SPECT Imaging of Dopamine D2 Receptors with 2′-Iodospiperone

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SPECT imaging of dopamine D2 receptors was performed using ^{123}I-labeled 2′-iodospiperone (2′-ISP). **Methods:** Iodine-123-2′-ISP was administered to 12 normal subjects. Serial SPECT scans were obtained in six normal subjects for kinetic studies and static SPECT images were obtained in four normal subjects. Two additional normal subjects had serial whole-body imaging to calculate radiation dosimetry. **Results:** Serial SPECT scans demonstrated rapid initial brain uptake followed by gradual washout from the cerebral cortex and cerebellum, while the activity in the basal ganglia was stable for 2 hr and gradually decreased thereafter. There was fast clearance of blood activity with rapid conversion of 2′-ISP to the hydrophilic metabolites. The basal ganglia-to-frontal cortex ratio increased with time and plateaued between 2 and 4 hr postinjection. The basal ganglia-to-frontal cortex ratio obtained during this period demonstrated an age-dependent decrease similar to other D2 ligands. **Conclusion:** 2′-ISP can be used for mapping dopamine D2 receptors in human brain with SPECT. The relatively high background activity, however, suggests that further modification of the compound may be needed prior to widespread clinical use.

Key Words: single-photon emission computed tomography; dopamine D2 receptor; iodine-123-2′-iodospiperone

J Nucl Med 1995; 36:1191–1195

Dopaminergic transmission has been reported to be involved in the pathogenesis of various neurological and psychiatric disorders, including movement disorders and schizophrenia. In recent years, in vivo imaging of the dopaminergic system in the human brain has been energetically examined. This system is mainly involved in the central motor control and is also subjected to the effects of neuroleptic agents. Pharmacological studies have shown that dopamine receptors are divided into several subtypes. Two major subtypes, D1 and D2, normally collaborate to control human physical movement and may be unbalanced in some abnormal conditions, including schizophrenia, Parkinson's disease, striatonigral degeneration, Huntington's chorea, dyskinesia and other neuropsychiatric disorders. Accordingly, in vivo imaging of dopamine D1 and D2 receptors is expected to make a great contribution both in the understanding of the human brain mechanism and the treatment of these neurological disorders.

Since the first successful imaging of dopamine D2 receptors in living human brain with PET (*1*), there have been extensive studies by many investigators using ligands labeled with short-lived positron-emitting radionuclides such as ^{11}C or ^{18}F (*2–5*). PET imaging, however, requires a small cyclotron for production of these radionuclides and on exquisite radiolabeling system, both of which limit its widespread application. An alternative approach was the use of ^{76}Br, another positron-emitting radionuclide with a longer half-life (*6, 7*) to enable tracer delivery to institutions without a cyclotron.

Because of SPECT's widespread use in clinical nuclear medicine, research efforts have focused on developing specific ligands radiolabeled with ^{123}I for dopamine receptor imaging (*8–12*). Among those, 2′-iodospiperone (2′-ISP) is one of the butyrophenone derivatives of dopamine D2 receptor antagonists applicable for SPECT imaging. In this article we report the kinetics of 2′-ISP in the blood and brain in normal volunteers.

MATERIALS AND METHODS

Subjects

The normal control subjects consisted of 12 healthy male volunteers (age range 20 to 51 yr) who had never suffered from any neurological or psychiatric disorders. SPECT studies were performed in 10 men; two men had serial whole-body imaging.

To prevent unnecessary radiation exposure to the thyroid, all subjects received potassium iodide (30 mg/day) for 4 days starting 2 days prior to the administration of ^{123}I-labeled 2′-ISP. The study was approved by the Ethical Committee of Kyoto University School of Medicine.

Synthesis of Radiolabeled Compound

Iodine-123-labeled 2′-ISP was synthesized by bromine-radioiodine exchange reaction. An aliquot (1 ml) of sodium [^{123}I]iodide (30 mCi/ml, 1.11 GBq/ml), produced by an indirect method using ^{127}I(p,5n)^{123}Xe reaction, was evaporated to dryness. To this residue was added 37.5 μl 80% dimethylformamide (DMF) solution containing 200 μg 2′-bromospiperone, 13 μg sodium iodide, 100 μg copper (II) sulfate pentahydrate and 180 μg l-naphthalene

Received Jun. 20, 1994; revision accepted Dec. 20, 1994.

