Synaptic Mitochondrial Sustain More Damage than Non-Synaptic Mitochondria following Traumatic Brain Injury and are Protected by Cyclosporine A

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Background: Currently there are no FDA-approved neuroprotective drugs for the treatment of traumatic brain injury (TBI). As central mediators of the secondary injury cascade, mitochondria are promising therapeutic targets for prevention of cellular death and dysfunction following TBI. One of the most promising and extensively studied mitochondrial targeted TBI therapies is inhibition of the mitochondrial permeability transition pore (mPTP) by the FDA-approved drug, cyclosporine A (CsA). A number of studies have evaluated the effects of CsA on total brain mitochondria following TBI; however, none have investigated the effects of CsA on isolated synaptic and non-synaptic mitochondria. Synaptic mitochondria are considered essential for proper neurotransmission and synaptic plasticity and their dysfunction has been implicated in neurodegeneration.

Purpose/Hypothesis: Synaptic and non-synaptic mitochondria have heterogeneous characteristics, but their heterogeneity can be masked in total mitochondrial (synaptic and non-synaptic) preparations. Therefore, it is essential that mitochondria-targeted pharmacotherapies, such as CsA, be evaluated in both populations.

Methods: Young adult male Sprague-Dawley rats (n = 20, Harlan, Indianapolis, IN, USA) weighing 300 to 350g were used for all studies. Animals were randomly assigned to experimental groups: sham (n = 6), controlled cortical impact TBI (CCI-TBI) + vehicle (n = 6), CCI-TBI + CsA (n=8). Animals were initially anesthetized with 4% isoflurane and placed in a stereotaxic frame, where they were maintained at 3% isoflurane for the duration of the procedure. A midline incision was made to expose the skull and a 6mm craniotomy was made lateral to the sagittal suture midway between lambda and bregma. The exposed brain with intact dura was injured using a computer controlled pneumatic impactor (TBI 03010; Precision Systems and Instrumentation, Fairfax Station, VA) fitted with a 5mm beveled tip set to impact at ~3.5m/sec, 2.2mm depth and 500msec dwell time. Following injury, surgicel was placed onto the dura and an 8mm plastic disk was affixed with tissue adhesive to close the craniotomy site. Body temperature was monitored and maintained at 37°C with a thermo-regulating heating pad. Sham animals underwent all procedures but did not receive an impact injury. The CsA concentration chosen was based on previously optimized concentrations for CCI-TBI. The CCI + CsA group was administered CsA obtained from the University of Kentucky Medical Center Hospital Pharmacy (Perrigo; Minneapolis, MN; 50 mg/ml) 15min following injury as a single intraperitoneal dose of 20mg/kg in saline/650mg cremophor/33.2%(v/v) ethanol diluted in saline to a final concentration of 10mg/ml. The injection volume was 0.2 ml/100g of body weight. CCI + Vehicle treated animals received an equivalent volume of saline/cremophor/ethanol 15min following injury. Cortical mitochondria were isolated as described previously with modifications to isolate synaptic and non-synaptic populations.

Results and Conclusions: Both non-synaptic and synaptic mitochondrial respiration are significantly impaired 24h following severe CCI-TBI. Acute (15min post-injury) CsA administration (20mg/kg, i.p.) improves non-synaptic and synaptic respiration, with a significant improvement being seen in the more severely impaired synaptic population. CsA remains a promising neuroprotective candidate for the treatment of human TBI.