

Hypothesis

The Mitochondrial Permeability Transition Pore Provides a Key to the Diagnosis and Treatment of Traumatic Brain Injury

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Summary

The pathological consequences of traumatic head injury result largely from the opening of the mitochondrial permeability transition pore (mPTP). The mPTP opens due to a decrease in brain phosphorylation energy resulting in a further decrease in brain ATP production and a measurable increase in brain heat generation and temperature. The increase in brain temperature can be measured transcranially by near infrared spectroscopy which can be used to diagnose traumatic brain injury (TBI) and to monitor treatment. Effective therapy of TBI can be achieved by closure of the mPTP by administration of cyclosporine A or by oral administration of ketone body esters. While ketosis has previously been known to prevent damage from TBI, the availability of oral ketone esters presents the first practical modality of achieving therapeutic levels of ketone bodies. © 2012 IUBMB

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INTRODUCTION

The term “traumatic brain injury” (TBI) embodies a heterogeneous array of brain pathologies affecting an estimated 1.5 million Americans per year and is a major cause of death and disability in children and young adults. TBI has been reported to afflict up to 20% of the veterans returning from deployment to Iraq and Afghanistan (1). At present, no treatment aimed at preventing chronic traumatic encephalopathy (CTE), a possible long-term consequence of TBI, is being implemented. CTE is a

chronic brain syndrome that can give rise to symptoms and signs of early-onset dementia, including impaired memory, confusion, depression, and suicide. In addition to the prevalence of TBI in military personnel, there is increasing awareness of TBI in athletic injuries. Evidence continues to mount that individuals who have experienced repeated concussive and subconcussive head injuries (such as professional boxers and football players) are also at enhanced risk of developing CTE. It has been estimated (2) that, each year, some 300,000 sports-related traumatic injuries, mostly concussions, occur in the United States. Concussions represented 8.9% of all high school athletic injuries and 5.8% of all collegiate athletic injuries, the rates being highest in football and soccer. There is growing concern that an appreciable proportion of such injuries may adversely affect brain function later in life.

PATHOPHYSIOLOGY

TBI is generally classified as focal damage consisting of anatomical lesions in brain tissue in the form of contusions, lacerations, and hemorrhages, which, in some cases, can be detected by computerized tomography (CT). TBI without overt anatomical lesions is only partially diagnosable with tensor magnetic resonance imaging (3). TBI (1) resulting from explosive blasts, auto accidents, or athletic injuries creates diffuse neuronal damage with or without loss of consciousness or impairment of cognitive function. TBI involves impairment of both cerebral blood flow and metabolism, with decreased cerebral O₂ uptake, increased lactate production, and depletion of brain high-energy phosphate stores. The drop in cellular energy leads, in turn, to an increase in intracellular Na⁺ and Ca²⁺, excessive release of neurotransmitters, and the initiation of apoptosis. Indeed, the magnitude of the deficit in cerebral energy metabolism after TBI has been shown to be the best predictor of outcome (4).

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Cyclosporine A, one of the earliest immunosuppressant drugs, acts to mitigate TBI's adverse effects in man and in laboratory animal models of TBI. This observation provides an important clue to the basic mechanism by which trauma to the brain causes diffuse neuronal damage. Cyclosporine has been found to bind specifically to mitochondrial cyclophilin-D and thereby close trauma-induced opening of the mitochondrial permeability transition pore (mPTP) (5). This observation, together with other information about mPTP's role in mitochondrial metabolism strongly suggests that opening of the pore sets in motion the complex series of acute and chronic pathologies associated with TBI. Indeed, this inference has been confirmed by the many reports that administration of cyclosporine A prevents longer-term pathological changes associated with TBI in animal studies (6, 7).

