# In-Vivo SPECT Imaging of D2 Receptor with Iodine-Iodolisuride: Results in Supranuclear Palsy

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We assessed the potential use of [$^{123}$I]iodolisuride (ILIS), a new iodine ergolene derivative, to study human striatal D2 dopamine receptors with SPECT. In normal subjects, we found that the tracer accumulated preferentially in striatum. This was prevented by high doses of haloperidol. The striatal accumulation was maximal between 60 and 180 min after injection. The striatum-to-cerebellum radioactivity concentration ratio as an index of specific binding, measured 60 min after injection, was 1.52 ± 0.19 (mean ± s.d.) in controls and 1.36 ± 0.11 in patients with supranuclear palsy ($p < 0.03$). Our results show that ILIS may be used to study D2 receptors with SPECT. In-vivo changes of D2 receptors in human brain may be detected with this method.

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Dopamine D2 receptors are studied in vivo with PET using ligands such as spiperone, benzamides or ergolenes derivatives labeled with $^{18}$F, $^{11}$C or $^{76}$Br (*1*). The results obtained in neurological and psychiatric diseases provide a template for similar SPECT studies (*2*). Two major advantages of SPECT are the lower cost and greater accessibility of this technique in comparison with PET. Recently, specific iodinated tracers have been developed to investigate D2 receptors with SPECT devices (*3,4*). The results obtained with [$^{123}$I]iodobenzamide showed that SPECT could be of clinical value for the diagnosis of Huntington's disease and for the monitoring of neuroleptic treatment (*5,6*). Among the ergolenes, [$^{123}$I]iodolisuride (ILIS), a 2-halogeno derivative of lisuride (*7*), showed binding characteristics similar to those of $^{76}$Br-promolisuride (BLIS), a specific D2 receptor PET tracer (*7,8*). In vitro, ILIS affinity for D2 receptors on rat striatal membranes is high (Kd = 0.27 nmol/liter). Biodistribution studies have confirmed that ILIS concentrated in rat striatum, the brain area with the highest density of dopamine receptors. The striatal binding was inhibited by spiperone, a specific D2 antagonist, but not by dopaminergic D1 (SCH23390), serotoninergic 5HT2 (ketanserine), alpha-1 adrenergic (prazosine) or alpha-2 adrenergic (yohimbine) ligands (*9*). This demonstrated the high selectivity of ILIS binding to D2 receptors. In addition, the high specific radioactivity of the tracer allows the administration of low drug amounts that results in very low D2 receptor occupancy with high pharmacological safety (*10*). Preliminary results obtained in a non-human primate showed that accumulation of ILIS in the basal ganglia region could be detected with SPECT.

The aim of the present work was to investigate whether: (1) ILIS could be used as an in-vivo D2 marker in the human brain using SPECT and (2) ILIS with SPECT could detect D2 receptor abnormalities. We found using a conventional SPECT device that ILIS accumulated preferentially in striatum and that specific binding was decreased in patients with progressive supranuclear palsy (PSP), an extrapyramidal disorder where loss of striatal D2 receptors has previously been reported in vitro (*11*) and in vivo (*12*).

## METHODS

### Radiopharmaceutical

ILIS was produced by a $^{123}$I electrophilic substitution in position 2 of the lisuride, a semi-synthetic ergolene derivative. ILIS labeling used iodogen with $^{123}$I t[$^{123}$I]S1B), Cis-Biointernational] with a radiolabeling yield of 90% (*7*). After HPLC purification on a silica gel column, both chemical and radiopharmaceutical purities were 99%. Radionucleidic purity was >98%, with $^{125}$I < 0.6% at injection time. Specific radioactivity was always >20 Ci/μmol. The radiochemical stability of the radiopharmaceutical solution was studied for 30 hr at 20°C by radio thin layer chromatography analysis.

