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Pioglitazone attenuates mitochondrial dysfunction, cognitive impairment, cortical tissue loss, and inflammation following traumatic brain injury

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ABSTRACT

Following traumatic brain injury (TBI) there is significant neuropathology which includes mitochondrial dysfunction, loss of cortical gray matter, microglial activation, and cognitive impairment. Previous evidence has shown that activation of the peroxisome proliferator-activated receptors (PPARs) provide neuroprotection following traumatic brain and spinal injuries. In the current study we hypothesized that treatment with the PPAR ligand Pioglitazone would promote neuroprotection following a rat controlled cortical impact model of TBI. Animals received a unilateral 1.5 mm controlled cortical impact followed by administration of Pioglitazone at 10 mg/kg beginning 15 min after the injury and subsequently every 24 h for 5 days. Beginning 1 day after the injury there was significant impairment in mitochondrial bioenergetic function which was attenuated by treatments with Pioglitazone at 15 min and 24 h ($p<0.05$). In an additional set of animals, cognitive function was assessed using the Morris Water Maze (MWM) and it was observed that over the course of 4 days of testing the injury produced a significant increase in both latency ($p<0.05$) and distance ($p<0.05$) to the platform. Animals treated with Pioglitazone performed similarly to sham animals and did not exhibit any impairment in MWM performance. Sixteen days after the injury tissue sections through the lesion site were quantified to determine the size of the cortical lesion. Vehicle-treated animals had an average lesion size of 5.09 ± 0.73 mm^3 and treatment with Pioglitazone significantly reduced the lesion size by 55% to 2.27 ± 0.27 mm^3 ($p<0.01$). Co-administration of the antagonist T0070907 with Pioglitazone blocked the protective effect seen with administration of Pioglitazone by itself. Following the injury there was a significant increase in the number of activated microglia in the area of the cortex adjacent to the site of the lesion ($p<0.05$). Treatment with Pioglitazone prevented the increase in the number of activated microglia and no difference was observed between sham and Pioglitazone-treated animals. From these studies we conclude that following TBI Pioglitazone is capable ameliorating multiple aspects of neuropathology. These studies provide further support for the use of PPAR ligands, specifically Pioglitazone, for neuroprotection.

Introduction

Traumatic brain injury (TBI) pathology results from both a primary injury and a secondary injury cascade. The primary injury is due to biomechanical damage which results in the shearing and compression of neuronal, glial, and vascular tissue. The cascade of secondary injury damage, which occurs in the hours and days following the initial insult, is due to activation of pathophysiological cascades, consisting of complex biochemical and cellular pathways that influence progression of the injury, such as alterations in excitatory amino acids (Yamamoto et al., 1999; Rose et al., 2002), increased reactive oxygen species (ROS) production (Marklund et al., 2001; Hall et al., 2004; Tavazzi et al., 2005), disruption of calcium homeostasis (Mattson and Scheff, 1994; Xiong et al., 1997; Sullivan et al., 1999b), post-traumatic neuroinflammation (Morganti-Kossmann et al., 2001; Vlodavsky et al., 2006) and mitochondrial dysfunction (Azbill et al., 1997; Xiong et al., 1997; Sullivan et al., 1998, 1999a,b). As a result of these secondary injury processes, there are significant reductions in ATP levels, increases in lipid peroxidation, release of cytochrome *c* and activation of apoptotic pathways (Sullivan et al., 1998, 2002), all of which can lead to the initiation of cell death pathways. Mitochondria are a major component of this secondary

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injury pathway because they function as a highly sensitive gatekeeper of cell death mechanisms and as the primary energy producer for the cell. As such, mitochondria play a pivotal role in cerebral energy metabolism, intracellular calcium homeostasis, and ROS generation and detoxification.

