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Differential susceptibility to the PPAR-γ agonist pioglitazone in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 6-hydroxydopamine rodent models of Parkinson's disease

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ABSTRACT

A growing body of evidence suggests that peroxisome proliferator-activated receptor (PPAR) agonists are valuable candidates as disease modifiers in Parkinson's disease (PD) and may thus enable neuroprotection and preserve motor function. The present study sought to evaluate the effect of the PPAR-gamma agonist pioglitazone in two different animal models of PD. The study was based on nigral dopaminergic neuron labelling and the assessment of motor behaviour in (i) the frequently investigated MPTP mouse model and (ii) the less well-known bilateral 6-OHDA rat model.

In MPTP-injected mice, pioglitazone reversed body weight loss and the reduction in rearing frequency and induced significant neuroprotection of the nigrostriatal dopaminergic pathway (by 24%, compared with vehicle). In contrast, pioglitazone did not have any effect on the 73.5% loss of dopaminergic neurons or motor impairments (a reduced rearing frequency and a loss of strength in the forepaws) in bilateral 6-OHDA rats.

The PPAR-gamma agonist pioglitazone had a significant neuroprotective effect in MPTP mice but not in bilateral 6-OHDA rats. The various effects of PPAR agonists in both models can be accounted by the different action mechanism of the 2 toxins or by the fact that 3 μg 6-OHDA injection was too harmful to be alleviated by the compound. This work supports PPAR-agonists to be relevant in the therapeutic strategy research in Parkinson's disease and highlights the importance in evaluating neuroprotective agent in different models.

1. Introduction

Given that the aetiology of Parkinson's disease (PD) is still not known, the drugs currently used in the clinic are mainly symptomatic in nature. Hence, preclinical research is now focusing on potentially disease-modifying compounds that could prevent (or at least slow) the degeneration of nigrostriatal dopaminergic neurons – the hallmark of PD. The cause of this neurodegeneration has not been fully elucidated; however, increased oxidative stress, inflammation, excitotoxicity, mitochondrial dysfunction and apoptosis have all been implicated as mechanisms that can mediate cell death in PD. In the last few years, peroxisome proliferator-activated receptors (PPARs) have been identified as promising targets for inducing neuroprotection in neurological diseases [1]. The PPAR isoforms α, β, and γ are ligand-activated transcription factors [2] that regulate cell functions such as lipid and glucose metabolism, cell growth, differentiation and inflammation [2–5]. In particular, PPAR-γ agonists have attracted significant scientific interest as agents that can protect against inflammation, oxidative stress and apoptosis [6]. As such, these compounds have been investigated in many different central nervous system diseases [7] and have shown neuroprotective activity in multiple sclerosis [8], cerebral ischemia [9–12] and Alzheimer's disease (for a review, see [13]). Gamma PPAR isoforms were found to be highly expressed in some regions of the brain – including the substantia nigra *pars compacta* (SNc) and the striatum [14] – and thus also constitute a potentially valuable drug target in PD. Pioglitazone (a synthetic PPAR-γ agonist from the thiazolidinedione family) has already been evaluated in this respect and was found to reduce nigral dopaminergic degeneration [15–18] and to limit motor impairments [17,19,20] in various animal models of PD.

Abbreviations: MAO-B, monoamine oxidase-B; PPAR, peroxisome proliferator-activated receptor; SNc, substantia nigra *pars compacta*; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA, 6-hydroxydopamine; VTA, ventral tegmental area; TH, tyrosine hydroxylase.

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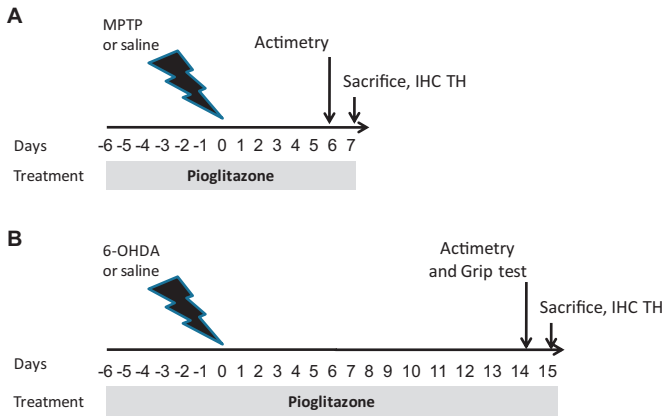

Fig. 1. Study design in mice (A) and rats (B). MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA: 6-hydroxydopamine.

It is important to use several different animal models to screen for pharmacologically active compounds. By investigating different species and different ways of inducing disease-like states, a drug's therapeutic effect can be validated more robustly. The PPAR-α agonist fenofibrate has already been tested in two rodent models of PD. It was found to have neuroprotective potential for the dopaminergic nigrostriatal pathways in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mouse model but, surprisingly, was less effective in a unilateral 8 μg 6-hydroxydopamine (6-OHDA)-injected rat model [21]. As mentioned above, the PPAR-γ agonist pioglitazone can also prevent MPTP-induced neurodegeneration in mice [15–17] but, to the best of our knowledge, has not yet been tested in 6-OHDA rats.