The mPTP has been seen to play a central role in cell death, apoptosis, and neurodegeneration. The mPTP is comprised of a complex of mitochondrial proteins, including the adenine nucleotide translocator in the inner mitochondrial membrane, the voltage-dependent anion channel in the outer mitochondrial membrane, cyclophilin-D, the F_1F_0 ATPase (8), and other proteins that collectively form a mega channel. The mPTP is essentially the same in all mitochondrial-containing tissues—liver, muscle, and brain. In liver, the mPTP is opened by a decrease in the ATP/ADP \times Pi ratio subsequent to the metabolism of short-chain fatty acids, and results in a massive uptake of CaMgPPi within the mitochondrial matrix (9, 10). Opening of the mPTP and intramitochondrial accumulation of CaMgPPi is facilitated by the presence of calcium mobilizing hormone and cyclic AMP (11). When TBI causes mPTP opening, acute cell death or initiation of slower cell death by apoptosis is likely to follow. Opening the pore destroys the mitochondrial proton gradient that drives mitochondrial ATP synthesis (12) and allows entry of cations (namely, Ca^{2+} and Mg^{2+}) into the negatively charged inner mitochondrial membrane space. Opening the outer mitochondrial membrane part of the pore allows escape of cytochrome C from the intermembrane space into cytoplasm, initiating the apoptotic process (13).

Recently, it has been shown that brain pyruvate dehydrogenase complex is significantly inhibited by TBI (14). The result is a 50% decrease in brain O_2 utilization, measured by O_2 PET scan, and an increase in the brain [lactate]/[pyruvate] ratio in cytoplasm—a hallmark of impaired mitochondrial ATP production and decreased cellular phosphorylation energy. These observed changes are also compatible with the decrease in the respiratory control ratio found in isolated brain mitochondria (15) and an increase in brain lactate and creatine observed by proton magnetic resonance spectroscopy (1H MRS). All these findings confirm a decrease in brain phosphorylation potential and a decrease in the ΔG of ATP hydrolysis. A decrease in brain ATP and an increase in brain Pi is associated with mPTP opening, while opposite changes favor mPTP closing (1, 16, 17).

DIAGNOSIS OF TBI

While CT and MRI cannot identify the majority of cases of TBI, recent studies have found that $\sim 30\%$ of military personnel with putative TBI resulting from exposure to blasts from explosive devices showed brain abnormalities when studied by means of diffusion tensor magnetic resonance imaging, a specialized application of MRI (3). Unfortunately, use of such sophisticated techniques is not practical under most field conditions leaving only a clinical evaluation for the diagnosis of concussion.

If one accepts the premise that TBI results in opening of the mPTP, then the energy of the proton gradient across the mitochondrial membrane could not be used to produce ATP but rather would produce heat. The total amount of energy released from oxidation of NADH to H_2O is given by the statement:



The energy of the oxidation of NADH to H_2O in the mitochondrial respiratory chain, ΔG_{Chain} , is given by the difference in the midpoint potential of O_2/H_2O couple and the $NAD^+/NADH$ couple in the statement: $\Delta G_{\text{CHAIN}} = -nf(E_h^7 O_2/H_2O - E_h^7 NAD^+/NADH)$ where the midpoint potential, E_h , at pH 7 of the O_2/H_2O couple is +0.814 V and for the $NAD^+/NADH$ couple -0.32 V; the number of electrons $n = 2$ and the Faraday, $f = 23.082$ kcal/mol/V (12). Therefore, the ΔG_{Chain} equals 0.05235 calories/ μmol of NADH oxidized to H_2O by using $1/2 \mu\text{mol}$ of O_2 . Confirming the hypothesis that TBI causes an opening of the mPTP with consequent production of heat, head injury patients have been shown to have brain temperatures about 1°C above core body temperature (18). The logic of the argument presented herein is confirmed in animal models of TBI by the observation that cyclosporine A, which closes the mPTP, decreased brain temperature monitored over the temporalis muscle by 1°C (19).

The generation of heat by opening of the mPTP offers a simple method for the provisional diagnosis of TBI and monitoring of its treatment. A method was developed in 1995 by Clyde Barlow and Britton Chance which allows the measurement of brain temperature at 970 nM transcranially by means of near infrared spectroscopy (NIRS) (20). A simple cheap and portable light-emitting diode and photoreceptor apparatus has been constructed which should permit—with some increase in sensitivity—transcranial measurement of the elevation of the $\sim 1^\circ\text{C}$ in brain temperature found in acute TBI (18). The simplicity of this NIR equipment should allow such measurement to be performed under field conditions.

