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Research report

Neuroprotective effects of peroxisome proliferator-activated receptor alpha and gamma agonists in model of parkinsonism induced by intranigral 1-methyl-4-phenyl-1,2,3,6-tetrahyropyridine

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HIGHLIGHTS

- Pioglitazone and fenofibrate protected against behavioral impairments caused by MPTP.
- Both drugs protected against dopaminergic neurons death caused by MPTP.
- Both drugs protected against the increase of caspase-3 in SNpc caused by MPTP.

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ABSTRACT

A large body of evidence suggests that peroxisome proliferator-activated receptor (PPAR) agonists may improve some of the pathological features of Parkinson's disease (PD). In the present study, we evaluated the effects of the PPAR-α agonist fenofibrate (100 mg/kg) and PPAR-γ agonist pioglitazone (30 mg/kg) in a rat model of parkinsonism induced by intranigral 1-methyl-4-phenyl-1,2,3,6-tetrahyropyridine (MPTP). Male Wistar rats were pretreated with both drugs for 5 days and received an infusion of MPTP. The experiments were divided into two parts. First, 1, 7, 14, and 21 days after surgery, the animals were submitted to the open field test. On days 21 and 22, the rats were subjected to the forced swim test and two-way active avoidance task. In the second part of the study, 24 h after neurotoxin administration, immunohistochemistry was performed to assess tyrosine hydroxylase activity. The levels of dopamine and its metabolites in the striatum were determined using high-performance liquid chromatography, and fluorescence detection was used to assess caspase-3 activation in the substantia nigra pars compacta (SNpc). Both fenofibrate as pioglitazone protected against hypolocomotion, depressive-like behavior, impairment of learning and memory, and dopaminergic neurodegeneration caused by MPTP, with dopaminergic neuron loss of approximately 33%. Fenofibrate and pioglitazone also protected against the increased activation of caspase-3, an effector enzyme of the apoptosis cascade that is considered one of the pathological features of PD. Thus, PPAR agonists may contribute to therapeutic strategies in PD.

1. Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative disorders, second only to Alzheimer's disease, in industrialized countries, with a prevalence of approximately 0.3% of the population [1]. It is characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), degeneration of dopaminergic and non-dopaminergic neurons, and presence of Lewy bodies [2,3]. Impaired motor function

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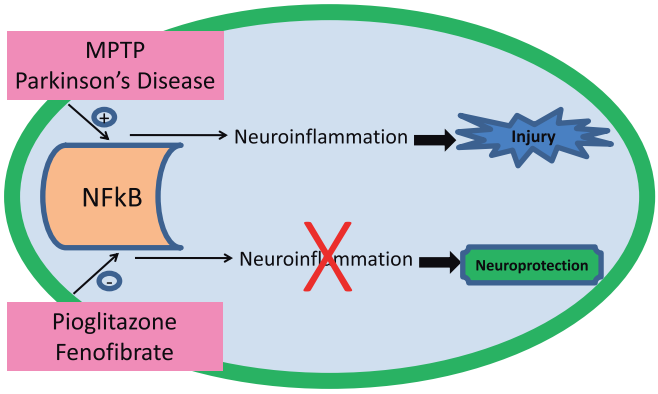

Fig. 1. The possible neuroprotective mechanism of action of fenofibrate and pioglitazone. Both MPTP and Parkinson's disease activate NF-κB, which is involved in neuroinflammation that leads to injury. Fenofibrate and pioglitazone inhibit the activation of NF-κB, consequently preventing the occurrence of neuroinflammation and exert neuroprotective effects.

