie restriction (albeit 3 times slower based on plasma concentrations of  $\beta$ hydroxybutyrate 0.57 mM vs 1.75 mM), was also neuroprotective in adult rats with traumatic brain injury. How the KD works has been reviewed elsewhere (Procaccio et al. 2014; Rho and Sankar 2008). The prominent mechanisms of KD includes increased mitochondrial biogenesis as seen by a combination of microarray analysis, electron microscopic estimation of mitochondrial profiles, levels of selected energy metabolites and enzyme activities (Bough et al. 2006). The ketogenic diet even altered gene expression in adolescent rat hippocampi. Among the components of KD medium-chain triglyceride decanoic acid (C10), a reported peroxisome proliferator activator receptor  $\gamma$  (PPAR $\gamma$ ) agonist mediated increase in mitochondrial content (Hughes et al. 2014).

In contrast to the beneficial effects of ketone metabolism, poor nutritional support can exacerbate TBI (Hoane et al. 2011). Currently, clinical studies are underway to determine the optimal method to induce cerebral ketone metabolism in the post-injury brain, and to validate the neuroprotective benefits of ketogenic therapy in humans (Prins and Matsumoto 2014).

Oxidation of nutrients culminates in the synthesis of high-energy compounds, particularly ATP, which works as the main chemical energy carrier in all cells. However, exogenous ATP administration after CNS injury is harmful. Intraperitoneal injections of ATP after stroke increased infarct volume, accompanied by seizures, hemorrhagic transformation, and higher mortality, perhaps due to cardioppression and hypoperfusion (Zhang et al. 2013).

As mentioned above, glucose is metabolized via several essential pathways that take place in mitochondria. Studies suggest that some forms of TBI may be amenable to exogenous energy supplementation, which if specifically delivered to CNS cells, may translate to function and structural recovery. The severity of mitochondrial dysfunction after mechanical traumatic injury will thus be an important factor in determining cell death or survival. Following traumatic brain injury, mitochondria sustain structural and functional impairment due to the direct shearing forces transmitted through the brain tissue, which then contribute to secondary damage that can continue for days after the initial injury (Gilmer et al. 2009). The state of hypovolemic shock immediately following TBI provides a complicating obstacle to therapy. The infusion of fluid as a resuscitation mechanism has been widely recognized as an appropriate primary treatment to increase blood pressure in trauma patients (Kortbeek et al. 2008). However, evidence has shown the deleterious effects of early fluid resuscitation (Clifton et al. 2011; Haut et al. 2011). Recent research has suggested that colloid-containing fluids are the most appropriate for infusion. This is due to the nature of these fluids to support plasma oncotic pressure. This results in the expansion of intravascular volume and allows proper resuscitation with smaller fluid volumes (Gantner et al. 2014). However, studies have shown that 4 % albumin is actually detrimental to brain injury (Investigators, S. S., Australian, New Zealand Intensive Care Society Clinical Trials, G., Australian Red Cross Blood, S., George Institute for International, H et al. 2007). Researchers have suggested that albumin may leak across the damaged BBB and cause a higher efflux of fluid to the interstitial spaces (Myburgh and Finfer 2009).

Proper fluid infusion is crucial in TBI treatment due to the critical role that cerebral edema plays in mitochondrial dysfunction. Most of the mitochondria in the enlarged astrocytic cytoplasm are swollen, including the mitochondrial matrix and the cristae. In contrast, cerebral dehydration was associated with mitochondrial shrinking in both astrocytes and neurons (Koizumi and Shiraiishi 1970). The morphological changes associated with edema may suggest that controlling edema is significant in maintaining the normal mitochondrial dynamics and quality control that allow for repair.

### **Evidence to support mitochondrial neuroprotection, in severe TBI**

#### **Cellular energetic failure in critical illness**

Cellular energetic failure caused by the inability to use oxygen at the cellular level has been increasingly recognized as a fundamental disorder contributing to organ dysfunction in the critically ill patient (Wallace 1999; Singer 2007; Carre



and Singer 2008; Kozlov et al. 2011; Galley 2011). Recent evidence in multiple organ failure suggested that altered mitochondrial biogenesis could affect the possibility to recover after a critical illness, thus affecting outcome (Carre and Singer 2008; Singer 2007; Carre et al. 2010; Piantadosi and Suliman 2012).

