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The PPAR gamma agonist Pioglitazone improves anatomical and locomotor recovery after rodent spinal cord injury

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Abstract

Traumatic spinal cord injury (SCI) is accompanied by a dramatic inflammatory response, which escalates over the first week post-injury and is thought to contribute to secondary pathology after SCI. Peroxisome proliferator-activated receptors (PPAR) are widely expressed nuclear receptors whose activation has led to diminished pro-inflammatory cascades in several CNS disorders. Therefore, we examined the efficacy of the PPARγ agonist Pioglitazone in a rodent SCI model. Rats received a moderate mid-thoracic contusion and were randomly placed into groups receiving vehicle, low dose or high dose Pioglitazone. Drug or vehicle was injected i.p. at 15 min post-injury and then every 12 h for the first 7 days post-injury. Locomotor function was followed for 5 weeks using the BBB scale. BBB scores were greater in treated animals at 7 days post-injury and significant improvements in BBB subscores were noted, including better toe clearance, earlier stepping and more parallel paw position. Stereological measurements throughout the lesion revealed a significant increase in rostral spared white matter in both Pioglitazone treatment groups. Spinal cords from the high dose group also had significantly more gray matter sparing and motor neurons rostral and caudal to epicenter. Thus, our results reveal that clinical treatment with Pioglitazone, an FDA-approved drug used currently for diabetes, may be a feasible and promising strategy for promoting anatomical and functional repair after SCI.

Keywords: Inflammation; Locomotion; Motor neurons; White matter sparing; Gray matter; Spinal contusion

Introduction

Peroxisome proliferator-activated receptors (PPARs), which exist as α, δ and γ isotypes, are ligand-activated transcription factors found in most eukaryotic cells (Desvergne and Wahli, 1999; Hihi et al., 2002). Upon ligand binding, the receptor/ligand complex can up-regulate or down-regulate transcription of genes containing a peroxisome proliferator response element (PPRE). Most ligands identified to date for PPARs are long-chain fatty acids and, accordingly, many PPAR gene targets are involved in fatty acid metabolism (Desvergne and Wahli, 1999; Hihi et al., 2002). PPAR functions extend beyond fatty acid metabolism, however, and include regulation of other important processes, such as inflammation (for reviews, see Landreth and Heneka, 2001; Moraes et al., 2006). PPARγ agonists in particular have been examined for their anti-inflammatory and anti-oxidant properties in several models of central nervous system (CNS) injury and disease, such as amyotrophic lateral sclerosis (Kiaei et al., 2005; Schutz et al., 2005), Parkinson's disease (Breidert et al., 2002; Dehmer et al., 2004), cerebral ischemia or hemorrhage (Ou et al., 2006; Victor et al., 2006; Zhao et al., 2006), and experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (Niino et al., 2001; Feinstein et al., 2002; Diab et al., 2002). These and other studies have shown that activation of PPARγ in CNS disorders consistently reduces iNOS levels, improves neuronal survival, and decreases expression of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNFα) and interleukin-1β (IL-1β) (Combs et al., 2000; Heneka et al., 2000; Niino et al., 2001; Breidert et al., 2002; Schutz et al., 2005; Zhao et al., 2006). A direct action within the brain or spinal cord is possible since upregulated PPARγ expression has been detected within the injured CNS (Diab et al., 2002; Ou et al., 2006; Victor et al.,

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2006). However, PPARγ agonists also can act directly on monocytes and T-cells to reduce pro-inflammatory cytokine release and decrease proliferation (Jiang et al., 1998; Ricote et al., 1998; Combs et al., 2000; Diab et al., 2002; Feinstein et al., 2002). Thus, activation of the PPARγ pathway appears to function as a potent anti-inflammatory mechanism in circumstances of CNS injury or disease.