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sulfonic acid dihydrate. In this reaction, nonradioactive iodide was added to increase the radiochemical yield. After being heated at 95°C for 1 hr, the resulting solution was applied to a reversed-phase high-performance liquid chromatography (HPLC) column (Lichrosorb RP-18; 8 × 300 mm) and eluted with methanol:triethylamine:water (75:1:50) at a flow rate of 2 ml/min. The fraction corresponding to 2'-ISP was collected and evaporated to remove the residual organic solvent. The resulting solution was adjusted to pH 4.5 with 0.25 N hydrochloric acid and sterilized by filtration through a 0.22-μm Milex filter.

The radiochemical purity of the product was determined by thin-layer chromotography (TLC) and analytical HPLC. For TLC analysis, a silica gel plate was used with a chloroform:methanol (6:1) solvent (Rf = 0.6 − 0.7). HPLC analysis was performed on a reversed-phase column (Lichrosorb RP-18; 7.5 × 300 mm) eluted with methanol:triethylamine:water (75:1:50) at a flow rate of 2 ml/min (retention time 40 min).

The total radiochemical yield after HPLC purification was approximately 20%–40%. The radiochemical purity of the product was greater than 98% and the specific activity (estimated by UV absorbance at 249 nm) was approximately 350 mCi/μmole (13 GBq/μmole).

Blood Metabolite Measurements

Venous blood samples were collected in heparinized tubes at 1, 2, 5, 15, 30 and 60 min after the injection of ^{123}I-labeled 2'-ISP. The blood samples were centrifuged at 1500 rpm for 10 min to collect plasma samples. These plasma samples were extracted three times with an equal volume of ethanol, and the combined ethanol extracts were analyzed by TLC (chloroform:methanol = 6:1). Approximately 80% of the radioactivity in the plasma was extracted by this organic solvent technique at each time of analysis.

Imaging Studies

Dynamic SPECT scanning was performed in six subjects using a multidetector SPECT scanner (SET-030W; Shimadzu Co., Kyoto, Japan) or a four-head brain SPECT camera (SPECT-2000H; Hitachi Medical Co., Tokyo, Japan). SET-030W had three fixed detector rings at 30-mm intervals, with a spatial resolution of 10 mm FWHM at the center of the field of view, as measured by a ^{99m}Tc line source (*12*). On the other hand, SPECT-2000H provided contiguous tomographic slices, with a spatial resolution of 8 mm FWHM. The resolution of the clinical ^{123}I images, however, was estimated as approximately 12 mm in FWHM for both systems. The high sensitivity of these SPECT systems enable acquisition of serial SPECT images every 5 min for 1 hr after injection of ^{123}I-2'-ISP (3–6 mCi), followed by static scanning for 20 min in the later period.

The initial serial SPECT images were reconstructed every 20 min after summation of raw data. With the SET-030W, the subject's head was positioned parallel to the canthomeatal line (CM line) and the plane of the lowest slice was adjusted to 2 cm above the CM line, providing three tomographic slices at 2, 5 and 8 cm above the CM line for acquisition. On the other hand, similar tomographic slices were selected after reorganization of the volumetric SPECT data obtained by SPECT-2000H. Several blood samples were also taken for measurement of radioactivity in five subjects.

In the other four subjects, SPECT images were obtained between 2 and 4 hr postinjection of ^{123}I-2'-ISP (3–6 mCi) using either the same multidetector SPECT scanner (SET-030W) or a single-head rotating gamma camera (RC-150E; Hitachi Medical Co., Tokyo, Japan). SPECT data with the single-head rotating gamma camera were acquired from 64 views over 360° with 40-sec acquisitions in each view using a low-energy, general-purpose collimator. The data of the limited field of the camera (25.6 × 25.6 cm) were stored as 64 × 64 matrix images (4 mm pixel size) and SPECT images of every three slices (12-mm thickness) were reconstructed. The spatial resolution of the final SPECT images of ^{123}I was approximately 15 mm FWHM with this system.

