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SHORT COMMUNICATION

SYNAPSE WILEY

Comparative analysis of striatal [^{18}F]FDOPA uptake in a partial lesion model of Parkinson's disease in rats: Ratio method versus graphical model

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Abstract

Animal models of Parkinson's disease are useful to evaluate new treatments and to elucidate the etiology of the disease. Hence, it is necessary to have methods that allow quantification of their effectiveness. [^{18}F]FDOPA-PET (FDOPA-PET) imaging is outstanding for this purpose because of its capacity to measure changes in the dopaminergic pathway noninvasively and in vivo. Nevertheless, PET acquisition and quantification is time-consuming making it necessary to find faster ways to quantify FDOPA-PET data. This study evaluated Male Wistar rats by FDOPA, before and after being partially injured with 6-OHDA unilaterally. MicroPET scans with a duration of 120 min were acquired and Patlak reference plots were created to estimate the influx constant K_c in the striatum using the full dynamic scan data. Additionally, simple striatal-to-cerebral ratios (SCR) of short static acquisitions were computed and compared with the K_c values. Good correlation ($r > 0.70$) was obtained between K_c and SCR, acquired between 80–120 min after FDOPA administration with frames of 10 or 20 min and both methods were able to separate the FDOPA-uptake of healthy controls from that of the PD model (SCR −28%, K_c −71%). The present study concludes that K_c and SCR can be trustfully used to discriminate partially lesioned rats from healthy controls.

KEYWORDS
Graphical analysis, Parkinsont's rat models, Patlak reference model, PET quantification, ratio method, [^{18}F]FDOPA

1 | INTRODUCTION

Chemical-induced and genetic Parkinson disease (PD) rat models are useful to test new therapies (Simola et al., 2007). One of the most widely used animal model for PD is the hemiparkinsonian rat in which rodents are unilaterally injected in the brain with the dopamine neurotoxin 6-hydroxydopamine (6-OHDA) to induce dopaminergic denervation of the striatum. The damage can be evaluated by many methods such as rotation, behavior, and immunohistochemistry among others (Truong et al., 2006). Positron emission tomography (PET) can also be used to evaluate this model noninvasively and in vivo, allowing the follow-up and evaluation of other procedures in the same animal. PET imaging of the dopaminergic system has been performed with different radiotracers in diverse species evaluating the turnover, binding to postsynaptic receptors (D1–D5), reuptake, and storage of dopamine. Despite the variety of dopaminergic radiopharmaceuticals, those based on fluorinated L-DOPA analogs are the most used because of their effectiveness to measure the loss of presynaptic dopaminergic neurons and their wholeness (Loane & Politis, 2011). 6-^{18}F-fluoro-L-dopa ([^{18}F]FDOPA) is by far the most widely used L-DOPA analog radiopharmaceutical. [^{18}F]FDOPA has not been extensively used in PD rat models because of many issues: robust PET quantification requires time-consuming scanning protocols, blood

sampling for metabolite analysis and premedication with inhibitors of catechol-O-methyl-transferase and aromatic L-amino acid decarboxylase (Walker et al., 2013). Furthermore, once the image is acquired, modeling of pharmacokinetics is also required. Simplification of the full kinetic model such as reference models has been developed to avoid the need of blood sampling, including the Patlak reference model (Sossi et al., 2003). When applying reference models in rats, the cerebellum is commonly used as reference tissue for dopaminergic radiopharmaceuticals (Kyono et al., 2011).

In the clinical setting, a simple ratio analysis of [^{18}F]FDOPA uptake in striatal subregions using a short 10-min scan acquisition has shown to be useful for the evaluation of PD, as an alternative to the Patlak model (Dhawan et al., 2002). On the other hand, Jokinen et al. (2009) showed that a simple striatal-to-occipital ratio of a short static scan 75 min after tracer injection is sufficient for reliably discriminating between patients with early PD from healthy controls. Such methodology has been successfully applied and validated in humans, but not in rodent PD models. The aim of this report was to compare the effectiveness of striatal-to-cerebellar ratio (SCR) and $K_{cerebellar}$ (K_c, Patlak reference model) to discriminate between partial 6-OHDA rat model and healthy control animals.

2 | MATERIALS AND METHODS

All animal experiments were performed observing technical specifications for the care and use of laboratory animals stated in the World Medical Association Declaration of Helsinki and were approved by the Animal Care and Use Committees of the Facultad de Medicina at UNAM.