Clinical studies in septic patients admitted to the intensive care unit (ICU) have demonstrated a decrease in mitochondrial respiratory chain complex protein subunits and activities on muscle biopsy as compared to controls. This decrease is seen to a greater extent in patients who had a poor outcome, demonstrating an association between mitochondrial dysfunction and outcome (Carre et al. 2010). Patients who survived showed early activation of the transcriptional program for mitochondrial biogenesis, while failure to activate it led to a reduction of mitochondrial content and determined cellular energetic failure (Carre et al. 2010). It may also be postulated that in the event of a prolonged systemic inflammatory insult, overproduction of cytokines and ROS, associated hypoperfusion and tissue hypoxia, the organ responds by switching off its energy-consuming biophysiological processes (Brealey et al. 2004), which would then need to be reactivated for recovery to take place. In this way, mitochondria can be instrumental both in failure and in recovery of cell and organ function.

Because mitochondria can directly or indirectly affect cell survival and organ function, clinical investigators in the ICU have focused on defining strategies that could be effective in preventing and reversing mitochondrial dysfunction in the critically ill patient (Kozlov et al. 2011).

As a key cellular event in critical illness, several types of mitochondrial targeted therapies have been proposed (Galley 2011) including mitochondrial substrate provision, mitochondrial cofactor provision, mitochondrial antioxidant quenching, mitochondrial ROS scavengers, and mitochondrial membrane stabilizers (Dare et al. 2009).

Since mitochondria are ubiquitous in all cells, a disruption in cellular energetic metabolism is implicated in almost all areas of critical illness. This is crucial especially in the neurocritical care arena, since mitochondrial dysfunction occurs both in acute and chronic neurodegenerative disorders (Mazzeo et al. 2009a; Soane et al. 2007). In particular, energetic failure is one of the most important mechanisms responsible for brain damage, early after a TBI. Consequently, the central question in the care of critically ill patient “Do ICU patients die from mitochondrial failure?” (Kozlov et al. 2011), should also be considered in the acute care of TBI patients.

The consequences of mitochondrial dysfunction after TBI are numerous including energetic and metabolic failure, loss of cellular calcium homeostasis, oxidative stress and promotion of apoptotic processes. These alterations contribute to secondary damage that can continue for days after the initial

injury and consequently mitochondria have become a natural target of neuroprotection, after TBI (Perez-Pinzon et al. 2012).

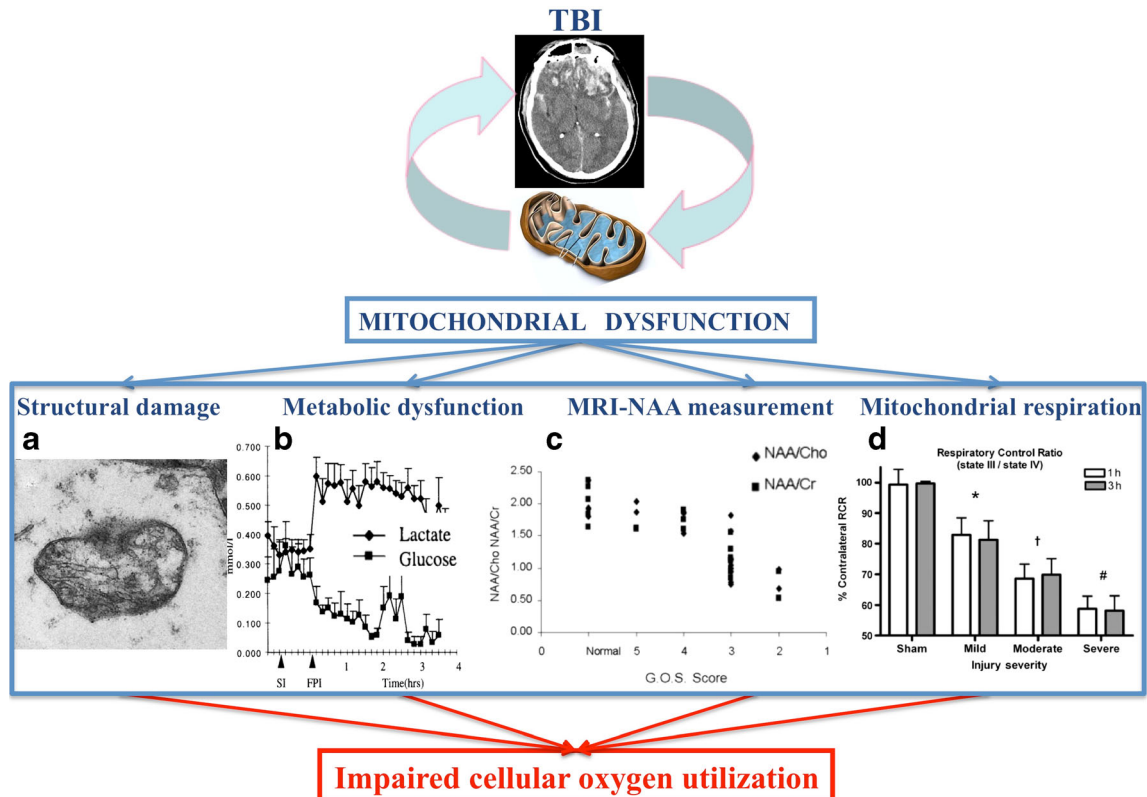
Evidence for mitochondrial dysfunction after severe TBI is enormous and ranges from altered mitochondrial morphology to depressed mitochondrial activities, loss of mitochondrial electron transport system (ETS), dissipation of membrane potential, release of mitochondrial proapoptotic proteins, and N-acetylaspartate (NAA) reduction on magnetic resonance (MR) spectroscopy (Fig. 5). Structural damage is expressed in term of swollen mitochondria, fragmented cristae, expanded matrix compartment and rupture of outer membrane, indicative of the onset of loss of  $\Delta\psi_m$ . In an experimental TBI model, mitochondrial swelling has been observed as early as 10 min after the injury (Lifshitz et al. 2004). Changes in cortical mitochondrial calcium buffering are also evident early after injury, with impaired calcium uptake by 3 h post injury (Singh et al. 2006).