TREATMENT

Cyclosporine A, which closes the mPTP, has been demonstrated to ameliorate TBI after its intrathecal injection (19). Although this agent penetrates the blood-brain barrier very poorly, injection of 20 mg/kg cyclosporine A into the peritoneal

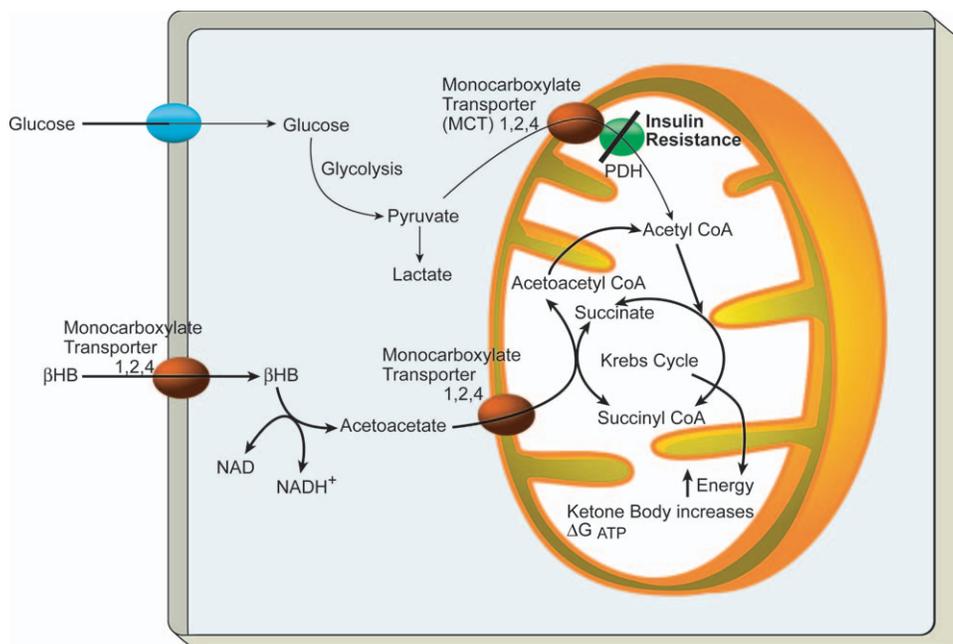


Figure 1. Correction of the underlying deficit in cerebral energy underlying TBI. The insulin resistance in brain causing a block in PDH can be by-passed by ketone bodies.

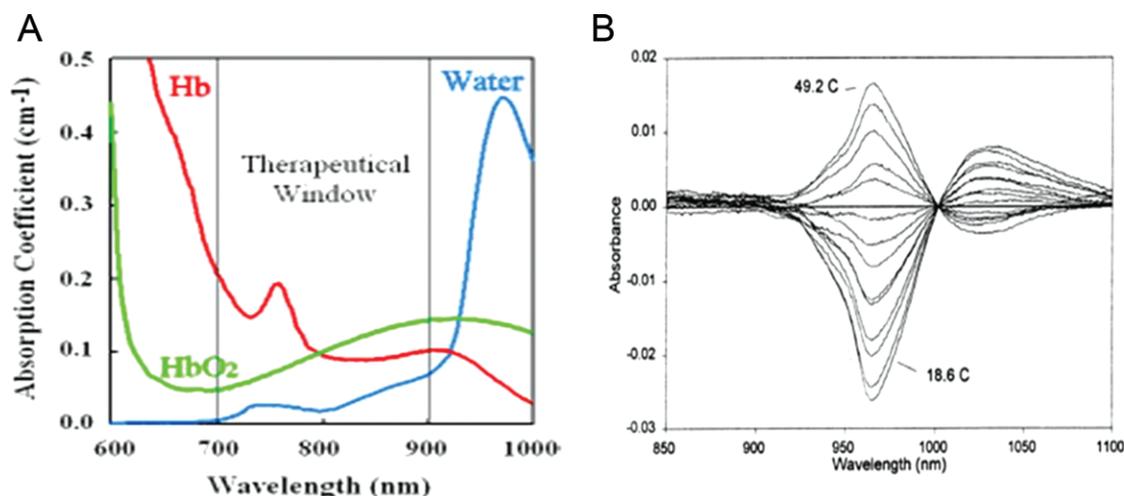


Figure 3. (A). Illustration of a merging of the hemoglobin and water absorption spectra to an arbitrary scale to show their spectral overlap and the optimal windows for minimal crosstalk of the three metrics to be studied. (B) Sensitivity of water absorption spectrum to temperature. Thermal difference spectra of water in a 1-mm path length, baseline 37°C with lowering to 17°C, and rising to 50°C showing that 970 nm is an appropriate measuring wavelength, and 1010 nm is a suitable reference wavelength. A signal increment of 0.010 optical density per degree for a 1-cm optical path is calculated.

space, followed 24 h later by a similar injection, significantly reduced cortical damage in animal models of TBI.

