Mitochondrial Dysfunction following TBI: Potential of Creatine as a Neuroprotective Strategy

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INTRODUCTION

Although traumatic brain injury (TBI) is a major healthcare problem in the United States, there are currently no pharmacological interventions approved for clinical treatment of this condition. TBI affects about 7 million individuals each year in North America. However, athletes—particularly in full-contact sports such as boxing, football, hockey and soccer—are exposed to single and repeated concussions at a much higher incidence than the general population, which can result in long-term neurological dysfunction and even death (Clark, 1998). Regardless of rule changes, improved protective equipment, and conditioning, approximately 300,000 people still experience sport-related TBI annually (Cantu, 1997; Thurman et al., 1998). Furthermore, accumulating clinical evidence, as well as experience in contemporary military operations, suggests that substantial short-term and long-term neurologic deficits can be caused without a direct contact to the head (Cernak et al., 1999; DePalma et al., 2005; Elder and Cristian, 2009; Ling et al., 2009; Trudeau et al., 1998). With an estimated 15 percent of troops serving in Iraq sustaining some level of neurological impairment following blast exposure, TBI is the signature injury of this war and makes troops another high incident population for TBI (Hoge et al., 2008).

Although the neuropathology of TBI is not completely elucidated, several lines of evidence have demonstrated that mitochondrial dysfunction is a major feature of TBI. Mitochondria have also been found to play a pivotal role in neuronal cell survival and death following injury. Mitochondria serve as the powerhouse of the cell by maintaining ratios of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) that thermodynamically favor the hydrolysis of ATP to ADP + Pi. Proton pumping by components of the electron transport system (ETS) generates a membrane potential (ΔΨ) that can then be used to phosphorylate ADP to ATP or used to sequester Ca\(^{2+}\) into the mitochondrial matrix. This allows mitochondria to act as Ca\(^{2+}\) sinks for the cell as well as to stay in tune with changes in cytosolic Ca\(^{2+}\) levels. However, excessive mitochondrial Ca\(^{2+}\) uptake following TBI can result in formation of the mitochondrial permeability transition pore (mPTP) (Sullivan et al., 2005). A consequence of mPTP formation is a loss of membrane potential, which causes the uncoupling of electron transport from ATP production. The release of pro-apoptotic molecules (i.e., cytochrome C, Smac/Diablo, and apoptosis-inducing factor) from the mitochondria is, in part, orchestrated by mPTP and leads to the activation of cellular death pathways. An additional consequence of mPTP formation is the production of reactive oxygen species (ROS), which contribute to cellular damage by oxidizing cellular proteins and lipids (Mazzeo et al., 2009). Thus, the fine line between cell survival and cell death relies on mitochondrial integrity and, ultimately, the state of mitochondria following TBI.

Creatine is a molecule that is produced both endogenously and acquired exogenously through diet where it plays a prominent role in buffering cellular energy stores by increasing le-

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vels of phosphocreatine. Thus, the creatine/phosphocreatine system can increase overall cellular bioenergetics following injury/insult by acting as an energy storehouse. Additionally, increases in creatine can stabilize creatine kinase which has been demonstrated to interact with components of the mPTP and inhibit permeability transition (Beutner et al., 1998; Beutner et al., 1996; O’Gorman et al., 1997). Inhibition of the mPTP has been demonstrated to reduce damage following TBI (Sullivan et al., 2005). Together, these data may point to creatine as a viable prophylactic treatment for certain populations engaged in activities that increase their chance for sustaining a TBI. However, limited preclinical data is available concerning the use of creatine following TBI, making this an untapped resource that should be further explored.