Furthermore, mitochondrial architectural profiles have recently been characterized in human tissue surgically resected within 1 week from injury from three contiguous cortical injury zones (Balan et al. 2013). Four mitochondrial structural patterns were distinguished: normal, normal reactive, reactive degenerating, and end-stage degenerating profiles. End-stage degenerating mitochondria were identified as spherical fission products, showing high amplitude swelling and swollen, collapsing mitochondria. Early after injury reactive/degenerative and end-stage degenerative changes in mitochondrial morphology were observed in the central or near zone of injury, while the more peripheral far zone and penumbra exhibit primarily normal and normal reactive mitochondrial morphotypes. Within the intermediate post-injury period (at approximately 36–42 h post-injury), the near zone of injury contains primarily reactive/degenerating and end-stage degenerating mitochondria, while penumbra and far zone exhibit all four mitochondrial profile categories. In the late phase post-injury, reactive/degenerating mitochondria were abundant in the penumbra and far zones, while the near zone showed end-stage degenerating mitochondria. The study also demonstrated that levels of irreversible (end-stage degenerating) and reversible (normal reactive) mitochondrial changes reflected regional levels of brain injury severity (Balan et al. 2013).

Furthermore, in an *in vitro* study of isolated brain mitochondria to measure concentration dependent effects of calcium on mitochondrial functions, it was observed that increasing calcium concentration directly reduced mitochondrial respiration in a dose dependent manner, as an immediate primary event following calcium exposure, while free radical production and oxidative stress can be considered secondary events after energetic failure (Pandya et al. 2013).

In order to provide an “image” of mitochondrial dysfunction after human severe TBI, the NAA level, a surrogate marker of mitochondrial status, has been measured using

## THE BIOENERGETIC FAILURE AFTER TRAUMATIC BRAIN INJURY



**Fig. 5** The Bioenergetic failure after traumatic brain injury (TBI). The figure illustrates some of the most powerful evidences for mitochondrial dysfunction after TBI: **a** Image of human brain mitochondria showing a swollen collapsing mitochondrion as evidence of mitochondrial structural damage (Balan et al. 2013). **b** Cerebral microdialysate lactate and glucose after fluid percussion injury (FPI) showing increased lactate and reduced glucose, as expression of metabolic dysfunction, after TBI. SI = saline injection (Chen et al. 2000). **c** Assessment of mitochondrial dysfunction by measuring N-acetylaspartate (NAA) levels in patients with severe head

injuries, using magnetic resonance (MR) spectroscopy, showing greater reduction of NAA/Choline (Cho) and NAA/Creatinine-containing compounds (Cr) ratios in patients with less favorable outcomes (Signoretti et al. 2008). **d** Changes in mitochondrial respiration measured using a Clark-type electrode in a Oxytherm System: Respirator control ratio (RCRs) significantly drops with increasing injury severity, suggesting that mitochondria are displaying an uncoupling of respiration from ATP production, with a decreased ability to produce ATP (Gilmer et al. 2009)

MR spectroscopy in patients with severe head injury. Significant reduction of NAA was observed in all patients compared to controls with more severe, irreversible reduction in patients with poor outcome. Comparatively higher levels of NAA were observed in patients who experienced a good outcome, suggesting minor, but recoverable mitochondrial impairment (Signoretti et al. 2008).