### Subjects

A total of 30 subjects were examined. Fourteen controls were either healthy subjects (n=10) or patients hospitalized for symptoms unrelated to the central nervous system (sciatica, n=3; peripheral neuropathy, n=1). All controls had normal neurological examination (except for mild sensory changes with reflex abnormalities of the lower extremities in the subjects with sciatica or peripheral neuropathy) and normal brain MRI. None of them had received dopamine agonists or antagonists. Fourteen patients

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(68.0 ± 5.7 yr; mean ± s.d.) with PSP were selected on the following criteria: (1) vertical voluntary gaze palsy, (2) akinesia and axial hypertonia, (3) falls, (4) frontal lobe syndrome, (5) onset after 50 yr, (6) progressive course of the disease, (7) duration less than 10 yr, (8) no evidence of a focal lesion on cerebral MRI. Eight PSP patients were treated with levodopa which was withdrawn at least 24 hr before the study; all were neuroleptic free. Two schizophrenic patients (27 and 29 yr) treated with high doses of haloperidol (10 mg/day and 50 mg/day) were studied. Informed consent was obtained from each subject before the study.

### Imaging Studies

An oral dose (2 g) of Lugol's solution (1%) was given to each subject 48 and 24 hr prior to the study to block the thyroid uptake of the tracer. T1- and T2-weighted MRI images of 5 mm thickness and parallel to the orbitomeatal (OM) line were previously obtained for all subjects. A dose of 3–5 mCi of pure, pyrogen-free and sterile ILIS was injected intravenously. SPECT studies were conducted with a rotating rectangular gamma camera (Sophy-camera DSX) equipped with a high-resolution (6.5 mm at 10 cm) and low-energy collimator with 20% window at 159 kev. For SPECT imaging, subjects were lying on a tomographic bed with their head positioned at the center of rotation using a laser beam. Accurate positioning was ensured by moving and rotating the camera head around so that the radius of rotation was reduced to a minimum with a body contour orbit. After head immobilization, all subjects were studied at rest with eyes closed. A planar image was first acquired for anatomical reference as follows: a lead marker was placed along the left OM line with the camera vertically placed before acquisition. Sixty-four frames of 64 × 64 matrix were subsequently acquired over a 360° rotation. A 32-min tomographic acquisition was initiated 60 min after injection in all subjects. Repeated acquisitions were obtained 5, 35, 60, 100, 160, 220 and 315 min after injection in two normal volunteers. The two initial acquisitions lasted 16 min (15 sec per frame). All others lasted 32 min (30 sec per frame).

Transaxial slices were reconstructed by filtered backprojection using a Butterworth 4/16 filter of the 4th order and a cutoff frequency of 0.25 cycles per pixel. In this study, we did not apply attenuation correction since we felt that the methods available at our center could not be confidently used at the cerebellar level. Hence, these methods are based on the assumption that the attenuation field of a given slice is convex and homogenous, while true attenuation coefficients at the cerebellar level are highly heterogenous due to the presence of the air of cavities of ears and nasopharynx, and to the variable thickness of the occipital bone. Elsewhere, no scatter correction was used. Sixteen reconstructed slices (9 mm thickness) parallel to the OM line were displayed. The OM level was located among these slices using the preliminary planar acquisition obtained with the lead marker. Subsequently, we selected a slice with the maximal striatal activity and a slice at cerebellar level. We always verified that these slices corresponded to striatum and cerebellum on MRI images obtained the same day at identical levels. Striatal and cerebellar selected SPECT slices were located ≈40 mm and ≈10 mm above the OM line, respectively. Two regions of interest (ROIs), 3.2 $cm^2$ in area, were centered on the striata in the area of highest activity. Two ROIs of 12.9 $cm^2$ were positioned in the cerebellar hemispheres, posteriorly and tangentially to a 50% computer-delineated isocontour of maximal activity on the cerebellar slice. To ensure the reproducibility of our method, ROI positioning was independently applied by two investigators, who were not informed as to the subject's condition.

In order to detect any pharmacological effect after the tracer injection, a neurological examination was performed by a certified neurologist (H.C.) just before and after the imaging session. Particular attention was paid to putative changes in motor performance, tremor, axial and limb tonus. Additionally, subjects were specifically asked during the second neurological examination for any subjective change in their mental or physical status.

### Data Analysis

Striatum and cerebellum radioactivity values were obtained by averaging right and left ROI activity. The striatum-to-cerebellum (S/C) ratio was then calculated. A linear regression analysis was used to test for significant effect of age on the S/C ratio in control and patient groups. PSP patients were compared with the seven control subjects who were older than 55 yr in order to obtain groups with similar mean age (68.0 ± 5.7 yr and 64.7 ± 7.6 yr in patients and controls, respectively; mean ± sd).

