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Levodopa induces long-lasting modification in the functional activity of the nigrostriatal pathway

Mario Riverol [a,b], Cristina Ordóñez [b], María Collantes [c,d], Carla DiCaudo [b], Iván Peñuelas [c,d], Javier Arbizu [d], Irene Marcilla [b], María R. Luquin [a,b,*]

[a] Department of Neurology, Clínica Universidad de Navarra, Pamplona, Spain
[b] Laboratory of Regenerative Therapy, Center for Applied Medical Research (CIMA), Pamplona, Spain
[c] Small Animal Imaging Research Unit, Center for Applied Medical Research (CIMA), Clínica Universidad de Navarra, Pamplona, Spain
[d] Department of Nuclear Medicine, Clínica Universidad de Navarra, Pamplona, Spain

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ABSTRACT

Much controversy exists concerning the effect of levodopa on striatal dopaminergic markers in Parkinson's disease (PD) and its influence on functional neuroimaging. To deal with this issue we studied the impact of neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and chronic levodopa administration on striatal ^{18}F-DOPA uptake (Ki) in an animal model of PD. The levels of several striatal dopaminergic markers and the number of surviving dopaminergic neurons in the substantia nigra (SN) were also assessed. Eleven *Macaca fascicularis* were included in the study. Eight animals received weekly intravenous injections of MPTP for 7 weeks and 3 intact animals served as controls. MPTP-monkeys were divided in two groups. Group I was treated with placebo while Group II received levodopa. Both treatments were maintained for 11 months and then followed by a washout period of 6 months. ^{18}F-DOPA positron emission tomography (PET) scans were performed at baseline, after MPTP intoxication, following 11 months of treatment, and after a washout period of 1, 3 and 6 months. Monkeys were sacrificed 6 months after concluding either placebo or levodopa treatment and immediately after the last ^{18}F-DOPA PET study. Striatal dopamine transporter (DAT) content, tyrosine hydroxylase (TH) content and aromatic L-amino acid decarboxylase (AADC) content were assessed. In Group II ^{18}F-DOPA PET studies performed at 3 and 6 months after interrupting levodopa showed a significantly increased Ki in the anterior putamen as compared to Group I. Levodopa and placebo treated animals exhibited a similar number of surviving dopaminergic cells in the SN. Striatal DAT content was equally reduced in both groups of animals. Animals in Group I exhibited a significant decrease in TH protein content in all the striatal regions assessed. However, in Group II, TH levels were significantly reduced only in the anterior and posterior putamen. Surprisingly, in the levodopa-treated animals the TH levels in the posterior putamen were significantly lower than those in the placebo group. AADC levels in MPTP groups were similar to those of control animals in all striatal areas analyzed. This study shows that chronic levodopa administration to monkeys with partial nigrostriatal degeneration followed by a washout period induces modifications in the functional activity of the dopaminergic nigrostriatal pathway.

Introduction

Radiotracer imaging techniques such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT) are able to assess *in vivo* the functionality of the presynaptic nigrostriatal dopaminergic system. 3,4-Dihydroxy-6-18F-fluoro-L-phenylalanine (^{18}F-DOPA), a PET tracer that measures the DOPA decarboxylase activity, and 2β-carbomethoxy-3β-(4-iodophenyl)tropane (^{123}I-β-CIT), a SPECT tracer that binds to the dopamine transporter (DAT), are the most widely used tracers in clinical practice. These two procedures seem to be very precise in the evaluation of the activity and integrity of the dopaminergic system and both have been considered useful tools for assessing disease progression, particularly in cases when drugs with potential neuroprotective effects are used (Maetzler et al., 2009). In fact, postmortem studies in humans and animals have shown that striatal ^{18}F-DOPA uptake correlates with both the number of nigral dopaminergic cells and the striatal dopamine levels (Pate et al., 1993; Snow et al., 1993). On the other hand, longitudinal studies in PD patients have shown a progressive reduction of the striatal uptake of these radiotracers along the evolution of the disease (Bruck et al., 2006; Hilker et al., 2005; Morrish et al., 1996; Nandhagopal et al., 2009; Nurmi et al., 2001; Vingerhoets et al., 1994), suggesting that functional neuroimaging techniques could also serve as a tool in the assessment of PD progression.

* Corresponding author at: Department of Neurology and Neuroscience Division, Clínica Universidad de Navarra and Center for Applied Medical Research (CIMA), Avenida de Pío XII, 36, 31008 Pamplona, Spain. Fax: +34 948 29 65 00.
E-mail address: rluquin@unav.es (M.R. Luquin).
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Two clinical trials, the CALM-PD and the REAL-PET studies, evaluated the possible neuroprotective effect of current symptomatic drugs in PD based on the changes produced in the striatal ^{123}I-β-CIT and ^{18}F-DOPA uptake respectively (Parkinson Study Group, 2002; Whone et al., 2003). Both studies suggested that either dopaminergic agonists slow down the loss of dopaminergic terminals, or levodopa accelerates the underlying neurodegenerative process. The ELLDOPA study, a levodopa placebo-controlled trial, was later conducted to evaluate the effect of levodopa on the rate of progression of PD (Fahn et al., 2004). The progression of PD was assessed by changes in the UPDRS scale, and in a reduced number of patients, by changes in the striatal ^{123}I-β-CIT uptake. Surprisingly, the results were contradictory. The clinical outcome suggested that levodopa could halt the progression of the disease, while the neuroimaging findings demonstrated a more progressive decline in the striatal tracer uptake in the levodopa-treated group than in the placebo group. The divergence between the clinical and imaging outcomes has been explained by the modification that levodopa and the progression of the disease could induce together in the targets of the radiotracers used. Accordingly, a non-progressive animal model of PD could be a good tool for evaluating whether levodopa by itself is able to modulate the activity of the presynaptic dopaminergic markers measured by neuroimaging studies and post-mortem analysis. Interestingly, an *in vivo* study in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-monkeys has shown that chronic levodopa administration does not modify DAT binding (Fernagut et al., 2010).

