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Full and partial peroxisome proliferation-activated receptor-gamma agonists, but not delta agonist, rescue of dopaminergic neurons in the 6-OHDA Parkinsonian model is associated with inhibition of microglial activation and MMP expression

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ABSTRACT

Background: Peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear hormone receptor that has been shown to have anti-inflammatory and matrix metalloproteinase (MMP) inhibitor properties. PPARγ agonists have been shown to have neuroprotective effects in various neurodegeneration models where inflammation is implicated, including models of Parkinson's disease. However, no studies have looked at the effects of partial PPARγ agonists.
Experimental approach: The neuroprotective effects of the PPARγ full agonist, pioglitazone (20 mg/kg), partial PPARγ agonist GW855266X (15 mg/kg) and PPAR-δ full agonist GW610742X (10 mg/kg) were investigated in the 6-hydroxydopamine (6-OHDA) model of Parkinson's disease when administered prior to or post 6-OHDA lesioning. The integrity of the nigrostriatal system was assessed by assessing the numbers dopaminergic neurons in the substantia nigra (SN) and by assessing striatal dopamine content. The degree of microglia activation in the SN was also immunohistochemistry assessed utilizing the marker OX-6 for activated microglia and CD-68 a marker for phagocytic microglia. Additionally we performed immunocytochemistry for MMP3 in the SN. Finally, we investigated whether a period of drug withdrawal for a further 7 days affected the neuroprotection produced by the PPARγ agonists.
Key results: Both Pioglitazone and GW855266X protected against 6-OHDA induced loss of dopaminergic neurons in the substantia nigra and depletion of striatal dopamine when administered orally twice daily for either 1) 7 day prior to and 7 days post lesioning or 2) for 7 days starting 2 days post lesioning when neurons will be severely traumatized. 6-OHDA lesioning was associated with an increase in microglia activation and in numbers of MMP-3 immunoreactive cells which was attenuated by pioglitazone and GW855266X. Neuroprotective effects were not replicated using the PPARδ agonist GW610742X. Subsequent withdrawal of both Pioglitazone and GW855266X, for a further 7 days negated any neuroprotective effect suggesting that long-term administration may be required to attenuate the inflammatory response.
Conclusions and implications: For the first time a partial PPAR-γ agonist has been shown to be neuroprotectory when administered post lesioning in a Parkinsonian model. Effects may be via the inhibition of microglial and MMP activation and support further research.

1. Background

Parkinson's disease (PD) is an age-related neurodegenerative disease, affecting 1–3% of the population over 50 years of age. Pathologically, the disease is primarily characterized by a progressive degeneration of the dopaminergic (DA) neurons of the substantia nigra pars compacta (SNc) resulting in motor symptoms such as bradykinesia, muscle rigidity, resting tremor and impairment of postural reflexes. At the present time there are no treatment strategies available that can stop or at least slow down the neurodegeneration process in PD. The degeneration of SNc DA neurons in PD is thought to result from a complex interplay of factors including oxidative stress, mitochondrial dysfunction and defects in the ubiquitin-proteasome

Abbreviations: HPLC-ECD, High performance liquid chromatography-Electrochemical detection; 6-OHDA, 6-hydroxydopamine; *mfb*, medial forebrain bundle; PD, Parkinson's disease; SNc, substantia nigra pars compacta; PPAR, Peroxisome proliferator-activated receptor; MMP, matrix metalloproteinase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP+, 1-methyl-4-phenylpyridinium; MAO-B, monoamine oxidase B, TNFα, tumor necrosis factor; iNOS, inducible nitric oxide synthase.

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system (Jenner and Olanow, 1998). However, recent evidence suggests that inflammation and microglial activation may also play a role in the neurodegenerative process in PD (Nguyen et al., 2002). Indeed, several studies have demonstrated that neuronal cell loss in the SNc in PD is accompanied by an inflammatory response with a proliferation of reactive microglial cells (McGeer et al., 1988; Mirza et al., 2000). In such PD patients, higher levels of tumor necrosis factor alpha (TNF-α), interleukin-1ß (IL-1ß) and interferon gamma (INF-γ) were detected in the striatum and cerebrospinal fluid (Mogi et al., 1994, 1996). Microglia, have also been reported to be locally activated not only in brains of patients with idiopathic PD (McGeer et al., 1988) but also upon 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) intoxication of humans (Langston et al., 1999). Activated microglia are also observed in the SNc and striatum in animal models of PD, such as MPTP (Kurkowska-Jastrzebska et al., 1999) lipopolysaccharide (LPS), rotenone (Gao et al., 2002) and medial forebrain bundle (*mfb*) axotomy (Sugama et al., 2003; Cho et al., 2003, 2006) and mfb 6-OHDA lesions (Marinova, 2009).

Peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear hormone receptor that was first reported to regulate glucose and lipid metabolism (Schoonjans et al., 1996; Vamecq and Latruffe, 1999). Subsequently, the PPARγ agonist pioglitazone, has been shown to have anti-inflammatory (Clark, 2002), matrix metalloproteinase (MMP) inhibitor properties (Hetzel et al., 2003; Zafiriou et al., 2005) and has neuroprotective effects in various experimental models of neurodegeneration, in which inflammation is implicated (Heneka et al., 2001; Breidert et al., 2002; Schutz et al., 2005). In vitro studies have demonstrated that PPAR-γ agonists act by blocking the differentiation of monocytes into macrophages (Ricote et al., 1998), the activation of microglia (Combs et al., 2000) and inhibition of the formation of cytotoxic factors from microglia. Previously, it was reported that pioglitazone and rosigltazone administered prior to and/or at the same time as MPTP intoxication, attenuated glial activation and prevented the DA cell loss in the SNc (Breidert et al., 2002; Schintu et al., 2009). However, pioglitazone had little effect on MPTP-induced dopamine depletion and no effect on the loss of tyrosine hydroxylase (TH) immunoreactivity, as a marker of dopaminergic terminals, and glial response in the striatum. This suggested that PPAR-γ agonist protects dopaminergic cell bodies but not terminals. More recently, another study in the mouse MPTP model demonstrated that neuroprotection observed with pioglitazone pretreatment was due to blockade of MPTP bioconversion to its active toxic metabolite MPP+, via inhibition of monoamine oxidase B (MAO-B) (Quinn et al., 2008) and not acting as an anti-inflammatory agent. However, recently another study has demonstrated the neuroprotective effects of the PPARγ agonist rosiglitazone when administered starting 24 days into the 30 day treatment strategy in the progressive MPTP plus probenecid model of PD (Carta et al., 2011) again supporting the potential neuroprotective effects of PPARγ agonists in PD.

Another member of the PPAR receptor family, PPARδ, has been reported to be the predominant PPAR isoform expressed in the CNS, relative to PPARα and PPARγ, and PPARδ mRNA is down regulated in neurodegenerative diseases such as Alzheimer's disease (de la Monte et al., 2006). A number of pre-clinical studies have confirmed the disease-modifying effects of PPARδ agonists within models of neuroinflammation, cognitive impairment and neurodegeneration. For example, L-165041 and GW501516 have been shown to attenuate the dopamine depletion and metabolite concentrations in the striatum following MPTP treatment to mice, suggesting that PPARδ agonists may be utilized in a number of neurodegenerative diseases, including PD (Iwashita et al., 2007).

These studies demonstrated for the first time that a partial and full PPAR-γ agonist, but not a PPARδ agonist, has neuroprotective properties when administered post lesioning in a 6-OHDA PD model thus indicating that PPAR-γ agonist can rescue degenerating neurons. Such effects may be mediated via the inhibition of microglial activation and MMP expression.

2. Materials and methods

2.1. Experimental design

The following agonists, developed at GlaxoSmithKline (GSK) were used: a partial PPAR-γ agonist (GW855266X; Spearing et al., 2008) and a PPAR-δ full agonist (GW610742X; Sznaidman et al., 2003). Pioglitazone hydrochloride, a full PPAR-γ agonist, was synthesized by ChemPacific (Baltimore, USA) and used as a reference compound. Dose selection for the present studies was based on the insulin-sensitizing profile achieved in animal models of diabetes or pharmacodynamic data generated with each of these compounds at GSK and from other studies utilizing PPAR agonist in animal models of PD (Schintu et al., 2009). Both GW855266X and GW610742X are potent PPARγ partial and PPARδ full agonists respectively (low nM range), with excellent selectivity over other PPAR isoforms (up to 1000 fold) and which exhibit good oral bioavailability in rodents (unpublished data). All PPAR agonists were dissolved in 0.5% methyl cellulose and administered orally via gavage twice daily.

In the first set of experiments, animals divided into groups (n = 6) were treated with pioglitazone (20 mg/kg), GW855266X (15 mg/kg), GW610742X (10 mg/kg), or drug vehicle for 14 consecutive days. The treatment was commenced 7 days before 6-OHDA lesion induction and continued for further 7 days post lesioning, similar to that used by Breidert et al. (2002) in the mouse MPTP model. Upon achieving encouraging results with some of the compounds, the compounds were administered using a more clinically relevant treatment regime i.e. after 6-OHDA lesion induction to see whether they can rescue degenerating neurons. Treatments with pioglitazone (20 mg/kg) or GW855266X (15 mg/kg) was given orally twice daily for 7 consecutive days starting 2 days after 6-OHDA lesion induction and animals were sacrificed on day 9. Treatment was initiated 2 days after the 6-OHDA lesion since this is the time point at which significant microglial activation begins to take place in our model (Marinova-Mutafchieva et al., 2009). In order to establish whether the neuroprotective effect of PPAR-γ agonists were long lasting the following experiment was carried out. Two days after the 6-OHDA lesioning, PPAR γ agonists pioglitazone (20 mg/kg) or GW855266X (15 mg/kg) or drug vehicle were administered twice daily for 7 days. 50% of the animals were sacrificed at the end of the 7 days of treatment while the remainder sacrificed after a further 7 days off drug.

2.2. Test systems used

Male Sprague–Dawley rats (Harlan, UK) weighing 220–240 g, were housed under a controlled temperature (21 + 1 °C), relative humidity (60%) and a 12 hour light/dark cycle (light on at 8.00 am) with free access to food and water. All scientific procedures were carried out in accordance Home Office Animals (Scientific procedures) Act, UK, 1986. 12 μg 6-hydroxydopamine hydrobromide (6-OHDA, free base, Sigma-Aldrich Ltd. UK) was injected in a total volume of 4 μl into the left *mfb* in isoflurane (Abbot, UK) anesthetized rats as previously described by the authors (Datla et al., 2006) using coordinates of 2.2 mm posterior, 1.5 mm lateral from bregma and 7.9 mm ventral to the dura, according to Paxinos and Watson (1986).

2.3. Assessment of the integrity of the nigrostriatal tract and degree of microglial activation

At the end of the study period rats were sacrificed, the brain was removed and cut at the level of the infundibular stem on a chilled platform, forming a hindbrain portion containing the SNc and a forebrain portion containing the striatum. The left and right striata were dissected out from the forebrain block and snap frozen. The hindbrain block was transferred to 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS), pH 7.4 and kept for 7 days, followed by