Recently, the role of mitochondrial polymorphisms on mitochondrial function have also been investigated, suggesting that mitochondrial DNA variation could factor into patient outcomes after TBI (Conley et al. 2014).

### Cyclosporine A for mitochondrial neuroprotection after severe TBI

Experimental and clinical studies support the concept that strategies aimed at preventing or reversing mitochondrial dysfunction and cellular energetic failure after acute brain

injury may represent a viable neuroprotective approach. Given the role of  $\Delta\psi_m$  in neuropathological events following TBI, it is a possible target for intervention (Kristal et al. 2004; Mazzeo et al. 2009a; Korde et al. 2005). Opening of  $\Delta\psi_m$  determines leaking of protons and massive entry of water and solutes with subsequent swelling of the mitochondria, dissipation of transmembrane mitochondrial potential, failure of oxidative metabolism and eventual breakage of the outer mitochondrial membrane (Soustiel and Larisch 2010). Cyclosporine A, for its ability to preserve mitochondrial integrity, thus inhibiting the opening of  $\Delta\psi_m$ , has been extensively investigated both in the experimental and in the clinical setting in the last two decades (Mazzeo et al. 2009a; Aminmansour et al. 2014; Hatton et al. 2008; Mazzeo et al. 2008; Okonkwo et al. 1999; Sauerbeck et al. 2011; Sullivan et al. 2000; Uchino et al. 1995).

In CCI rats, administration of CsA 15 min post-injury was able to significantly attenuate mitochondrial dysfunction in

several measured parameters of mitochondria integrity and energetics, restoring mitochondrial membrane potential, reducing intra-mitochondrial calcium levels, and reducing reactive oxygen species production following TBI (Sullivan et al. 1999). Furthermore, the continuous administration of CsA early post-injury was able to ameliorate cortical damage following TBI, with significant reduction in lesion volume in animals (Sullivan et al. 2000). CsA was able to ameliorate mitochondrial dysfunction, preserving mitochondrial bioenergetic state even in immature models of focal and diffuse TBI in rats and piglets (Kilbaugh et al. 2011), suggesting a possibility for use in adolescents.

Furthermore, in an experimental model of TBI investigating the effect of CsA upon NAA reduction and ATP loss, the drug was able to blunt a 30 % NAA reduction and restore 26 % of ATP loss, thus demonstrating significant neuroprotection (Signoretti et al. 2004).

In addition, improvement in cognitive performance, amelioration of acute motor deficit, and attenuation of the decrease in  $O_2$  consumption have been documented when the drug is given after fluid percussion injury in rats (Alessandri et al. 2002).

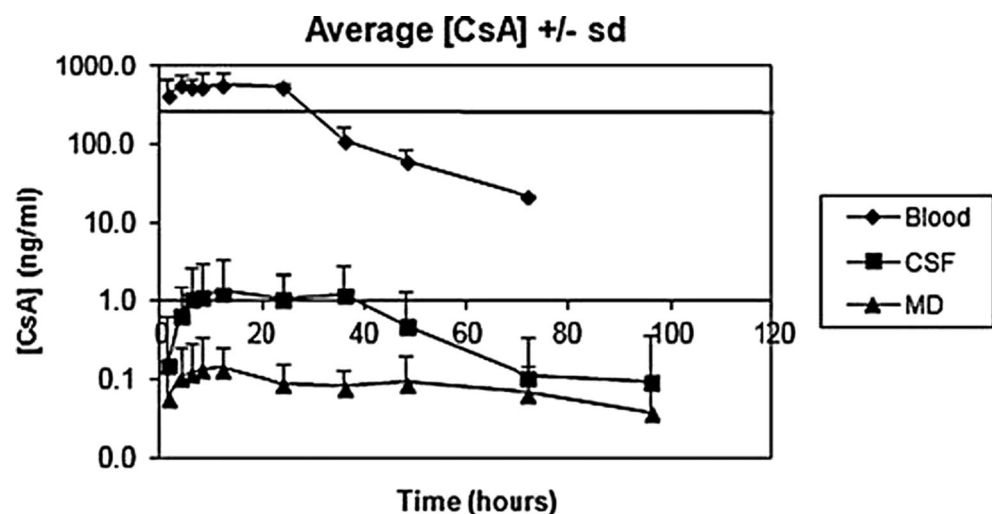
Besides its use in preclinical models, CsA was also proven to reduce reperfusion injury in a randomized control trial in patients with acute ST-elevation myocardial infarction, with a reduction in infarct size of approximately 40 % (Piot et al. 2008).

