Effect of the 5α-reductase enzyme inhibitor dutasteride in the brain of intact and parkinsonian mice

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A B S T R A C T

Dutasteride is a 5alpha-reductase inhibitor in clinical use to treat endocrine conditions. The present study investigated the neuroprotective mechanisms of action of dutasteride in intact and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned mice using a low dose of MPTP not affecting motor activity modeling early stages of Parkinson's disease (PD). We hypothesized that dutasteride neuroprotection is due to altered steroids levels. Dutasteride pre-treatment prevented loss of striatal dopamine (DA) and its metabolite DOPAC. Dutasteride decreased effects of MPTP on striatal dopamine transporter (DAT), vesicular monoamine transporter 2 (VMAT2) and D2 DA receptor specific binding while D1 receptor specific binding remained unchanged. Dutasteride enhanced DAT specific binding and the glycosylated form of DAT in intact mice. MPTP-lesioned mice had plasma and brain testosteron levels and dihydrotestosterone levels lower than control mice whereas progesterone and its metabolites (dihydroprogesterone, isopregnanolone and tetrahydroprogesterone) pathway showed increases. Dutasteride treatment by inhibiting transformation of progesterone and testosterone to its metabolites elevated plasma and brain concentrations of testosterone compared to MPTP mice and decreased DHT levels in intact mice. Plasma and brain estradiol levels were low and remained unchanged by MPTP and/or dutasteride treatment. Dutasteride treatment did not affect striatal phosphorylation of Akt and its downstream substrate GSK3β as well as phosphorylation of ERK1/2 in intact and MPTP lesioned MPTP mice. Striatal glial fibrillary acidic protein (GFAP) levels were markedly elevated in MPTP compared to control mice and dutasteride reduced GFAP levels in MPTP mice. Treatment with dutasteride post-lesion left unchanged striatal DA levels. These results suggest dutasteride as promising drug for PD neuroprotection.

1. Introduction

It is well documented that Parkinson's disease (PD) starts several years before its diagnosis, the disease becoming noticeable when motor symptoms such as bradykinesia, rigidity, resting tremor, and postural instability appear \cite{1}. Neuropathological hallmarks of PD are the selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) resulting in depletion of striatal dopamine (DA) and presence of intraneuronal protein inclusions composed mainly of alpha-synuclein known as the Lewy bodies \cite{2}. In the early stages of PD, patients do not present clinical symptoms but when they emerge, about 50–70% of the dopaminergic neurons have degenerated \cite{1}.

Treatments available for PD are only symptomatic and as disease progresses, their beneficial effects are eventually hampered by the emergence of drug-resistant symptoms. These include various motor complications such as Levodopa (L-DOPA)-induced dyskinesias (LID), limiting the quality of life of PD patients; these motor complications can be very difficult to manage \cite{3}.

Therefore it appears necessary to target neuronal death in order to halt, delay or slow disease progression. Accordingly, effective neuroprotective or disease-modifying therapies should be initiated in the presymptomatic phase of the disease.

\textsuperscript{Abbreviations:} 3α-diol, 5α-androstane-3α, 17β-diol; 3β-diol, 5α-androstane-3β, 17β-diol; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; DA, dopamine; DAT, dopamine transporter; DHEA, dehydroepiandrosterone; DHP, 5α-dihydroprogesterone; DHT, dihydrotestosterone; DOPAC, 3,4-dihydroxyphenylacetic acid; ERK, extracellular signal-regulated kinase; GFAP, glial fibrillary acidic protein; GSK3β, glycogen synthase kinase 3β; HPLC, high performance liquid chromatography; HVA, homovanillic acid; Iba1, ionized calcium binding adapter molecule 1; ISOPREG, isopregnanolone; L-DOPA, Levodopa; LID, Levodopa-induced dyskinesias; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson’s disease; PROG, pregnenolone; PREG, pregesterone; PVDF, polyvinylidine difluoride; RIPA, radioimmunoprecipitation assay; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase; TPH, tetrahydroprogesterone or allopregnanolone; VMAT2, vesicular monoamine transporter 2

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Evaluation of SNpc neurons and striatal DA terminals indicates that loss of striatal terminals exceeds loss of DA neurons in the SNpc [4]. Increasing evidence suggests that dopaminergic degeneration in PD starts in striatal DA terminals progressing retrogradely to the DA cell body in the substantia nigra (dying-back degeneration) (reviewed in [5]).

A dose of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) inducing a partial lesion was used in the present study to model early stages or prodromal PD. Under these conditions DA neurons are impaired showing more damage to terminals in the striatum with spared cell bodies in the SNpc. Our mice MPTP model focused on axons of the DA system involved early in PD offering new approaches to protective and restorative therapeutics [5].

In the present study, mice lesioned with MPTP were used to assess the neuroprotective effects of dutasteride, an inhibitor of the 5α-reductase enzyme involved in neuroactive steroids synthesis [6]. 5α-reductase is an enzyme catalyzing the saturation of the 4,5 double bond of the A ring of A4-3 ketosteroids such as testosterone and progesterone (PROG) into dihydrotestosterone (DHT) and 5α-dihydroprogesterone (DHP) respectively [7]. The 5α-reduced metabolites are involved in the regulation of different biological effects in the nervous system including neuroprotection and response to injury [7]. Disturbances in neuroactive steroid synthesis as well as changes in 5α-reductase activity are reported in plasma and cerebrospinal fluid of PD patients [6]. Modulation of 5α-reductase activity may prevent neurodegeneration by increasing the levels of the neuroprotective steroids PROG as well as 17β-estradiol via aromatization of testosterone [8]. Inhibition of 5α-reductase was reported to suppress neuroactive steroid synthesis and reversed psychotic-like effects induced by DA receptor agonists [9] as well as improved symptoms related to DA hyperactivity such as observed in Tourette syndrome [10]. Also, therapeutic effects of the 5α-reductase inhibitor finasteride were observed in patients displaying L-DOPA-induced pathological gambling [11].

