Application Note

Assessing therapies for traumatic brain or spinal cord injuries

XF technology provides insight into treatment interventions for mitochondrial dysfunction

RESEARCH AREA

Neurodegeneration

ASSAY TYPES

XF Cell Mito Stress Test
Respiratory Complex Assay

KEYWORDS

Mitochondria, oxidative phosphorylation, TBI, SCI, molecular hydrogen, N-acetylcysteine amide (NACA)

Sudden injury or trauma that causes damage to the brain or spinal cord is categorized as traumatic brain injury (TBI), and traumatic spinal cord injury (SCI), respectively. These types of injuries result from impacts such as blasts, falls, and automobile accidents. Within moments of mechanical impact the balance between free radical production and antioxidant capacity is upset, resulting in oxidative stress, which plays an important role in the pathophysiology of nervous system injury. Moreover, TBIs and SCIs include secondary pathophysiological events that can result in neuronal death in and around the injury site. Due to the speed at which these mechanisms begin and perpetuate, any TBI or SCI treatment would need to be of low toxicity and easily administered.

A key feature of TBI and SCI, from mild to severe, is mitochondrial dysfunction. With mitochondria at the center of generating cellular energy metabolism, intracellular signaling, and regulating cell death and survival, compromised function strains the cellular metabolic network. Several studies have shown that mitochondrial dysfunction plays a critical role in the pathogenesis of several diseases including neurodegeneration (Semple *et al.,* 2014). Therefore, focusing on mitochondrial dysfunction may provide viable therapeutic targets to mitigate many of the deleterious and lasting effects of neurotrauma. This Application Note describes publications that use XF Technology to investigate potential therapies that target mitochondrial dysfunction to attenuate the effects of TBI and SCI.

A recent study published by Dohi *et al.* (2014) examined the effects of molecular hydrogen given in drinking water, using a TBI animal model and conditionally immortalized mouse cerebral pericyte cells (ImBPC). Pericyte cells, a constituent of the blood brain barrier (BBB), regulate several functions of the BBB, including blood flow within and into the BBB. The authors hypothesized that the chemical

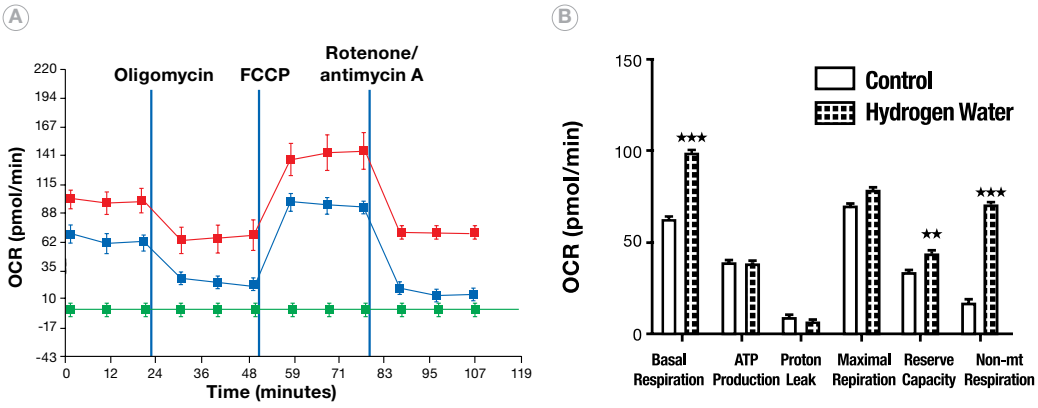

Figure 1 | Molecular hydrogen treatment increases mitochondrial respiration in ImBPCs

Following treatment of molecular hydrogen, ImBPCs underwent an XF Cell Mito Stress Test.

A: Cells pre-treated with molecular hydrogen (red trace) compared to control cells (blue trace).

B: Respiration parameter analysis from the XF Cell Mito Stress Test. Figures presented serve as a representative of 3 experiments, presented as mean ± SEM. **= p<0.01 and ***= p < 0.001.

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characteristics of molecular hydrogen, including being uncharged and of low molecular weight, and having the ability to penetrate biological membranes, as well as the blood brain barrier (BBB), would lend itself well to be used as a potential therapy.

Using the XF Cell Mito Stress Test (Figure 1), the authors demonstrated a difference in the oxygen consumption rate (OCR) between cells pretreated with molecular hydrogen water and controlled-distilled water. As illustrated in Figure 1A, cells pretreated with molecular hydrogen have a higher basal respiration rate (red trace), compared to untreated cells (blue trace). Subsequent analysis of mitochondrial parameters (Figure 1B) revealed a significant increase in the basal respiration, reserve capacity, and non-mitochondrial respiration, but not ATP production, maximal capacity, or proton leak. These results indicate that in molecular hydrogen-treated cells, ATP production is independent of oxygen use.

The authors used an XF24 Extracellular Flux Analyzer (Seahorse Bioscience), in conjunction with the XF Cell Mito Stress Test, to measure changes in mitochondrial respiration. Based on their observations, they concluded that molecular hydrogen might help overcome the ATP deficit in cells undergoing TBI and be a non-toxic treatment for acute TBI.

Discussion

Researchers use XF Stress Tests and kits, reagents, and consumables to uncover relevant functional metabolic data. As described in these publications, XF Technology is being used to assess potential new therapies for the treatment of traumatic brain and spinal cord injuries.