Many of the inflammatory-related molecules reduced by PPARγ activation are also thought to contribute to pathology following spinal cord injury (SCI). Because of the success of PPARγ agonists in multiple studies of CNS injury, we hypothesized that PPARγ activation would be beneficial in an animal model of spinal contusion that mimics a large portion of human SCI. Thus, we examined the effect of Pioglitazone given during the first week post-injury on chronic recovery from spinal contusion in rats. Pioglitazone is an FDA approved PPARγ agonist used clinically for the treatment of diabetes; it is also currently being tested in multiple sclerosis patients (Pershadsingh et al., 2004). Importantly, if it proved advantageous to the outcome of experimental SCI, the drug would be readily available to SCI patients. Indeed, our results reveal that Pioglitazone treatment during the first week post-injury promoted several aspects of anatomical repair, including white matter and gray matter sparing and motor neuron survival at the lesion margins. In addition, several aspects of locomotion were improved in animals receiving Pioglitazone treatment. Thus, this therapeutically available agent appears to hold promise for the treatment of spinal cord injury.

Methods and materials

These studies were divided into three main experiments: Study I, II and III. The purpose of Study I was to compare two different doses of Pioglitazone on anatomical and locomotor outcome at 5 weeks post-injury. Study II was a replication study using the more efficacious dose of Pioglitazone. The third study was an acute experiment in which rats were treated with Pioglitazone as in Study II then sacrificed on day 7 at the end of treatment. Adult female Sprague–Dawley rats (214–245 g) were used for all studies. All procedures were carried out in accordance with the Ohio State University IUCAC guidelines.

Study I

Rats received a moderate contusion injury using the OSU electromagnetic spinal cord injury device as previously described (McTigue et al., 2006). Briefly, rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg), and then a partial laminectomy was performed at vertebral level T_8. A computer controlled impact probe was slowly lowered to the dural surface. Next, the probe rapidly impacted the spinal cord to a depth of 0.7 mm over 23 ms, producing a closed-dural contusion injury. The injury site was surgically closed and the animal was hydrated and placed in a warmed recovery cage. Animals received antibiotics for 5 days post-injury (dpi) and manual bladder expression until return of automatic voiding, typically 10–14 dpi.

Rats were randomly assigned to one of three groups ($n=6$/group): 10 mg/kg Pioglitazone, 1 mg/kg Pioglitazone or vehicle (sterile phosphate buffered saline (PBS)). The high dose Pioglitazone solution was prepared as a suspension by dissolving one pulverized 45 mg Pioglitazone tablet (Takeda Pharmaceutical Company, Japan; purchased at OSU Hospital pharmacy) into 9 ml of PBS (37 °C) for a concentration of 5 mg/ml. The low dose was prepared by diluting 1 ml of the high dose to 0.5 mg/ml. Drug solutions were made fresh for each administration; drug or vehicle injections (i.p.) were given 15 min post-injury and, beginning on day 1 post-injury, every 12 h for 7 days. Rats survived for 5 weeks post-injury.

Study II

Rats underwent laminectomy surgery as above. Because a faulty force transducer on the injury device was replaced between Study I and II, the displacement for injuries in Study II had to be increased to 0.8 mm in attempt to match the injury severity of the first study. Rats were randomly assigned to a Pioglitazone group (10 mg/kg; $n=6$) or vehicle group (PBS; $n=6$), and injections were delivered as in Study I. Rats survived for 5 weeks post-injury.

Study III

Rats underwent laminectomy surgery as above and an 0.8 mm displacement spinal contusion injury was delivered as in Study II. Animals were randomly divided into a Pioglitazone group (10 mg/kg; $n=7$) or vehicle group (PBS; $n=7$) as in Study II. Rats survived for 7 dpi.

Behavioral evaluation

BBB

All animals were gentled prior to injury so that they were comfortable being handled and walking in the open field apparatus. Using the Basso–Beattie–Bresnahan (BBB) locomotor rating scale (Basso et al., 1995), rats were tested prior to injury to ensure that all animals began with a normal score of 21. Then on days 1, 3, 5, 7, 10, 14, 21, 28 and 35 post-injury rats were scored by two observers blinded to the treatment groups. Scores for each hindlimb were averaged, and then used to create group means at each day. Groups were compared using two-way repeated measures ANOVA followed by Bonferroni post-hoc analysis.

BBB subscores

BBB scores were further analyzed by calculating subscores (Popovich et al., 1999; Lankhorst et al., 2001; Basso, 2004), which allows for characterization of the individual aspects of locomotion, alone or in combination. To calculate subscores, individual categories of BBB outcomes were quantified as shown in Table 1. For stepping, toe clearance and paw placement, each limb was scored separately and then summed for a final score in each category. For example, the maximum possible score for stepping is 6, which equates to consistent