2.1 | Animals

Rats used were male Wistar ($n = 10$, 6–7 weeks old, 230–250 g), housed in a 12-h light–dark cycle at 22 ± 2°C, with free access to food and water. A baseline PET scan was acquired to each rat ($n = 10$) 10 days before the 6-OHDA lesions were performed. Ten days after the lesion process, a second PET scan was acquired to six rats.

2.2 | Lesion procedures

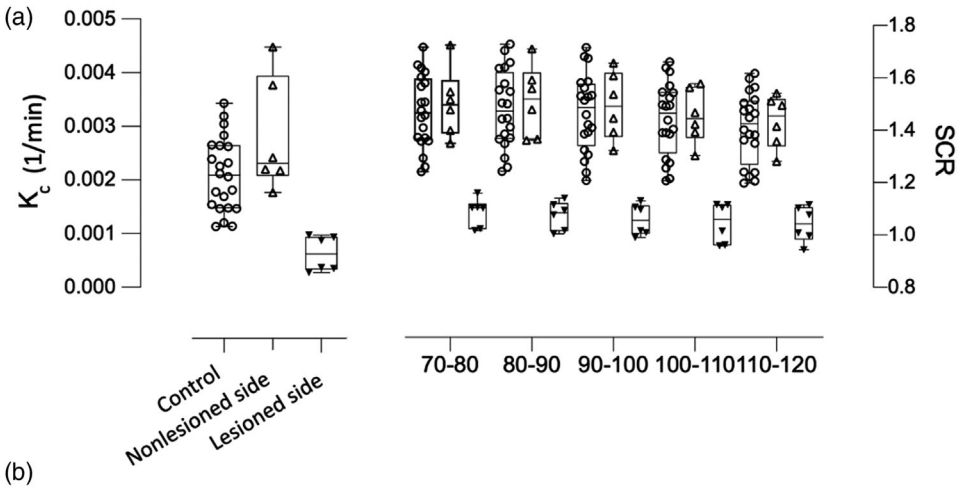
Lesions were performed as previously reported (Arias-Carrión et al., 2004). Briefly, rats were intraperitoneally (i.p.) anesthetized with ketamine, xylazine, and acepromazine (66 mg, 0.26 mg, and 1.3 mg/kg, respectively) and placed in a stereotaxic instrument. The injection site was located according to the coordinates −3.5 mm anteroposterior to bregma, −1.5 mm sideline, and −8.8 mm ventral skull surface, and then injected with 4 μl of 6-OHDA (2 μg/μl of 6-OHDA in physiological saline solution, plus 0.5% of ascorbic acid).

2.3 | Behavioral testing

On day 7, after injury with 6-OHDA, rats were injected with apomorphine (0.02 mg/kg dissolved in a 0.1% ascorbate saline solution). Rats were fixed in a harness and linked to mechanical sensors connected directly to a computer. Each 360° clockwise or counterclockwise turn was automatically recorded for 30 min and the total turns were determined (Truong et al., 2006)

2.4 | Micro PET imaging

Imaging was performed in a Focus 120 microPET scanner. [^{18}F]FDOPA (37 ± 8 MBq) was administered intravenously as a slow bolus injection via the tail vein, while rats were under anesthesia (2–3% isoflurane). Brain PET data were acquired for 120 min from the moment of dose injection. Animals were maintained under gaseous anesthesia for the duration of scans. Images were acquired in dynamic mode (frames: 10 × 60 s; 10 × 120 s; 10 × 180 s; and 6 × 600 s) and reconstructed with a 2D-OSEM algorithm on a matrix of 128 × 128 pixels, including corrections for scanner normalization, detector dead time, as well as random and scattered events. Carbidopa (Psicopharma) (10 mg/kg i.p. in sterile water) and entacapone (Sigma–Aldrich) (40 mg/kg i.p., in 0.3 ml of DMSO) were administered to the anesthetized rats 60 and 90 min before [^{18}F]FDOPA administration, respectively.



(b)

	CONTROL	NONLESIONED SIDE	LESIONED SIDE	CONTROL VS LESIONED Δ%	NONLESIONED VS LESIONED Δ%
K_c MEAN	0.0021±0.0007	0.0028±0.0011	0.0006±0.0003	-71	-78
SCR 70-80	1.47±0.14	1.50±0.13	1.09±0.05	-26	-27
SCR 80-90	1.48±0.15	1.51±0.14	1.07±0.05	-27	-29
SCR 90-100	1.47±0.15	1.49±0.13	1.06±0.06	-28	-29
SCR 100-110	1.44±0.14	1.45±0.10	1.05±0.08	-28	-28
SCR 110-120	1.41±0.13	1.43±0.10	1.04±0.07	-26	-27