## RESULTS

The mean injected dose of radioactivity administered to all 30 subjects was 182.78 ± 35.52 MBq, corresponding to 0.35 ± 0.34 nmoles of ILIS. None of the subjects showed any clinically detectable change at neurological examination. None of them reported any subjective change in their mental or physical status.

In controls, three-dimensional reconstructed images showed that ILIS accumulated preferentially in the striatum (Fig. 1). The kinetic analysis performed in two controls showed that the S/C ratio reached a maximal value at 60 min after injection (Fig. 2). The S/C value was measured on images acquired at this time in all subjects (Table 1). In controls, the S/C ratio ranged from 1.28 to 1.98 with a mean value of 1.59 ± 0.22 (n = 14). A trend for the S/C to decrease with aging was observed (not statistically significant). In PSP patients, the S/C ranged from 1.19 to 1.55 with a mean value of 1.36 ± 0.11. This was significantly lower (Student's t-test; p = 0.026) when compared to controls of similar mean age (S/C = 1.52 ± 0.19). The PSP patients treated with levodopa prior to the study had similar S/C values (S/C = 1.35 ± 0.11) than those who had never been treated (S/C = 1.36 ± 0.13). In the subjects treated with high doses of haloperidol, S/C ratios were 0.95 and 0.99. Interobserver concordance in

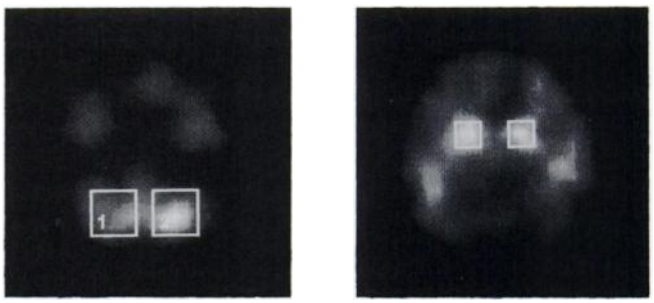


**FIGURE 1.** Striatal and cerebellar ROIs.

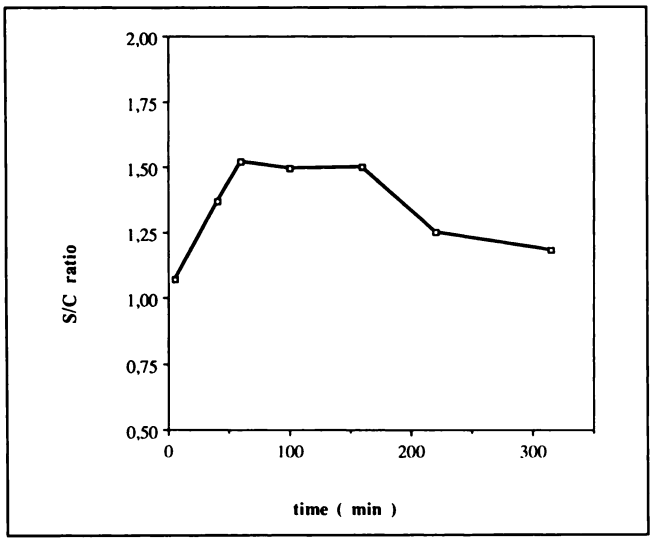


**FIGURE 2.** Striatum-to-cerebellum ratio versus time postinjection calculated with repeated tomographic acquisitions in two controls (mean values).

**TABLE 1**
Striatum to Cerebellum Radioactivity Ratios in Controls and PSP Patients

| Controls | | Patients | |
|---|---|---|---|
| Age | S/C | Age | S/C |
| 23 | 1.79 | 59 | 1.48 |
| 27 | 1.73 | 61 | 1.54 |
| 30 | 1.81 | 61 | 1.31 |
| 34 | 1.98 | 63 | 1.23 |
| 51 | 1.51 | 65 | 1.31 |
| 52 | 1.32 | 66 | 1.55 |
| 55 | 1.48 | 68 | 1.36 |
| 57 | 1.48 | 69 | 1.50 |
| 57 | 1.45 | 70 | 1.37 |
| 59 | 1.28 | 71 | 1.36 |
| 63 | 1.51 | 74 | 1.21 |
| 70 | 1.69 | 74 | 1.37 |
| 76 | 1.87 | 77 | 1.28 |
| All: 51.8±17.0 | 1.59±0.22 | | |
| >55 yr: 64.7±7.6 | 1.52±0.19 | 68.0±5.7 | 1.36±0.11* |

S/C ratios were significantly decreased in PSP patients in comparison to controls who were more than 55 yrs old.
* p=0.026.

the determination of the S/C ratio was excellent ($r = 0.97$, $df = 29$, $p < 0.0001$).