TRAUMATIC BRAIN INJURY

Although treatment options designed to improve survival of their injuries are limited to minimizing acute brain edema, decreasing intracranial pressure, and the prevention of peripheral complications, there is no current treatment aimed at the loss of neural tissue that occurs following TBI. Perhaps the most insidious aspect of TBI is that it can occur without any obvious signs of injury to the patient’s body. Medical reports dating back to World War I have recorded medical incidences of mysterious neurological disorders. Physicians in the British armed forces began to label the bulk of these phenomenon with the term “shell shock” (SS) (Jones et al., 2007). Although some cases were attributed to psychosis, SS was responsible for 14 percent of all discharges from the British armed forces, and accounted for over one-third of all discharges of nonwounded soldiers by 1917. The controversial definition of the disorder, its method of treatment, public controversy and stigma over diagnosis delayed the development of treatment protocols and eventually caused the British army to ban the use of the term “shell shock” from reports. However, with the start of the Second World War it became readily apparent that disavowing the existence of this disorder did not prevent another epidemic.

In response to the army regulations, alternative terminology arose in its place, such as postconcussional syndrome (PCS) or posttrauma concussion state. Physicians began realizing many of the soldiers that suffered from this concussed state had been in a close proximity to an explosion, and thus, leading them to speculate that some force was affecting neural tissue without affecting the rest of the body. It was also realized that patients with a severe head injury would present with immediate neurologic symptoms that would trend toward recovery; whereas PCS would have delayed onset of neurologic symptoms with a trend toward worsening symptoms (Jones et al., 2007). Since soldiers and civilians can often suffer immense psychiatric morbidity without realizing the need for medical treatment that normally stems from a physical injury, this delayed development of symptoms in mild to moderate TBI patients is perhaps the most unfortunate aspect of this condition. A recent online poll indicated that 42 percent of respondents who suffered a TBI failed to seek medical care (Setnik and Bazarian, 2007); a rate that is considerably higher than the Centers for Disease Control and Prevention estimate of 25 percent. It has been observed clinically that even mild or moderate TBI can require neurosurgical intervention, and any delay in treatment could prove to be costly in terms of cognitive and functional recovery (Setnik and Bazarian, 2007).

Of the more than 1.5 million military personnel deployed since 2001 to the Middle East, approximately 25% of the injured service members have reported brain injury. Given the statistic from the poll above, this is probably an extreme underestimate with regards to military personnel. Unpublished data from the Department of Defense indicates that blast injuries are the leading cause of TBI in war zones; consequently, TBI has been labeled as a signature injury of the cur-
rent Middle Eastern conflicts (Hoge et al., 2008). In addition to cognitive deficits, this injury population also has an increased predisposition to the development of post traumatic stress disorder (PTSD).

Within the civilian population of the United States about 2 percent of the population (5.3 million) is currently living with disabilities that are the direct result of TBI (Langlois et al., 2006). TBI has a bimodal age distribution of incidence such that the peaks are found in young (<25) and elderly (>75) populations (Langlois et al., 2006; Rutland-Brown et al., 2006). Due to the high incidence and the development of chronic symptoms associated with TBI, the medical costs within the U.S. alone have been estimated at over $50 billion dollars per year. These dismal figures do not factor in the cost to social and family dynamics that occur following TBI. Despite being obvious that TBI is a devastating military and civilian health care problem in the U.S., there are currently no pharmacological treatments approved for clinical treatment of this condition. Several lines of evidence have indicated that mitochondrial dysfunction is a prominent feature of TBI, and mitochondria are known to play a pivotal role in neuronal cell survival and death following injury. As such, there is a clear need for the development of mitochondrial-targeted neuroprotective therapies for the treatment of TBI.