FIGURE 1 (a) (Left) striatal K_c values of [^{18}F]FDOPA obtained for control (baseline study), nonlesioned, and lesioned sides. (Right) Striatum-to-cerebellar ratios (SCR–FDOPA) evaluated at different time intervals (control, nonlesioned, and lesioned sides, respectively). All lesioned sides are statistically different from control sides ($F = 43.7$, $p < .001$). (b) K_c and SCR values for [^{18}F]FDOPA uptake (mean ± SD) in rat striatum of controls, lesioned and nonlesioned sides, and their percentage difference

2.5 | Image data processing

Processing of reconstructed images was performed with PMOD software v3.7 (PMOD Technologies LLC). Time activity curves were obtained by drawing volume of interest (VOI) into the striatum (left and right) and into the cerebellum using spheres of 33.5 mm^3 drawn on the baseline scan, and once images were aligned, the VOIs of the first scan were copied to the second scan. Patlak reference analysis was performed from 0 to 120 min with a t^* of 10 min in order to obtain K_c for each striatum (Patlak & Blasberg, 1985). SCRs were determined at different time-points postinjection (between 54 and 110 min) and intervals (from 8 to 30 min) from the same acquired data. A parametric image of K_c was also made with PXMOD tool in order to illustrate visual differences between methods.

2.6 | Statistical analysis

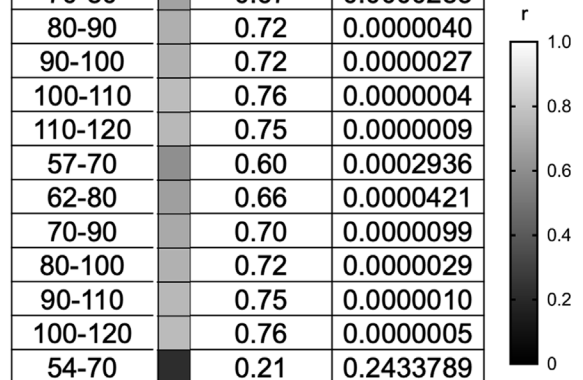
A correlation matrix comparing K_c versus SCR at different times was made to find the best K_c–SCR correlation match and a Pearson correlation ($\alpha = 0.05$) was performed. For comparison of controls, lesioned, and nonlesioned sides, nonparametric one-way analyses of variance followed by a Dunn's test were made. Statistical analysis was performed using GraphPad Prism 8.0.

3 | RESULTS

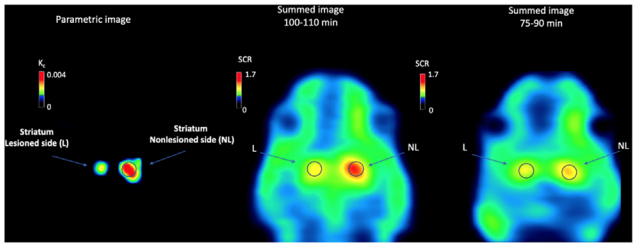
The rats showed a rate of rotation of 389 (±151) turns/30 min contralateral to the injured side. Both measures, K_c and SCR, allow to discriminate controls from lesioned side as showed in Figure 1(a); although K_c separate better lesioned from nonlesioned side (−78%), SCRs also allow to discriminate them (27–29%), as shown in Figure 1(b). PET data showed that starting at 80 min after [^{18}F]FDOPA administration, the semi-quantification achieves confident SCR values ($r \geq 0.70$). For this reason, only times after 1 h postinjection (p.i.) were evaluated to compare SCR versus K_c. Figure 2(a) shows a correlation matrix between the computed K_c values and the SCRs obtained at different time points-intervals postinjection of the tracer;

(a)

Time (minutes)	Kc vs SCR r	p
62-70	0.64	0.0000851
70-80	0.67	0.0000283
80-90	0.72	0.0000040
90-100	0.72	0.0000027
100-110	0.76	0.0000004
110-120	0.75	0.0000009
57-70	0.60	0.0002936
62-80	0.66	0.0000421
70-90	0.70	0.0000099
80-100	0.72	0.0000029
90-110	0.75	0.0000010
100-120	0.76	0.0000005
54-70	0.21	0.2433789
57-80	0.26	0.1519758
62-90	0.32	0.0778563
70-100	0.34	0.0535530
80-110	0.36	0.0431991
90-120	0.30	0.0932435



(b)



(c)