## DISCUSSION

The SPECT imaging studies obtained in normal subjects after ILIS injection showed that the tracer penetrated rapidly into the brain and accumulated preferentially in the basal ganglia area, the region with the highest dopamine D2 receptors density in human brain (*13*). In contrast, this preferential accumulation of the tracer did not occur in subjects treated with high doses of haloperidol. Since haloperidol is a specific D2 blocker and does not affect 5HT2 binding (*14*), the ILIS striatal accumulation observed in-vivo with SPECT indicated very likely specific binding of the tracer to available D2 receptors.

In a recent PET study performed with BLIS, a ligand that shared many pharmacokinetic properties with ILIS, Delforge et al. (*15*) have shown that the accurate D2 receptors density (B'max) and Kd quantification imposed three injections of labeled and unlabeled ligand and a very long duration of the experiment because of the low association rate constant of the tracer. Identical limitations might be raised for ILIS and should be inappropriate to clinical studies. Therefore, we chose to calculate the S/C ratio for estimating the magnitude of specific binding in

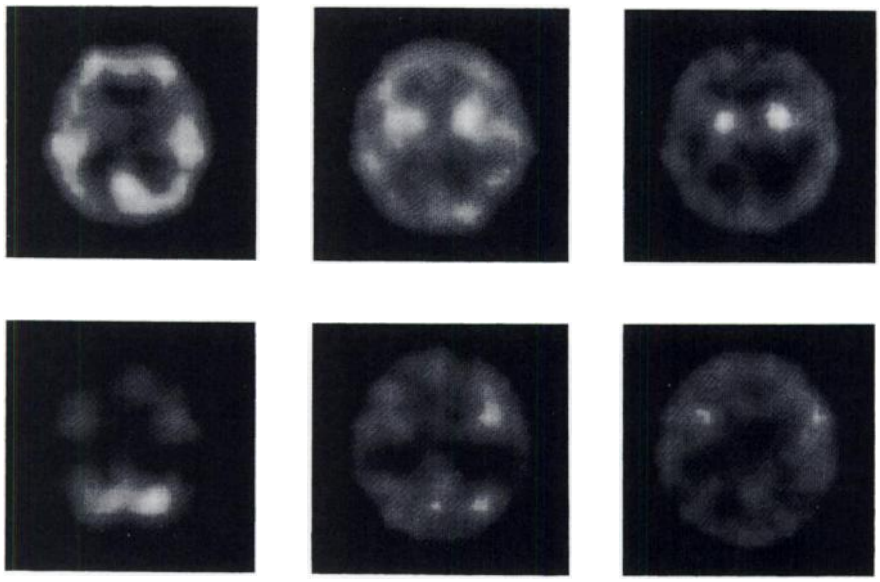

**FIGURE 3.** ILIS images of a normal subject (left), a PSP patient (middle) and a schizophrenic patient treated with haloperidol (right). The top images are parasagittal sections. The bottom images are transversal sections.