We recently reported in MPTP mice that dutasteride prevented MPTP-induced DA depletion [12]. The objectives of the present study were to evaluate the mechanisms of action implicated in the neuro-modulatory and neuroprotective effects of dutasteride treatment in MPTP mice modeling early stage of PD. The dose- and time-response effects of dutasteride on striatal DA terminals in MPTP mice were evaluated. A pre-treatment with dutasteride was compared to a post-lesion treatment. We assessed markers of DA terminals integrity namely DA levels and its turnover, DA transporter (DAT) and vesicular monoamine transporter 2 (VMAT2). The activities of cellular pathways included the regulation of dihydrotestosterone (DHT) and dihydroxytestosterone (DHT) respectively [7]. The 5α-reduced metabolites are involved in the regulation of different biological effects in the nervous system including neuroprotection and response to injury [7]. Disturbances in neuroactive steroid synthesis as well as changes in 5α-reductase activity are reported in plasma and cerebrospinal fluid of PD patients [6]. Modulation of 5α-reductase activity may prevent neurodegeneration by increasing the levels of the neuroprotective steroids PROG as well as 17β-estradiol via aromatization of testosterone [8]. Inhibition of 5α-reductase was reported to suppress neuroactive steroid synthesis and reversed psychotic-like effects induced by DA receptor agonists [9] as well as improved symptoms related to DA hyperactivity such as observed in Tourette syndrome [10]. Also, therapeutic effects of the 5α-reductase inhibitor finasteride were observed in patients displaying L-DOPA-induced pathological gambling [11].

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (Charles River Canada, Montreal, QC, Canada) aged 10 weeks were used. All animals were given food and water ad libitum and housed in cages under controlled condition at 22°C with a 12 h light/12 h dark cycle. Each experiment included 7 to 15 mice per treatment group. To avoid contamination, mice that received MPTP were housed separately from saline-treated control mice. The Laval University Animal Care Committee approved these animal studies. All efforts were made to minimize animal suffering and to reduce the number of mice used.

2.2. Pre-treatment experiment

For the pre-treatment experiment, 63 mice were included following the same acute MPTP model as previously used [12] to induce a decrease of approximately 50% of striatal DA contents as well as in DAT- and VMAT2-specific binding in the striatum without affecting tyrosine hydroxylase (TH) expression in the substantia nigra. For the pre-treatment experiment, groups of mice received treatments with dutasteride (5 or 12.5 mg/kg once daily intraperitoneal (i.p.)) or vehicle (0.9% saline with 0.3% gelatin, once daily i.p.) for 10 days. On day 5, mice received 4 i.p. injections of MPTP (5.5 mg/kg) at 2-h intervals, whereas the control group received a saline solution. On day 11, mice were decapitated, and the brains were quickly removed and frozen in isopentane (−40°C). The doses of dutasteride (5 and 12.5 mg/kg) investigated in the present study were as in our previous experiments [12] and lower than those reported in some brain studies. Hence, dutasteride was investigated at doses not to affect motor behavior. Indeed, finasteride (60 or 100 mg/kg i.p.) and dutasteride (40 or 80 mg/kg i.p.) were reported to reduce hyperlocomotion induced by D-amphetamine and attenuate stereotyped behaviors induced by the DA agonist apomorphine [9].

2.3. Motor behavior experiment

Thirty mice were used to assess the effect of the acute MPTP regimen and dutasteride treatment on motor behavior. They were separated in three groups of mice namely control, MPTP and MPTP + dutasteride 12.5 mg/kg and were lesioned and treated as in the pre-treatment experiment. Motor coordination and postural stability were estimated with the open-field test and the rotarod test (Rotarod LE8200, Panlab, Harvard apparatus, USA). Mice were first evaluated with the open-field test. The animals were gently placed in the center of the open-field and were allowed to freely explore the area for 5 min. The parameters quantified with this test were the mean speed and the distance travelled. The open-field was washed with a 5% water–ethanol solution before behavioral testing to eliminate possible bias due to odors left by previous mice.

The experimental design with the rotarod consisted of 3 consecutive trials of 1 min in which mice learned to remain on the rod (30 mm diameter). This was followed by a second session where mice were placed on top of the beam revolving at an initial speed of 4 rpm in each 3-min trial. The body orientation was opposite to beam movement in the longitudinal axis, so that forward locomotion was necessary to avoid a fall. Latencies before falling were measured in one 3-trial session with a 15-min inter trial interval. The mean of scores was calculated.

2.4. Post-treatment experiment

For the post-treatment experiment, 57 mice were used. Mice received 4 intraperitoneal injections of MPTP (5.5 mg/kg) at 2-h intervals, whereas the control group received saline solution. Three doses of dutasteride (5, 12.5 and 25 mg/kg) were investigated in this experiment. One hour after the first MPTP injection, mice received one intraperitoneal injection of dutasteride then one hour after the last injection of MPTP they received another intraperitoneal injection of dutasteride. During the following 5 days, these mice received one daily intraperitoneal injection of dutasteride. On day 6, mice were decapitated, and the brains were quickly removed and frozen in isopentane (−40°C).

2.5. Preparation of brain tissue and assay of biogenic amines contents

The left anterior striata were dissected and prepared for assay of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) as well as serotonin (5-HT) and its metabolite...