In the present study, we decided to observe and compare the effects of the PPAR-γ agonist pioglitazone on motor activity and nigral dopaminergic neuron loss in MPTP-treated mice and the bilateral 6-OHDA rat model. Although there are many different MPTP mouse models, the acute model used in the present study has been widely studied and extensively described in the literature. The unilateral 6-OHDA rat model is also widely used but its strictly unilateral nature has a number of disadvantages, since the undamaged hemisphere can exert compensatory effects and thus interfere with the results [22–26]. Likewise, in pharmacological studies, it is difficult to determine whether a compound's effects are due to a neuroprotective effect on the damaged side or potentiation of neuronal activity on the undamaged side. Moreover, bilateral lesions more closely approximate the human disease (even though the degree of damage in each hemisphere is not always equivalent in patients). Relatively symmetric neurodegeneration is of particular importance when studying the behavioural effects of a systemically injected pharmacologically active compound. Although the severe damage caused by bilateral 6-OHDA lesions and the correspondingly intensive nursing care have discouraged extensive investigation of this model [27,28], we decided to re-examine in the present context.

2. Methods

The study design is shown in Fig. 1.

2.1. Animals and treatments

All experiments were carried out in accordance with the "Principles of Laboratory Animal Care" (NIH publication 86-23, revised in 1985) and the current French and European Union legislative and regulatory frameworks on animal experiments (The Council of the European Communities Directive 86/609). Animals were group-housed (10 per cage) in a temperature-controlled room (22 ± 2 °C) with a 12/12-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. Mice

Five-month-old male C57Bl/6J mice (Elevage Janvier, Le Genest St Isle, France) weighing 28–30 g were used in the experiments. The mice received four intraperitoneal injections (with 2 h intervals) of a saline solution containing 0 or 20 mg/kg of MPTP ("saline mice" and "MPTP mice", respectively) (Sigma–Aldrich, St Louis, MO, USA). Pioglitazone (50 mg/kg in a vehicle solution containing 1% carboxymethylcellulose and 0.01% Tween) was administrated by oral gavage once a day for 14 days (starting 6 days before the MPTP or saline injections and continuing for 7 days thereafter) based on the administration protocol of a previous work [21,33]. Three different groups were set up: the saline-vehicle group ($n = 8$, saline mice treated with vehicle), the MPTP-vehicle group ($n = 10$, MPTP mice treated with vehicle) and the MPTP-pioglitazone group ($n = 11$, MPTP mice treated with pioglitazone). Body weight was measured at the start of the experiment (D-6) and at the end of treatment period, before sacrifice (D7).

2.1.2. Rats

Six-week-old male Wistar rats (Elevage Janvier, Le Genest St Isle, France) weighing 200–220 g were used in the experiments. The rats received a bilateral, intracerebral injection of saline solution containing 0.05% ascorbic acid ("vehicle rats") or 3 μg of 6-hydroxydopamine in 0.05% ascorbic acid ("6-OHDA rats") (Sigma–Aldrich, St Louis, MO, USA). Pioglitazone (50 mg/kg in a vehicle solution containing 1% carboxymethylcellulose and 0.01% Tween) was administrated by oral gavage once a day for 21 days (starting 6 days before 6-OHDA or vehicle injections and continuing for 14 days thereafter) based on the administration protocol of a previous work [21]. Three different groups were set up: the vehicle–vehicle group ($n = 8$, vehicle rats treated with vehicle), the 6-OHDA–vehicle group ($n = 8$, 6-OHDA rats treated with vehicle) and the 6-OHDA–pioglitazone group ($n = 6$, 6-OHDA rats treated with pioglitazone). Body weight was measured at the start of the experiment (D-6), on the day of surgery (D0) and at the end of the treatment period, before sacrifice (D15).

2.2. Surgery on rats and 6-OHDA injections

Rats were anaesthetized with chloral hydrate (300 mg/kg, Sigma–Aldrich) and placed in a stereotaxic frame. Briefly, after incision of the scalp and skull drilling, 3 μg of 6-OHDA was infused (over a 4-min period) into the left and then the right median forebrain bundle, using a Hamilton syringe. The stereotaxic co-ordinates provided by the Paxinos and Watson brain atlas [29] were adapted to fit young animals, as described previously [30]. After the infusions, the scalp was sutured and animals were allowed to recover under a warm lamp until waking. Daily post-surgery care was performed throughout the experiment.

2.3. Behavioural assessments

2.3.1. Actimetry

The spontaneous motor activity of mice and rats was recorded over a 10-min period in an actimeter (Panlab, Barcelona, Spain). The apparatus was a 45 cm × 45 cm × 35 cm transparent Plexiglas enclosure equipped with two frames of infrared beams for measuring horizontal motor activity (distance travelled, speed and type of movement) and vertical motor activity (rearing). The chosen