is classically used to make a clinical diagnosis of PD. The main features include bradykinesia, rigidity, tremor, and postural instability, with an asymmetric onset that spreads to become bilateral with time [1,4]. Importantly, PD is also associated with numerous debilitating non-motor features, such as cognitive deficits that range from memory impairment to dementia, emotional changes (*e.g.*, depression and anxiety), sleep perturbations, autonomic dysfunction, and gastrointestinal symptoms [5–8]. Depression is one of the most common of these symptoms, occurring in approximately 35% of patients [9]. The causes of neuronal death in PD remain unclear. Many mechanisms of neuronal death in PD have been proposed, including the formation of free radicals, oxidative stress, mitochondrial dysfunction, excitotoxicity, trophic factor deficiency, inflammatory processes, genetic factors, environmental factors, the toxic action of nitric oxide, and apoptosis. All of these factors interact with each other, inducing a vicious cycle of toxicity that leads to neuronal dysfunction, atrophy, and finally cell death [10,11]. The currently available treatments for PD comprise palliative symptomatic therapies [3,12]. Pharmacological interventions seek to increase dopamine (DA) levels through the increased production of DA or inhibition of DA metabolism by orally administering dopamine precursor levodopa, although many patients develop levodopa-induced dyskinesias and motor fluctuations [3,13]. Peroxisome proliferator-activated receptors (PPARs) are ligand-dependent transcription factors. The activation of the PPAR-γ subtype by an agonist, such as pioglitazone, increases insulin sensitization and modulates glucose and lipid metabolism. It is currently approved as an oral monotherapy and adjunctive therapy for patients with type 2 diabetes mellitus [14–16]. PPAR-α is activated by various hypolipidemic fibrates, such as fenofibrate [17,18], which are lipid-lowering drugs that are used for patients with hypercholesterolemia and hypertriglyceridemia [19]. PPARs are able to regulate inflammatory pathways (Fig. 1) through the transrepression of transcription factors (*e.g.*, nuclear factor-κB [NF-κB]), which is crucial for inflammation reactions [20] and regulates the expression of apoptotic genes [21]. PPAR-α activation induces the expression and activation of antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase. With regard to inflammatory pathways, PPAR-α activation prevents the synthesis and release of cytokines (*e.g.*, interleukin-6 and tumor necrosis factor-α). PPAR-γ activation also reduces the expression of inducible nitric oxide synthase and cyclooxygenase-2 (COX-2) and production of proinflammatory cytokines. These mechanisms explain how PPAR activation induced by synthetic ligands reduces inflammation in different tissues. Postmortem analyses have provided evidence of the activation of NF-κB in PD [22,23]. Furthermore, neurotoxins, such as 1-methyl-4-phenyl-1,2,3,6-tetrahyropyridine (MPTP), rotenone, and 6-hydroxydopamine (6-OHDA), are used in experimental models of PD and stimulate the activation of NF-κB [24–26] (Fig. 1). MPTP induces the selective but widespread loss of striatal DA through the selective retrograde degeneration of SNpc cells [27], which can reduce striatal DA levels by up to 70% in mice and monkeys [28]. In the present study, we compared the effects of pretreatment with the PPAR-γ agonist pioglitazone and PPAR-α agonist fenofibrate for 5 days before performing bilateral intranigral infusions of MPTP. We performed behavioral tests (*i.e.*, open field test to determine motor activity, two-way active avoidance task to assess memory, and forced swim test [FST] to assess depressive-like behavior). We also examined the levels of DA and its metabolites using high-performance liquid chromatography (HPLC). Immunohistochemistry was performed to determine tyrosine hydroxylase (TH) activity. To assess the participation of apoptosis in the mechanism of neuronal death in the MPTP model of PD, fluorescent detection was used to determine caspase-3 activation. This evaluation was important for determining whether fenofibrate and pioglitazone inhibit toxin-induced neuronal death in the PD model.

2. Methods

2.1. Animals and treatments

All of the experiments were performed in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animals, United States National Institutes of Health. The protocol complied with the recommendations of the Federal University of Paraná and was approved by the Institutional Ethics Committee (protocol no. 470). The animals were group-housed (10 per cage) in a temperature-controlled room (22 ± 2 °C) with a 12 h/12 h light/dark cycle. Food and water were freely available in the home cage. After delivery, the animals underwent a 7-day habituation period with no handling.

2.1.1. Rats

Male three-month-old Wistar rats, weighing 280–320 g, were used in the experiments. The rats received a bilateral intracerebral injection of saline solution ("vehicle rats") or 100 μg MPTP (Sigma–Aldrich, St. Louis, MO, USA) in 1 μl of 0.9% sterile saline ("MPTP rats"). Pioglitazone (30 mg/kg) or fenofibrate (100 mg/kg) in a vehicle solution that contained 1% carboxymethylcellulose (CMC) was administered by oral gavage once per day for 5 days before MPTP or vehicle infusions. Six different groups were formed: CMC + sham (n = 8–10, vehicle rats infused with vehicle), CMC + MPTP (n = 8–10, vehicle rats infused with MPTP), pioglitazone + sham (n = 8, pioglitazone rats infused with vehicle), pioglitazone + MPTP (n = 8–10, pioglitazone rats infused with MPTP), fenofibrate + sham (n = 8–10, fenofibrate rats infused with vehicle), and fenofibrate + MPTP (n = 8–10, fenofibrate rats infused with MPTP). One day after surgery, some of the animals were euthanized, and their brains were dissected to determine caspase-3 in the SNpc, perfused to immunohistochemically to determine TH or were decapitated to dissect the striatum and evaluate DA and its metabolites. One, 7, 14, and 21 days after surgery, motor behavior was assessed in the open field test. The FST and two-way active avoidance test were conducted 21 days (training session) and 22 days (test session) after MPTP administration.

2.1.2. Surgery and MPTP injections

Rats were anesthetized with equitesin (chlornembutal, 0.3 ml/kg, i.p.). Bilateral infusions of MPTP HCl (100 μg in 1 μl