In a study by Pandya *et al.* (2014), the authors examined the effects of N-acetylcysteine amide (NACA), the amide form of its parent N-acetylcysteine, a precursor of glutathione. They noted that using this modified antioxidant holds great promise for treating neurological traumas, as glutathione plays an essential role in scavenging excess reactive oxygen species (ROS; Shah *et al.*, 2013). They hypothesized that NACA would attenuate the cellular damage following TBI. The XF24 Analyzer was used to assess mitochondrial respiratory chain complexes in isolated mitochondria following an induced TBI. Increased mitochondrial respiration was observed in the isolated mitochondria taken from NACA-treated injured animals, and NACA-treated control subjects. Based on these observations, the authors concluded that not only is NACA a viable option for treating TBI, but is also nontoxic to naive or uninjured animals.

A critical component of TBI, SCI, and general neurotrauma research, is characterization of potential therapeutics, as well as an understanding of the mechanism of action of the potential candidate. Therefore, targeting the mitochondria instead of the product of neurotrauma (e.g. oxidative damage and neuronal death), may help to overcome the limited success observed with previous therapeutic strategies (Semple, 2014).

Patel *et al.* (2014) also recently published a study using NACA for treatment of SCI, in which the authors hypothesized that the antioxidant nature of NACA would also confer its protective effects to acute mitochondrial function. An XF24 Analyzer was used to study mitochondrial respiratory chain complexes using synaptic (predominately neuronal sources), nonsynaptic (including neuronal stoma and glial sources), and total mitochondria isolated from injured and naïve Sprague-Dawley rats. A dose-dependent increase in mitochondrial respiration was observed, with 300 mg providing the maximum effects. The researchers, based on their observations, concluded that acute SCI resulted in reduced mitochondrial function, and that treatment with NACA significantly maintained each tested population of isolated mitochondria.

Materials and Methods

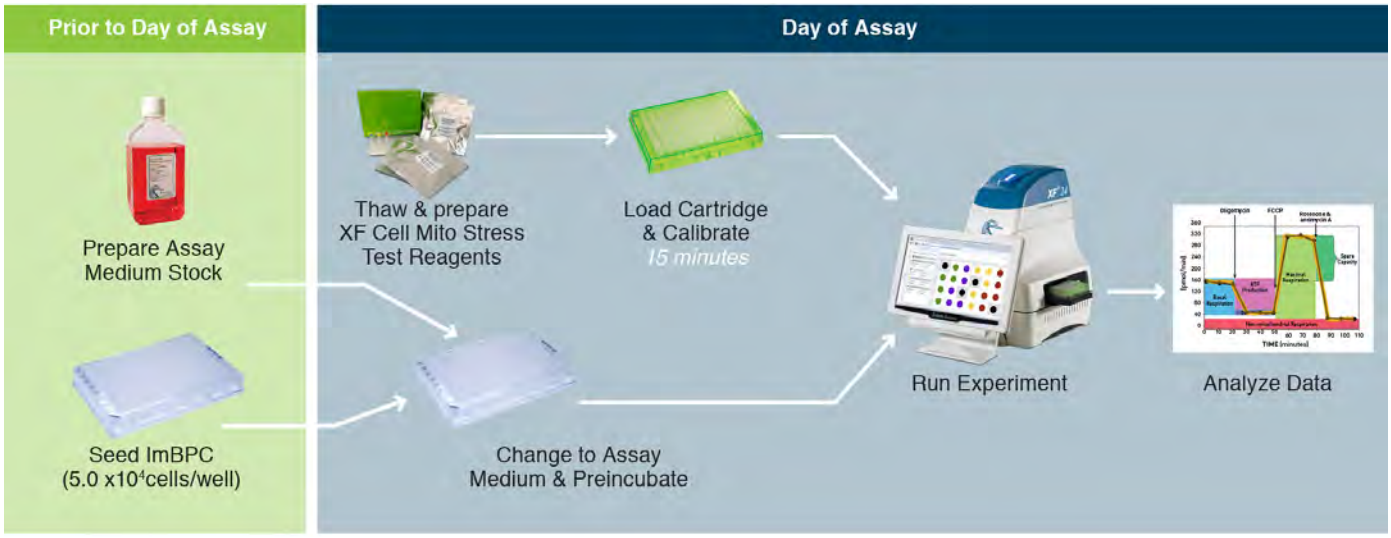
Conditionally immortalized mouse cerebral pericyte cells (ImBPC) were cultured in 5.5 mM glucose DMEM supplemented with 10% fetal bovine serum (FBS), non-essential amino acids, interferon-γ (44 U/mL), penicillin (100 U/ mL), and streptomycin (0.1 mg/mL). Cultures were maintained at 33°C and 5% CO_2.

XF Analysis

Metabolic analysis of ImBPC were performed using an XF24 Analyzer, which enables real time, simultaneous measurement of oxygen consumption and extracellular acidification rates (OCR and ECAR, respectively), via a transient microchamber in specialized tissue culture microplates.

ImBPC were passaged and seeded onto XF24 microplates at $5.0x10^4$ cells/well in growth medium and incubated for 24 hours at 33°C; 5% CO_2. Following incubation the growth medium was exchanged for XF DMEM, supplemented with 5 mM glucose and 1 mM sodium pyruvate (pH 7.4). For the XF Cell Mito Stress Test, following basal respiration, the mitochondrial inhibitors oligomycin, FCCP, and a mixture of rotenone and antimycin A were injected sequentially.

Figure 2 | Flow Chart of XF Assay



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