Although opening of the mPTP appears to lie at the heart of the pathophysiology of TBI, and even though cyclosporine A can close the mPTP, it is not an ideal agent for treatment because of its inhibition of immune function and its many

adverse side effects, including nephrotoxicity, hepatotoxicity, and neurotoxicity. As an alternate therapeutic approach, elevation of blood ketone bodies, either by fasting or feeding high-fat ketogenic diets has been shown to be neuroprotective in animal models of TBI (21, 22). However, preparation of an appropriate ketogenic diet is complicated and patients find such diets



Figure 2. Diagnosis of TBI. Clyde Barlow demonstrated that temperature could be evaluated through tissue by NIR at 970 nM. Opening of the mPTP during TBI would produce heat rather than ATP and might be diagnosable by NIR. A simple, portable NIR spectroscopy apparatus suitable for the measurement of various NIR wavelengths through skull under field conditions, demonstrated by Britton Chance pictured here. (Reproduced from Ref. 20.)

unappetizing and difficult to consume. The major problem with the ketogenic diet approach is the time lag of 3+ days entailed in achieving therapeutic levels of blood ketone bodies. These difficulties have now been overcome by the development of a food supplement (D- β -hydroxybutyrate-R 1,3 butanediol mono-ester) for oral administration, that is regarded as “Generally Recognized As Safe” (GRAS) by the FDA. The supplement raises blood ketone body concentrations to 5–7 mM/L during the first hour after oral administration, obviating the need to feed a slow acting, difficult-to-prepare, and unpalatable ketogenic diet. The ketone ester can be administered as a liquid at a dose of 0.3 g/kg body weight as soon as possible following TBI in conscious patients or by nasogastric tube in patients who are unconscious. The ester is promptly hydrolyzed and converted to ketone bodies in the small intestine. The blood levels that result are easily monitored in finger-stick samples by use of commercially available analysis kits. The metabolism of ketone bodies achieves neuroprotection by increasing the cellular phosphorylation potential (12, 23), thereby closing the mPTP and restoring ionic balance and neuronal volume regulation, both of which depend on physiologically normal $\Delta G'$ of ATP hydrolysis (24).

Severe injury of any sort, including TBI, results in insulin resistance (25). In brain and other tissues, insulin resistance results in inhibition of the activity of pyruvate dehydrogenase, the key step in converting pyruvate, the end-product of glycolysis, into acetyl CoA, necessary to operate the Krebs cycle, which produces the reducing power required for ATP synthesis by the electron transport system. The metabolism of ketone bodies provides a readily available source of acetyl CoA, bypassing the block caused by the inhibition of PDH (Fig. 1).

CONCLUSIONS

Cyclosporine A has been shown to decrease the pathological changes in brain resulting from TBI. Cyclosporine A closes the mPTP by combining with the mitochondrial protein, cyclophilin. The opening of the mPTP results in the diversion of the energy generated by transport of electrons in the respiratory chain from ATP production to heat production. The heat produced by TBI resulted in an increase of brain temperature by about 1°C above core body temperature (18). Administration of cyclophilin A in animal models of TBI has been shown to decrease temperature over the temporalis muscle by about 1°C (19). Monitoring of brain temperature by NIR thus provides a simple method for the diagnosis of TBI and the monitoring of therapeutic interventions (Figs. 2 and 3).

Ketogenic diets or fasting, both of which elevate blood ketone bodies, have both been shown to decrease the pathological changes resulting from TBI (22). The availability of ketone esters makes the achievement of ketosis after TBI practical. Opening of the mPTP allows small ions to enter mitochondrial matrix space resulting in a further decrease in the ΔG of ATP (9). A drop in the phosphorylation potential must inevitably lead to an increase in intracellular brain Na⁺ and Ca²⁺ with resultant swelling (24). Brain swelling is, therefore, an inevitable consequence of the drop in ΔG ATP. The ΔG of ATP can be increased by the metabolism of ketone bodies (12, 23). The oral administration of ketone body esters would thus provide a practical and less toxic method than cyclosporine A for the treatment of TBI.

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