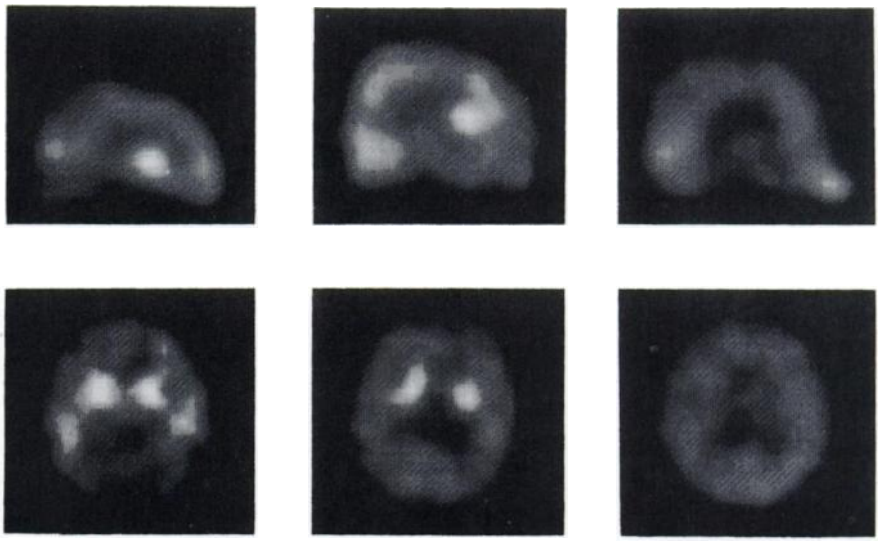

**FIGURE 4.** Horizontal images were reconstructed at the striatal (upper row) and cerebellar level (lower row). Images scanned between 5–20 min after injection had a perfusion-like regional distribution (left). The 60–90-min images (middle) and 160–190-min images (right) showed the preferential accumulation of ILIS in the striatal area.

the D2 receptor-rich basal ganglia relative to the cerebellum devoid of specific D2 binding sites (*13,16*). This easily measurable index has been widely used in PET studies (*17–19*). Wienhard et al. (*22*) recently compared the S/C values to the binding potential in a PET study using fluoroethylspiperone. Their conclusions reflected the reliability of this index for clinical use on the basis of its sensitivity and low variability. Furthermore, Delforge et al. also demonstrated with BLIS that S/C ratio and B'max obtained using the kinetic analysis were positively correlated. Therefore, the S/C ratio may be considered a reliable index of striatal D2 receptor density, assuming that nonspecific binding, affinity and access to the receptors are stable. Since this assumption may be difficult to establish in practice, we wish to stress that the exact biological significance of any detected abnormality of S/C ratio should be ultimately verified by comparisons with validated quantitative measurements in PET and/or postmortem studies.

The mean S/C ratio obtained with ILIS in our controls was close to the ratio obtained in normal subjects using PET with 3N-[$^{11}$C]-methylspiperone (*17*) or [$^{76}$Br]bromospiperone (*12*). The intersubject variability was low (coefficient of variation = 13%) and comparable to the corresponding values obtained in PET with bromolisuride (10%) or bromospiperone (11%). Additionally, the interobserver concordance indicated that our data analysis was reliable and reproducible. The significant 10% decrease in the S/C ratio that was detected in supranuclear palsy with ILIS shows the ability of our method to detect D2 receptor changes, since this result agrees with Ruberg's postmortem finding of a striatum D2 receptor loss in PSP (*12*). However, the ILIS S/C decline was lower than the 26% decrease of the S/C ratio measured by Baron et al. in supranuclear palsy using PET with bromospiperone (*23*). Moreover, we found only a nonsignificant trend of the S/C ratio to decrease with age in our normal subjects (4% per decade), while the age-related effect was highly significant in PET studies with spiperone derivatives (*17,22,23*) and ranged from 4% (*23*) and 20% (*16*) per decade. These discrepancies with PET findings may have three explanations. First, technical limitations, such as the lack of an accurate attenuation correction, may explain that our SPECT imaging remains a less sensitive imaging method compared with PET (*24,25*). However, the development of faster SPECT systems with high sensitivity and resolution and research efforts being done on attenuation and scatter correction are very promising. Second, the loss of D2 receptors may be effectively less marked in our PSP patients than in those studied by Baron et al., although they were selected on very similar clinical criteria (*23*). Similarly, the lack of a statistically significant age-related decrease in the S/C ratio in our study may also result from the different size and age structure of our control sample. Third, changes in the S/C ratio observed with PET may be related to variations in both the striatal tracer uptake and receptors changes (*16,21*). Wienhard et al. suggested that this may explain the discrepancy between postmortem studies reporting minor (*26,27*) or no changes (*14,28*) in human striatal D2 density and in-vivo findings demonstrating a clear-cut, major decline of the S/C ratio with aging (*17,22,23*). ILIS S/C may be less sensitive to tracer uptake than some D2 PET tracers (*23,26*) because the ILIS S/C was calculated at its maximal value (i.e., in an apparent equilibrium state). In summary, ILIS combined with conventional and widely-used SPECT devices may be used for clinical in-vivo investigations of D2 receptors in the human brain. Optimally, we feel that PET and postmortem studies should be performed to verify the estimation of D2 receptors obtained with SPECT.

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