MITOCHONDRIA AND TBI

Several studies in recent years have shown that mitochondria play a pivotal role in neuronal cell survival in addition to mitochondrial dysfunction being considered an early, prominent event in central nervous system (CNS) injury that can cause neuronal cell death (Fiskum, 2000; Sullivan et al., 2005; Sullivan et al., 2004). Experimental data also indicates that excitotoxicity may be the initial upstream mechanism that leads to TBI-induced neuronal cell death (Choi et al., 1990; Faden et al., 1989). In order to discuss mitochondrial dysfunction, however, we must first address normal mitochondrial function. Mitochondria are double-membraned organelles that orchestrate oxidative phosphorylation. Specifically, mitochondria act as the “powerhouses” of cells by taking products from the Krebs cycle (citric acid cycle), fatty acid oxidation, and amino acid oxidation and producing most of the cell’s supply of ATP—the energy source used to power virtually all cellular functions. In fact, in the cells of evolutionarily ‘higher animals,’ greater than 95 percent of all ATP is produced by oxidative phosphorylation within mitochondria. Mitochondrial function is dependent upon the generation and maintenance of the mitochondrial ΔΨ, which is used to drive ATP production. ΔΨ is generated by the translocation of protons across the inner mitochondrial membrane via the ETS, culminating in the reduction of O2 to H2O. This store of potential energy (the electrochemical gradient) can then be coupled to ATP production as protons flow back through the ATP synthase and complete the proton circuit. The potential can also be used to drive Ca2+ into the mitochondrial matrix via the electrogenic uniporter when cytosolic levels increase (Gunter et al., 1994). When cytosolic levels decrease, mitochondria pump Ca2+ out to precisely regulate cytosolic Ca2+ homeostasis.

During excitotoxic insults, such as the result of TBI, Ca2+ uptake into mitochondria has been shown to increase ROS production, inhibit ATP synthesis, and induce mitochondrial permeability transitions. It is important to note that inhibition of mitochondrial Ca2+ uptake by reducing ΔΨ (chemical uncoupling) following excitotoxic insults is neuroprotective, emphasizing the pivotal role of mitochondrial Ca2+ uptake in TBI-induced neuronal cell death (Pandya et al., 2007; Sullivan et al., 2004). Studies from our group have demonstrated that changes in mitochondrial Ca2+ levels/cycling are coupled with increases in oxidative damage and significant mitochondrial dysfunction, which occurs acutely and is progressive for up to 48 hrs post-injury.
The opening of the mitochondrial permeability transition pore (mPTP) is suggested to be a key mediator in this process.

While several studies have demonstrated mitochondrial failure in rodent TBI models over the past 15 years, only recently have careful time course studies been carried out to better understand the temporal profile of bioenergetic failure. In the mouse controlled cortical impact (CCI) model of TBI, we have shown that mitochondrial failure is significant by 3 hrs within the cortical tissue surrounding the injury site and follows a progressive failure that peaks at 12 hrs (Singh et al., 2006). The onset of mitochondrial dysfunction has been demonstrated to be even more rapid in the tissue considered to be the injury core and penumbra following CCI. In these studies it is apparent that a significant loss of mitochondrial bioenergetics begins as early as 1 hr post-injury and continues for up to 48 hrs post-injury (Gilmer et al., 2009; Pandya et al., 2007; Pandya et al., 2009). Furthermore, mitochondrial Ca\(^{2+}\) overload, which directly initiates mPTP formation, was found to coincide with the loss of mitochondrial bioenergetics. However, both mitochondrial bioenergetics and Ca\(^{2+}\) loading were most amendable to treatment with a mitochondrial uncoupler administered within a 6 hr post-injury window (Pandya et al., 2009). Thus, these data sets show that a critical time for intervention occurs at \(t < 6\) hrs post-injury. In fact, in order for any mitochondrial–targeted compound to maximally rescue mitochondria at the epicenter of the injury, administration within a 3 hr post-injury window would be needed; while administration within the first 6 hrs would prevent mitochondrial failure in the cortical tissue surrounding the epicenter. Given these findings, a prophylactic approach with a safe compound is very logical for persons at an increased risk for TBI, such as athletes and military personal in active war zones.