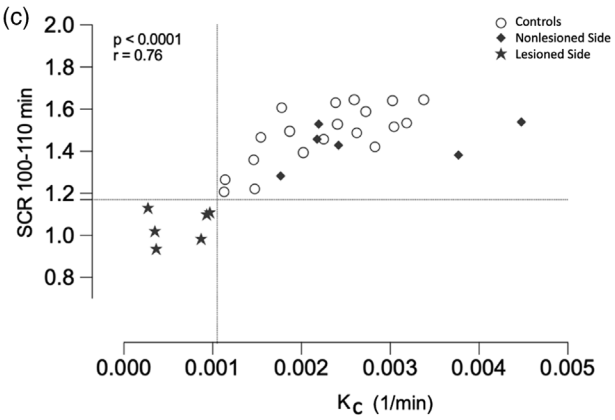


FIGURE 2 (a) Correlation matrix of K_c versus SCR evaluated at different time intervals, ordered by length of evaluated time interval. The color scale bar represents the *r* value from Pearson correlation and *p* is the test probability. (b) Typical parametric images (transverse plane) in terms of K_c (0–120 min, $t^* = 10$ min) and SCRs at two evaluated time intervals obtained with [^{18}F]FDOPA using a partial lesion model of PD in rats. Circles are the VOIs used for measurement, centered in the striatum. (c) Striatal K_c versus SCR plot of control (baseline study), lesioned and nonlesioned sides. Lines indicate the threshold of both measurements that separate the lesioned side from the control and nonlesioned sides

this matrix was used to infer the optimal time interval and frame duration. As an example, parametric images in terms of K_c and SCRs were created and are presented in Figure 2(b); SCRs images in this figure are representative for the best time interval found in this work for rats (100–110 min) and a typical acquisition time used for humans (75–90 min). Since the correlation matrix showed a better correlation between SCR and K_c at around 90–120 min p.i., a regression analysis for SCR at 100–110 min p.i. versus K_c (Figure 2(c)) was performed as an example finding a good correlation ($r = 0.76$, $p < 0.0001$) between both measures.

4 | DISCUSSION

Reference tissue models have proven quantitative accuracy, noninvasively, obtaining similar results of K_c in striatum (0.0005–0.0013 1/min) for PD models (Becker et al., 2017; Kyono et al., 2011). Unfortunately, K_c still requires the acquisition of time-consuming dynamic scans. Acquisition of long PET scans is impractical for many reasons, hence a method capable of balancing quantitative accuracy and scan time reduction is desirable. Ratio methods allow a significant reduction in total acquisition time and the values obtained by these methods are only an approximation of the true K_i value obtained from a full dynamic scan (Tantawy et al., 2009). In this report, it was found that a short 10 min scan acquired at least 80 min p.i. can effectively separate partially lesioned striatum sides from controls. The decrease of FDOPA uptake in partially lesioned striatum in terms of SCR was around 30% compared with that of controls.

This research also showed that SCR values are more dependent on the evaluated p.i. time than on the duration of the acquisition; for example, correlation between the K_c and SCRs for static images acquired at 100 min p.i. is almost the same for scans with a duration of 10 and 20 min, showing even similar statistical values (*r* and *p*), as shown in Figure 2(a). For the time interval 80–120 min p.i., all SCR showed a strong correlation ($r > 0.70$) with the gold standard K_c. Furthermore, around 100–110 min, the correlation between SCR and K_c is stronger ($r = 0.76$, $p = .000004$) (Figure 2) and has the capability to separate controls from partially lesioned rats, dividing both groups by a dotted line. For humans, the optimal time for ratio analysis with [^{18}F]FDOPA is 75–90 min but in this research, it was found that for rats it is not; differences could be due to diverse physiological processes and dissimilar conversion rates of DOPA between species, affecting the pharmacokinetics of the radiopharmaceutical. For the above, a simple ratio of an image acquired at 100–110 min after radiopharmaceutical injection can be used as an optimal time for [^{18}F]FDOPA acquisition, but other time intervals as 100–120 min, 100–115 min, 110–120 min, are also useful.

5 | CONCLUSIONS

A simple ratio method analysis in terms of SCR computed around 80–120 min p.i., with a frame duration between 10 and 20 min, has a good correlation ($r > 0.70$) with the influx constant K_c of [^{18}F]FDOPA in rat striatum, allowing to obtain reliable values to test the PD rat model in an easy way without the need of long acquisition image times and Patlak modeling. A single short scan of 10 min around 100 min after [^{18}F]FDOPA injection is enough to separate partially lesioned rat models of PD from healthy controls.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

All authors planned the experiments and wrote the article; A. A. E. and M. A. A. R. performed the acquisition and analysis of MicroPET imaging and L. V. D. provided the animal model.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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