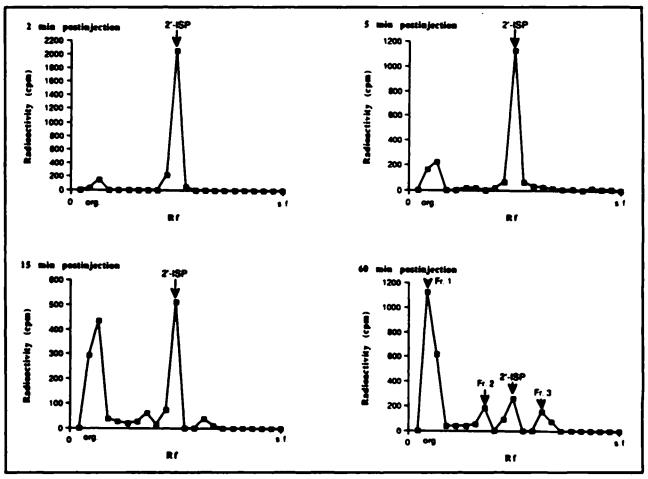

FIGURE 1. Representative TLC profiles of the ethanol-extractable fraction in plasma at different time points.

For the SPECT systems used in this study, attenuation correction was performed by a simple postcorrection method which assumes that the head has an ellipsoid shape with uniform attenuation. Data analysis was performed by placing irregular-shaped regions of interest (ROIs) on the bilateral frontal cortex and the basal ganglia in the SPECT image. The average value of the right and left ROIs was used for the analysis.

To determine 2'-ISP kinetics for dosimetric calculations, serial whole-body imaging along with urine collection was performed in two normal subjects 20 hr after the injection of 1.5 mCi ^{123}I-2'-ISP.

RESULTS

Blood Analysis

Figure 1 shows the typical TLC profile of plasma activities at various time courses. Immediately after the injection, most of the activities were observed in the original 2'-ISP position but then gradually degraded into three major fractions of metabolites. At 60 min postinjection, only a small amount of the original 2'-ISP remained in the blood, and the major activity was seen in fraction 1, corresponding to the hydrophilic metabolites. Figure 2 shows the plasma clearance and 2'-ISP activity 1 hr postinjection. Only 40%–50% of the total activity was 2'-ISP at 15 min and less than 20% at 1 hr. These blood data suggested that most of the input of 2'-ISP into the brain is determined in the initial several minutes after the injection.

SPECT Studies

Figure 3 demonstrates a typical example of serial SPECT images corresponding to the slice of the basal ganglia and shows rapid initial uptake in the whole brain

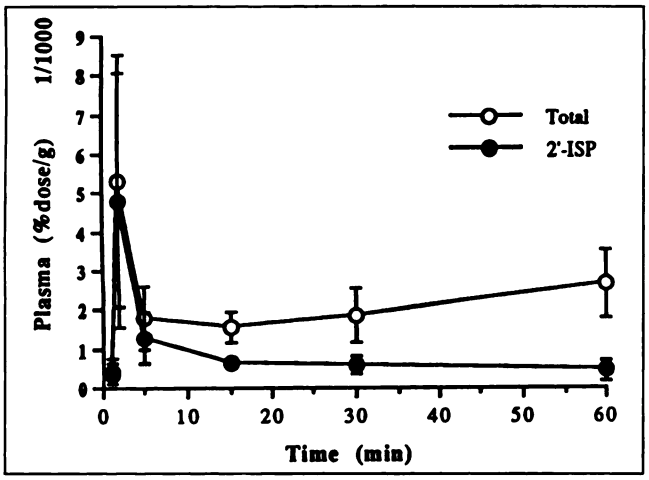

FIGURE 2. Temporal changes in total radioactivity and 2'-ISP in plasma. The values are shown as mean and standard deviation of five normal subjects.