Following TBI, a significant disruption of mitochondrial homeostasis has been documented that results in a decline in cellular bioenergetics, increased mitochondrial ROS production and a decline in synaptic equilibrium (Azbill et al., 1997; Xiong et al., 1997; Sullivan et al., 1998, 1999a,b). Therefore, following TBI, the degree of mitochondrial injury or dysfunction can be an important determinant of cell survival or death (for reviews see Robertson, 2004; Sullivan et al., 2005; Robertson et al., 2006) and therapeutic treatments designed to protect and stabilize the mitochondria have demonstrated the ability to reduce injury in preclinical studies (Sullivan et al., 2000a; Pandya et al., 2007). Although preclinical research has identified neuroprotective agents which target mitochondrial function, inflammation, and oxidative damage, attempts to move therapies into clinical usage have so far been unsuccessful (Schouten, 2007). Given the complexity of the secondary injury, it has been suggested that drugs which target multiple pathological pathways may yield more effective therapeutic approaches for TBI. The PPARγ agonist Pioglitazone has been shown to reduce inflammation (Besson et al., 2005; Chen et al., 2007; Park et al., 2007; Hyong et al., 2008; Kapadia et al., 2008) and oxidative damage (Chen et al., 2007; Yi et al., 2008), attenuate mitochondrial dysfunction (Hunter et al., 2007), and reduce cell death (McTigue et al., 2007; Park et al., 2007) following CNS injury. Pioglitazone's ability to target multiple injury mechanisms may provide it with an advantage over other therapeutics for TBI which target a single secondary injury cascade. The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily, which regulate gene expression using various ligand-dependent and independent molecular processes. Three different isoforms of the PPARs exist, which are encoded by separate genes: PPARγ (NR1C3), PPARα (NR1C1), and PPARδ (NR1C2, β, or NUC-1) (Dreyer et al., 1992; Michalik and Wahli, 1999; Torra et al., 2001). While these isoforms have similar protein sequence and structure, they differ in their ligand-binding domains and have different ligand specificity, tissue distribution, and biological actions (Guan et al., 2002). The PPARs regulate inflammation mainly through their transrepression capabilities although the transactivation of certain target genes can occur. Several inflammatory signaling systems may be affected by PPAR-mediated transrepression such as nuclear factor-kappa B (NFκB), activator protein-1 (AP-1), signal transducer and activator of transcription (STAT), or nuclear factor of activated T cells (NFAT) (Ricote et al., 1998; Delerive et al., 2001; Park et al., 2003; Bernardo and Minghetti, 2006). These pathways immunoregulate macrophages, microglia, dendritic cells, endothelial cells, B cells, and T cells (for reviews see Clark, 2002; Daynes and Jones, 2002; Hunter and Guoying, 2007). Treatment with Pioglitazone following LPS induced brain inflammation has shown the ability to prevent both mitochondrial impairment and neuronal cell loss (Hunter et al., 2007).The therapeutic use of various PPARs has shown a benefit in multiple CNS injury models including spinal cord injury (SCI) (McTigue et al., 2007; Park et al., 2007), traumatic brain injury (TBI) (Besson et al., 2005; Chen et al., 2007, 2008; Yi et al., 2008), and stroke (Collino et al., 2006; Allahtavakoli et al., 2009). Of the three known PPAR isoforms, PPARα and PPARγ have been the most well studied in CNS injury and have been shown to reduce lesion size both in SCI (McTigue et al., 2007; Park et al., 2007) and TBI (Yi et al., 2008), reduce inflammation (Besson et al., 2005; Chen et al., 2007; Park et al., 2007; Hyong et al., 2008; Kapadia et al., 2008) , minimize oxidative damage (Chen et al., 2007; Yi et al., 2008), spare neurons (McTigue et al., 2007; Park et al., 2007), and preserve behavioral function (Chen et al., 2007; McTigue et al., 2007; Park et al., 2007; Chen et al., 2008).

The PPARγ agonists Pioglitazone and Rosiglitazone are both FDA approved drugs for diabetes treatment (for review see Sood et al., 2000) and have been utilized as therapeutics in animal models of CNS injury (Besson et al., 2005; Kiaei et al., 2005; Schutz et al., 2005; Collino et al., 2006; Chen et al., 2007, 2008; McTigue et al., 2007; Park et al., 2007; Feng et al., 2008; Hyong et al., 2008; Sun et al., 2008; Yi et al., 2008; Allahtavakoli et al., 2009). Rosiglitazone has been previously shown to have a higher binding affinity for the PPARγ receptor (Young et al., 1998), however, Pioglitazone has been shown to more readily cross the blood brain barrier (BBB) (Berger and Moller, 2002) as well as partially activate the PPARα receptor (Sakamoto et al., 2000). Pioglitazone's increased brain penetration and activation of two separate PPAR pathways may yield a greater therapeutic potential for the treatment of TBI (for review see Kapadia et al., 2008). Currently evidence exists showing that activation of either the PPARα (Chen et al., 2007, 2008) or PPARγ (Yi et al., 2008) pathways are protective in models of TBI, however, no studies currently exist showing the effect of Pioglitazone following TBI. Because of the success of PPARγ agonists in multiple models of CNS injury and their offer of a broad range of potentially protective properties, we hypothesize that PPARγ activation by Pioglitazone will be beneficial in an animal model of controlled cortical impact (CCI) that has hallmarks of human TBI. The current project addresses the hypothesis that Pioglitazone will offer significant neuroprotection leading to the maintenance of mitochondrial function, sparing of cortical tissue, attenuation of inflammation, and preservation of cognitive function following TBI.

Results

Pioglitazone protects mitochondria from injury-induced mitochondrial dysfunction

Following TBI there is significant damage to the mitochondria resulting in impaired in bioenergetic function. In order to elucidate the effect of Pioglitazone on mitochondrial bioenergetic function, two different treatment paradigms for Pioglitazone administration were utilized. In the first set of animals, Pioglitazone was administered 15 min after the injury (10 mg/kg) and animals were sacrificed 25 h after the injury. In the second set of animals Pioglitazone was administered at 15 min and 24 h after injury (10 mg/kg/injection) and animals were sacrificed at 25 h after the injury. Following injury in both sets of animals, reductions in pyruvate/malate (PM), ADP, and FCCP (uncoupled) respiration rates were seen in vehicle-treated animals (Fig. 1, $^{*}p<0.01$). With only a single 15 min injection there was a slight but non-significant increase in respiration rates following Pioglitazone treatment (Fig. 1A); however, when Pioglitazone was given at both 15 min and 24 h after the injury a significant increase in mitochondrial function was observed ($^{*}p<0.01$, Fig. 1B), indicating that under these conditions Pioglitazone treatment leads to preservation of the mitochondria's ability to produce ATP.

Pioglitazone treatment improves Morris Water Maze performance following TBI

Cognitive impairment is a significant pathological outcome with both human and rodent cortical impact TBI. In order to assess the ability of Pioglitazone to reduce cognitive impairment following injury, animals were administered vehicle, Pioglitazone, or Pioglitazone plus the PPARγ antagonist T0070907 with an initial injection at 15 min post-injury and subsequent injections at 24 h, 48 h, 72 h and 96 h after the first injection. Pioglitazone and T0070907 were administered at 10 mg/kg at every dose. MWM assessments were assessed 10 days post-injury and consisted of 4 days of trials. Following repeated measures analysis of the MWM results, a significant effect of day ($p<0.0001$) and a trend towards effect of treatment (latency $p=0.0693$; distance $p=0.0648$)