The main objective of this study was to assess whether chronic levodopa treatment induces changes on striatal DOPA-decarboxylase activity measured by the striatal ^{18}F-DOPA uptake in MPTP-monkeys and its reversibility after a long washout period. In addition, we also performed a histological analysis of the substantia nigra *pars compacta* (SNpc) and the quantification of the striatal presynaptic dopaminergic markers DAT, tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase (AADC) by Western blot in all animals to evaluate the impact of levodopa and a washout period on these parameters.

Materials and methods

Animals

Eleven male macaques (*Macaca fascicularis*; 3 years old, weight: 3–4.5 kg) were included in the study. Animals were housed in an animal room under standard conditions of air exchange (16 l/min), humidity (50%), and light/night cycles (8 a.m. to 8 p.m.), and were fed fresh fruit and commercial pellets, with free access to water. Experimental protocols were in accordance with the European Communities' Council Directive of 24 November 1986 (86/609/EEC) regarding the care and use of animals for experimental procedures, and under the guidance of the Ethics Committee for Animal Experimentation of the University of Navarra.

Experimental protocol

Eight monkeys were rendered parkinsonian by systemic administration of MPTP. The other 3 animals served as control for the histological and biochemical studies. MPTP (Sigma, St Louis, MO) was dissolved in sterile saline and given intravenously (0.25 mg/kg) under light sedation with a mixture of ketamine (5 mg/kg) and midazolam (1 mg/kg). Injections were repeated every week for 7 consecutive weeks (Fig. 1). This MPTP schedule was chosen on the basis of previous studies in our laboratory showing that this regimen elicits a dopaminergic nigral cell loss of approximately 50%, which allows us to perform an adequate analysis and quantification of the ^{18}F-DOPA PET images (Ordoñez et al., 2013; Vázquez-Claverie et al., 2009). One month after the last MPTP dose, parkinsonian monkeys were randomly allocated to 2 groups. Four animals (Group I) received orange juice with no active drug and the other 4 monkeys (Group II) were administered levodopa (30 mg/kg/day obtained from Madopar®) dissolved in orange juice. Both treatments were given three times a day and maintained for 11 months. Brain magnetic resonance imaging (MRI) was performed on the MPTP-treated animals at baseline and also one year later (Fig. 1). ^{18}F-DOPA PET scans were also performed at baseline, one month after the last MPTP dose, before initiating placebo or levodopa treatment, the day right after the last dose of levodopa or placebo (11 months after initiating treatment), and after a washout period of 1, 3 and 6 months (Fig. 1). Monkeys were sacrificed 24 h after the last ^{18}F-DOPA PET scan and 6 months after the last placebo or levodopa dose.

Behavioral assessment

Motor deficits induced by MPTP were assessed according to a non-human primate disability rating scale, which independently scores from 0 (normal) to 3 (maximum disability) parkinsonian features such as tremor (intensity and duration), balance, feeding and freezing; from 0 (normal) to 4 (maximum disability) bradykinesia and posture, and from 0 (normal) to 5 (maximum disability) the reduction in spontaneous activity, thus giving a total maximum score of 28 (Luquin et al., 1999). All motor assessments were performed by the same investigator (M.R.). Evaluations were made before MPTP intoxication, one month after the last dose of MPTP, and once a month until the animals were sacrificed. Motor activity induced by placebo and levodopa was assessed by direct observation every 15 min for 3 consecutive hours, which is the estimated active period of the levodopa. Motor activities were also video-recorded.

Neuroimaging studies

Neuroimaging studies, either MRI or PET, were performed under slight anesthesia induced by ketamine (10 mg/kg, im) and midazolam (1 mg/kg, im). Sedation was maintained during the performance of the scans with a mixture of ketamine (5 mg/kg) and midazolam (0.5 mg/kg), administered every 30–45 min, when necessary.

MRI acquisition

Brain MRI was performed on a 1.5 T Siemens Symphony scanner (Erlangen, Germany). T_1 weighted axial images were acquired using a Magnetization Prepared Rapid Gradient Echo (MPRAGE) sequence with the following acquisition parameters: TE = 5.03, TR = 2140, flip angle = 15°, slice thickness = 1 mm, image matrix = 192 × 192 × 88 and pixel size = 1 × 1 mm^2. These images were used to create an MRI template to spatially normalize and quantify the PET studies and also to perform voxel-by-voxel statistical analysis.

Brain PET scans

PET imaging was performed in a small animal Philips Mosaic tomograph (Cleveland, OH, USA), with 2 mm resolution, 11.9 cm axial field of view (FOV) and 12.8 cm transaxial FOV. Animals were fasted overnight and the following day, 1 h prior to the procedure, 50 mg of carbidopa was given orally to block peripheral decarboxylation of ^{18}F-DOPA. The standard acquisition protocol has previously been described in detail (Collantes et al., 2009). In brief, the animals were placed on the bed in prone position with the head centered in the FOV. A transmission study prior to the emission scan was carried out with an external ^{137}Cs source (370 MBq). ^{18}F-DOPA was injected intravenously through the saphenous vein and a list mode study of 100 min was immediately started. The mean (standard deviation [SD]) injected activity of ^{18}F-DOPA was 72.9 (0.9) MBq. For each study, a summed sinogram of the whole emission study and dynamic sinograms were created. From these sinograms, images were reconstructed in a 128 × 128 matrix with a 1 × 1 × 1 mm^3 voxel size using the 3D RAMLA algorithm with