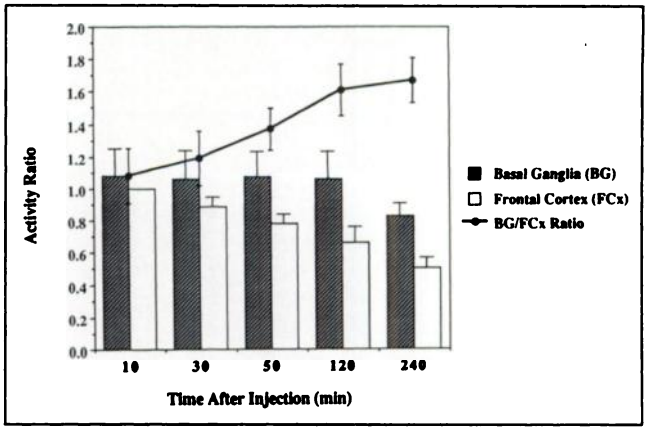

FIGURE 4. Serial changes in radioactivities of basal ganglia and frontal cortex, as well as basal ganglia to frontal cortex ratio (BG/FCx) in six normal subjects (mean and standard deviation). The activities in basal ganglia and frontal cortex were expressed as relative counts to those of frontal cortex in the initial SPECT images (10 min).

according to blood flow. The activities in the cerebral cortex were washed out gradually. On the other hand, basal ganglia activity was stable for 2 hr postinjection, and then decreased by time, resulting in residual hot spots in the basal ganglia during the later imaging period. Figure 4 shows the relative activity changes in the basal ganglia and frontal cortex in six normal subjects, where the SPECT counts were normalized to the activity of the frontal cortex in the initial SPECT image (0–20 min) in each subject. The activity in the frontal cortex decreased somewhat rapidly, while that in the basal ganglia was stable for 2 hr and then washed out gradually. The basal ganglia-to-frontal cortex ratio calculated from these data showed a gradual increase for 2 hr and almost plateaued between 2 and 4 hr postinjection, suggesting a pseudo-equilibrium state during this period.

Figure 5 shows the relationship of the basal ganglia-to-frontal cortex ratio obtained between 2 and 4 hr postinjection and age. The data demonstrated an age-dependent decrease of the basal ganglia-to-frontal cortex ratio similar to other D2 ligands.

Radiation Dosimetry

Table 1 shows the estimated absorbed dose of ^{123}I-labeled 2'-ISP. Approximately 84% of the activity was excreted to the urine 20 hr after administration. The critical organs were the large intestine and the urinary bladder.

DISCUSSION

Characteristics of 2'-ISP

We have demonstrated that ^{123}I-labeled 2'-ISP, an iodinated spiperone analog, can be used for SPECT imaging of dopamine D2 receptors in the human brain. Previous in vitro and in vivo studies demonstrated specific binding of 2'-ISP to D2 receptors. In contrast to ^{11}C-labeled N-methylspiperone (NMSP), which is a representative D2 receptor ligand of butyrophenone derivatives used for PET measurement, 2'-ISP has slightly less affinity for D2 receptors and negligible affinity to serotonin S2 receptors (*13*). One significant difference from NMSP is a gradual decrease of radioactivity in the striatum 2 hr postinjection. This is

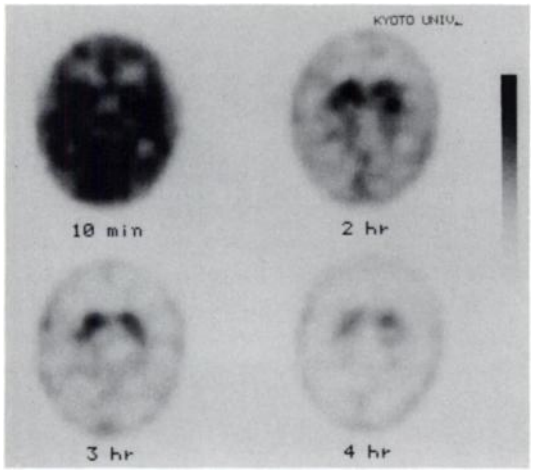

FIGURE 3. Serial SPECT images of 2'-ISP in a normal subject.