Almost 10 years ago our research group at Virginia Commonwealth University in Richmond designed a prospective randomized double-blind, placebo-controlled study to evaluate safety, tolerability and pharmacokinetics of a single intravenous infusion of CsA in patients with severe TBI. Patients were assigned to receive, within 12 h of injury, either an intravenous infusion of 5 mg/Kg/day of CsA administered over 24 h, or placebo. It was observed that the administration of CsA early after injury resulted in a significant increase in

extracellular fluid glucose and pyruvate, important energy substrates after TBI, representing the most robust result of the study. Drug administration also resulted in higher levels of brain extracellular lactate than in placebo, which could be explained by a more complex metabolic response in which lactate increase could reflect higher glycolytic rate and hyper-metabolism (Mazzeo et al. 2008). Lactate increase could also provide a metabolic substrate for neurons, to aid in ionic restoration, helping in recovery following TBI (Chen et al. 2000). A significant increase in mean arterial pressure and cerebral perfusion pressure was also demonstrated in patients receiving CsA than in the placebo group, which could help in the case of a reduced cerebral blood flow after TBI. No evidence of suppression of immune function was seen (Mazzeo et al. 2006), while a good safety profile of the drug was reported (Mazzeo et al. 2009b). Our research group also described the pharmacokinetics of CsA and reported the exposure of CsA in multiple biofluids following neurotrauma, by assessing drug concentrations in whole blood, cerebrospinal fluid (CSF), and dialysate brain extracellular fluid (ECF) (Brophy et al. 2013). Average CsA concentrations in the blood, CSF, and ECF dialysate over time are shown in Fig. 6. CSF exposure achieved 0.37 % of whole blood area under curve (AUC), while ECF dialysate exposure achieved 0.04 % of whole blood AUC. We could demonstrate that CsA, given via a 24 h continuous infusion, penetrates into the CSF, ECF as measured via brain microdialysis, as well as brain tissue. Measurement of concentration of a putative neuroprotectant in the ECF dialysate can provide a valuable feedback method to infer the concentration of the drug in the brain and thus to understand the in vivo dosage necessary to provide the desired neurochemical effect (Brophy et al. 2013).

The possibility of combination therapy, targeting more than one of the affected pathways should also be considered when dealing with mitochondrial neuroprotection, and this could open future clinical investigations.

**Fig. 6** Cyclosporine A concentrations in the blood, cerebrospinal fluid and brain extracellular fluid dialysate in brain injured patients. Average concentrations are log transformed and error bars represent the standard deviation (Brophy et al. 2013)



## Conclusions

Normal mitochondrial function is essential to maintain cerebral homeostasis. Following TBI, mitochondria sustain structural and functional impairments that are responsible for metabolic dysfunction, cellular energetic failure and eventually contribute to secondary brain damage. The consequent metabolic crisis persists even after adequate restoration of substrates of energy metabolism, lasting for days. Targeting mitochondrial function has been proposed as a new strategy for neuroprotection, alone or in combination with other treatments.

This review summarized the data on normal mitochondrial function in central nervous system and impairment of cerebral oxygen metabolism following TBI and penetrating TBI, and discussed old and new therapeutic strategies with an aim to clean up dysfunctional mitochondria and rebuild metabolic capacity via mitochondrial biogenesis after injury.

Restoration of cerebral tissue oxygenation by use of hyperbaric oxygen, normobaric hyperoxia, and perfluorocarbons has been evaluated, after TBI, but their attractive role carries several limitations and inability to use supplied oxygen is likely due to mitochondrial dysfunction. Previously, preclinical and clinical studies on TBI induced mitochondrial dysfunction have focused on opening of the mitochondrial permeability transition pore, consequent neurodegeneration, and attempts to mitigate it with cyclosporine A or analogous drugs unsuccessfully. A promising future approach to TBI treatment could be to relieve the metabolic load placed upon the damaged mitochondria, to promote repair “mitochondrial pit-stop (Liu et al. 2009)” via mitophagy and to enhance mitochondrial biogenesis via ketogenic diet or novel PINK agonists such as ATP analog kinetin triphosphate (KTP) (Hertz et al. 2013).

**Acknowledgments** This work was supported by Department of Defense Grant # #PT074521W81XWH-08-1-0419 to RB. The authors acknowledge Daniela Bruce and Joseph Prieto for illustrations

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