**CREATINE**

Creatine (N-(aminoiminomethy)-N-methyl glycine) is an amino acid endogenously produced from glycine, methionine and arginine in the liver, kidney, and pancreas and is also supplied in diets containing meat products. While as much as 95 percent of the total pool of creatine is contained in muscle, high levels of creatine have also been demonstrated in the brain (Mujika and Padilla, 1997). For athletes, creatine is used to increase levels of phosphocreatine (which serves as a phosphate donor to generate ATP) and thereby decrease muscle fatigue during—and improve recovery after—repeated bouts of high intensity exercise (Mujika and Padilla, 1997). Importantly, creatine is also the main shuttle to transport energy from the mitochondria to locales in the cytosol via phosphocreatine. This is accomplished by generation of phosphocreatine from mitochondrial ATP in the intermembrane space via phosphocreatine kinase. Phosphocreatine can then be shuttled to various sites within the cell and used to regenerate ATP from ADP. This allows phosphocreatine to serve as a spatial/temporal buffer for ATP produced by oxidative phosphorylation in mitochondria. Higher levels of phosphocreatine therefore result in a higher reserve of ATP that is available for cells following injury and may account for the neuroprotection afforded by creatine supplementation. In fact, creatine supplementation has been placed into several human clinical trials for various CNS disorders including amyotrophic lateral sclerosis, Charcot-Marie-Tooth disease, Huntington’s disease, and Parkinson’s disease with mixed results (see Gualano et al., 2010 for review). In an effort to boost neuronal ATP and bioenergetics, all these trials used started creatine treatment after the disease state had been reached (Adhihetty and Beal, 2008).
It is also apparent that many of the neuroprotective functions that creatine has been shown to afford cannot be attributed to changes in cellular bioenergetics. One of the most striking examples is the anti-apoptotic effect, which has been attributed to the prevention or delay of the mPTP, that elevated creatine levels have been reported to produce (Adhihetty and Beal, 2008; Andres et al., 2008). Additionally, creatine kinase is now recognized as a component of the mPTP, and its activation inhibits the induction of the mPTP (Beutner et al., 1998; Beutner et al., 1996; O’Gorman et al., 1997). Given that bioenergetic failure and mPTP activation have been documented as key players in TBI-induced neuropathology, creatine supplementation would be expected to offer neuroprotection following experimental TBI (Sullivan et al., 2005).

CREATINE SUPPLEMENTATION AND TBI

Creatine supplementation has been shown to be neuroprotective following TBI in both mice and rats. Our laboratory demonstrated in 2000 that chronic administration of creatine ameliorated cortical tissue damage by 36 percent in mice and 50 percent in rats depending upon the regimen and dosage used during the pretreatment (Sullivan et al., 2000). In mice, pretreatment with 3g/kg (intraperitoneal injections) for a minimum of 3 days prior to injury was required to demonstrate significant neuroprotection. In rats, animals that were fed a dietary supplementation of 1 percent creatine for 4 weeks demonstrated significant neuroprotection that was linked to improved mitochondrial bioenergetics, increased ATP levels, and an increased threshold for activation of the mPTP. Further experiments have reported that 2 weeks of dietary supplementation of creatine (0.5% and 1%) prior to injury was sufficient to significantly reduce lactate and free fatty acid levels following TBI (Scheff and Dhillon, 2004). Additionally, all animals fed a creatine supplemented diet had significantly less cortical tissue damage compared to non-supplemented controls. To date these are the only studies assessing the use of creatine for the treatment of TBI.

CLOSING REMARKS

Creatine may be a viable prophylactic treatment for TBI based on its proposed target mechanisms including stabilization of cellular bioenergetics and inhibition of mPTP activation. Yet, a Medline search using the terms “traumatic brain injury” and “creatine supplementation” yields only six hits, of which only one is relevant. This may seem surprising considering the robust neuroprotective effects demonstrated by creatine pretreatment. However, the need to preload the system with creatine (or with any other compound) has historically reduced enthusiasm for funding this line of research as it relates to the treatment of TBI. This, of course, has left many unanswered questions:

- What is the optimal dosage of creatine (i.e., dose-response)?
- What is the minimum amount of pretreatment needed, or the therapeutic window of opportunity?
- Is post-injury treatment beneficial in combination with pretreatment?
- What is the optimal route of administration?
- Can having creatine onboard enhance or hinder other neuroprotective treatments (in other words, does prophylactic creatine alter the TBI patient profile)?

Based on the safety profile of creatine and current experimental data, it is obvious that the potential for using creatine following TBI has not been explored sufficiently; however, creatine
supplementation may offer a much needed therapeutic approach for targeting TBI in specific populations.
REFERENCES