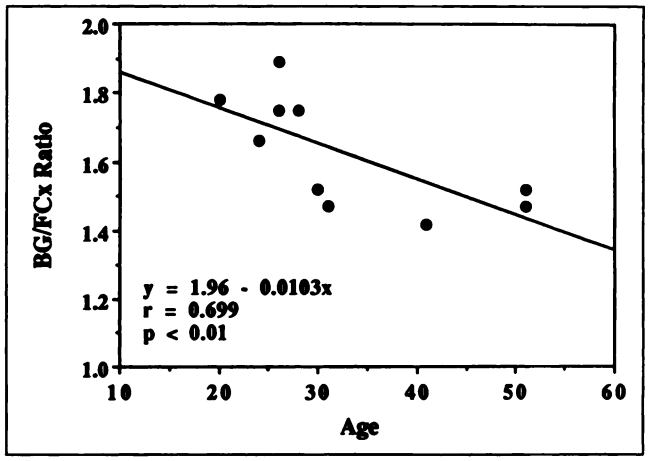

FIGURE 5. Basal ganglia-to-frontal cortex ratio (BG/FCx) of 2'-ISP plotted against age.

TABLE 1
Estimated Absorbed Dose for Iodine-123-2′-ISP

Organ	rad/mCi	mGy/MBq
Brain	0.041	0.011
Upper large intestine	0.370	0.100
Lower large intestine	0.094	0.025
Liver	0.180	0.049
Lungs	0.063	0.017
Kidneys	0.200	0.054
Spleen	0.290	0.079
Red marrow	0.075	0.020
Bone surface	0.062	0.017
Ovaries	0.084	0.023
Testes	0.041	0.011
Urinary bladder wall*	0.310	0.085
Total body	0.061	0.016

*Calculation is based on a 4-hr voiding interval.

probably due to the lower affinity of 2′-ISP for D2 receptor (K_d = 0.25 n*M*) (*13*) than that of NMSP (K_d = 0.05 n*M*) (*3*). Although 2′-ISP kinetics were slower than ^{123}I-labeled iodobenzamide (IBZM), which has already been used in clinical SPECT studies (*14,15*), or the more recently reported iodobenzofuran (IBF) (*16*), 2′-ISP also can reach a pseudo-equilibrium state (*17*) within a measurable time with SPECT. This pseudo-equilibrium condition enables semiquantitative evaluation of the specific binding to the receptors as a simple parameter of the specific-to-nonspecific binding ratio.

Quantitative Analysis

In a kinetic study, the estimation of nonspecific binding is particularly important. For the analysis of PET data, the cerebellum has been commonly used as a nonspecific reference region (*17,18*). In SPECT measurements, however, the accuracy of radioactivity measurement in the cerebellum might be limited because of incomplete attenuation correction (*16*). In addition, the sensitivity varies among the slices obtained with the SET-030W due to the effects of radiation scatter from the lungs. Because 2′-ISP showed negligible binding to the cortical S2 receptors (*13*), we used the frontal cortex as a reference region and calculated the activity ratio of basal ganglia-to-frontal cortex as a reference region and calculated the activity ratio of basal ganglia-to-frontal cortex as a simple semiquantitative index for the specific binding of 2′-ISP (*14*).

The basal ganglia-to-frontal cortex ratio of 2′-ISP obtained in this human study was lower than that found in previous animal experiments with 2′-ISP (*19*) and human data obtained with 2′-NMSP (*20*) or IBF (*16*), but it was similar to the values obtained with IBZM (*14*). Those values can be attributed, in part, to the limited spatial resolution and undesired radiation scatter in SPECT measurements, but the physical and chemical characteristics of 2′-ISP itself may also be responsible for this observation. We used a multidetector SPECT scanner because of its high sensitivity, which is a suitable quality for dynamic SPECT imaging. Poor axial resolution and a limited number of tomographic slices, however, interfered with the accurate quantification of radioactivity in small structures such as the basal ganglia in this study.

