The progression of dopaminergic depletion in unilateral 6-OHDA-lesioned rats: PET imaging and histopathologic studies

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Introduction

Parkinson’s disease (PD) is a neurodegenerative disorder characterized by progressive death of dopaminergic neurons in the substantia nigra causing dopamine depletion in the striatum, which is associated with metabolic compensatory changes. The role of a 4-hydroxydopamine (6-OHDA)-induced lesion in one hemisphere has been widely used as a model of PD. The so induced neurochemical and histopathological changes have been extensively characterized in this model. However, the pathophysiological and compensatory mechanisms associated with the lesion are not well understood.

The aim of this neuroimaging study is to define and characterize the time-course of the metabolic changes and striatal dopaminergic depletion associated with the 6-OHDA unilateral lesion. Two different dose of the neurotoxic and in vivo PET images studies, using the [11C]Fluorodeoxyglucose ([11C]FDG, metabolism marker) and [11C]Dihydroaldehydebenzene ([11C]DBZ, dopaminergic system marker) radiotrigands were employed in the study along with immunohistological post-mortem evaluation of the lesion.

Materials and Methods

Animals and experimental groups

Twenty-six male Sprague-Dawley rats (250-300 gr) were used and distributed in the following experimental group:

I. Sham (n=7)
II. Low dose of 6-OHDA (n=12)
III. High dose of 6-OHDA (n=7)

6-OHDA induced lesion

Rats were unilaterally lesioned using either 4µg/4µl (low dose) or 8µg/4µl (high dose) of 6-OHDA by intrastriatal injection in the median forebrain bundle (coordinates from Lombara: +4.00 mm anterior, +1.3 mm lateral, -8.4 mm from skull, both bar: +4.5 mm). Sham animals received 4µl saline with 0.92% ascorbate. The rate of infusion was 1 µl/min.

Rotational screening

Three weeks after the 6-OHDA lesion, apomorphine-induced rotational behavior of the animals was measured for 1 h (0.5 µg/kg, s.c.).

PET imaging

PET imaging was performed using monomericine (11C-DSTBZ) and metabolic (11C-FDG) radiotrigands and conducted 1 day before surgery, and 1 day and 1, 2, 3 and 6 weeks after the lesion in each animal.

Analysis based on regions of interest was done in the striatum for [11C-DSTBZ] PET images using FMODS software (version 3.2, FMOD Technologies Ltd., Zurich, Switzerland). A voxel-based statistical analysis was performed in the whole brain for [11C-FDG] studies with statistical parametric mapping (SPM, Institute of Neurology, London, UK) using MATLAB software (version 7.6, MathWorks Inc.).

Histological analysis

All animals were sacrificed 6 weeks after 6-OHDA lesion. Dopamine transporter (DAT) and Vesicular Monoamine Transporter type-2 (VMAT2) expression in striatum was analyzed in coronal 20 µm thick sections obtained using a cryostate. Specific primary antibodies (DAT: 1:300, Santa Cruz Biotechnology; VMAT2: 1:100, Phoenix Pharmaceuticals) were used along with the ABC staining method and DAB development. Optical density (O.D.) of immunolabelling in the striatum of both hemispheres was measured using a computer system of imaging analysis (Image). NIH, USA. Ten rostro-caudal sections were examined for each animal. Statistical analysis using SPSS 18.0 set significant changes at p<0.05. p<0.01, p<0.001

Results

11C-DTBZ microPET images

Images of coronal and horizontal brain sections of sham and lesioned rats with a low (4µg/µl) and a high (8µg/µl) dose of 6-OHDA. The lesioned side in all animals is the left hemisphere. Graph showing the percent reduction of 11C-DSTBZ intensity in the striatum of the lesion side respect to the intact side in the sham group and in different time-points both before and after the 6-OHDA induced lesion in the animals that received either a high or a low dose of the neurotoxin. 11C-DSTBZ PET values obtained in the first and sixth weeks are similar for each dose of 6-OHDA, but the reduction of the intensity is higher in the group of animals that received the high dose of the neurotoxin (p<0.05).

18F-FDG microPET images — SPM analysis

Images of coronal and horizontal brain sections of sham and lesioned rats with a low (4µg/µl) and a high (8µg/µl) dose of 6-OHDA. Images are coronal and horizontal brain sections regions showing significant changes compared with baseline. Significant contralateral activation and ipsilateral hypoactivation are shown in both lesioned rats groups (p<0.05 FWE) but with a different metabolic pattern. Remarkably, the analysis revealed the both doses of 6-OHDA causes a hypometabolism in the periaqueductual gray (3 weeks) and in contralateral regions (subcortical: 1 day and 6 weeks).

Conclusions

PET images of 11C-DSTBZ show that the 6-OHDA lesion is not associated with a progressive dopaminergic striatal depletion, suggesting that it occurs within the first days after the neurotoxin administration. These results were corroborated by histological analyses. Dynamic metabolic patterns shown with 18F-FDG PET are evident in both groups of animals. Accordingly, the 6-OHDA-lesioned rat model could provide useful in vivo information about basal ganglia compensatory mechanisms.

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