Target-to-Nontarget Ratios of 2′-ISP

We injected 3–6 mCi 2′-ISP with a specific activity of 350 mCi/μmole. The amount of administered 2′-ISP was less than 0.1 μg/kg, and striatal uptake of 2′-ISP in mice was not influenced by dose ranges below 10 μg/kg (*19*). Therefore, the relatively low target-to-nontarget ratio was not attributable to low specific activity but may be caused by other factors. The washout of 2′-ISP from the nonspecific binding site was slow, probably due to the high lipophilicity of 2′-ISP (*21*). N-alkylation of 2′-ISP further increased the lipophilicity from 2.3 to 2.9, as expressed by the logarithm of the octanol-phosphate buffer (pH 7.4) partition coefficient, but it did not increase the specific-to-nonspecific binding ratio in mice, although it did increase the blood-brain barrier permeability (*21*). High lipophilicity of iodinated radioligands also increases lung uptake which then decreases the delivery of 2-ISP into the brain (*22*). Another possible disadvantage of 2′-ISP may be the considerable amount of activity in the extracranial tissues, including soft tissues and salivary glands, which could degrade image quality and decrease the contrast between specific binding in the striatum and nonspecific binding in the surrounding brain tissues. This is also probably caused by the high lipophilicity of 2′-ISP.

The parent 2′-ISP is rapidly metabolized after it is given intravenously and is degraded into three major metabolites. The concentration of these metabolites gradually increased and reached 80% of the injected 2′-ISP after 1 hr. Most of these metabolites were hydrophilic and cannot be taken up by the brain. Blood metabolite analysis suggested the presence of a small fraction of lipophilic metabolites (Fraction 3 in Fig. 1). This compound also shows very little brain uptake because of the strong binding to plasma protein and erythrocytes (unpublished data). Therefore, the major fraction of arterial input of 2′-ISP into the brain is determined in the initial minutes after injection, although a small fraction of 2′-ISP activity remains in the blood in the later period. The activity in the basal ganglia was stable for 2 hr and washed out gradually thereafter. Because we only analyzed blood samples for 1 hr, exquisite kinetic analyses such as compartment modeling could not be performed. Instead, we used a target-to-nontarget basal ganglia-to-frontal cortex ratio as a simple semiquantitative parameter of specific binding in the basal ganglia.

Clinical Implications

We also observed an age-dependent decrease in the basal ganglia-to-frontal cortex ratio, although the present results were limited in the range of age. The slope of the decrease obtained in this study was less steep compared with NMSP (*21*), but it was equivalent to the values obtained with ^{76}Br-spiperone (*7*) or IBZM (*16*). Reduced binding of radioligands to dopamine D2 receptors has also

been reported in a few neurological disorders, including Huntington's chorea (*16*) and progressive supranuclear palsy (*7,10*). Thus, this method might be useful for differentiating various diseases with movement disorders.

CONCLUSION

Among the various radioligands for D2 receptor imaging with SPECT, 2′-ISP is a representative radioligand of butyrophenone derivatives, but it has no affinity to serotonin receptors. The kinetic behavior of 2′-ISP is slower than IBZM and IBF. Both benzamide derivatives are significantly influenced by endogenous dopamine levels. This can be avoided by using a much higher affinity ligand, such as iodolisuride, but then the equilibration condition would hardly be reached. It is necessary to use a ligand with appropriate affinity and kinetics for quantitative receptor imaging. Although both benzamides and butyrophenones bind to D2 receptors, each tracer group may have its own respective clinical uses, especially in monitoring therapeutic drug effects. Because butyrophenones have been commonly used as neuroleptics, a radiolabeled analog should have potential use in monitoring the effects of these drugs. As demonstrated in this study, a relatively low target-to-nontarget ratio may limit their clinical use and further modification of the compound would be needed to develop the ligands with better kinetic characteristics for clinical use.

ACKNOWLEDGMENTS

The authors thank Mr. Toru Fujita for his valuable technical assistance. This work was supported in part by a Japan Ministry of Health and Welfare research grant for nervous and mental disorders and a grant-in-aid for Scientific Research (06404031) from the Japan Ministry of Education, Science and Culture.

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