Simultaneous SPECT Studies of Pre- and Postsynaptic Dopamine Binding Sites in Baboons

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The central nervous system dopamine transporters (DATs) and dopamine D_2/D_3 receptors are implicated in a variety of neurological disorders. Both sites are also targets for drug treatment. With the successful development of [^{99m}Tc]TRODAT-1, single-isotope imaging studies using this ligand for DAT imaging can be complemented by additional use of ^{123}I-labeled D_2/D_3 receptor ligand co-injected to assess both pre- and postsynaptic sites of the dopaminergic system simultaneously. **Methods:** Twelve SPECT scans of the brain were obtained in two baboons after intravenous administration of 740 MBq (20 mCi) [^{99m}Tc]-TRODAT-1 (technetium, [2-[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3,2,1]oct-2-yl]methyl](2-mercaptoethyl) amino]ethyl]-amino]ethanethiolato (3-)]- oxo-[1R-(exo-exo)]) and 185 MBq (5 mCi) [^{123}I]iodobenzamide or [^{123}I]iodobenzofuran. SPECT data were acquired by a triple-head gamma camera equipped with ultra-high-resolution fanbeam collimators (scan duration = 210 min). Two sets of SPECT data were obtained using energy windows of 15% centered on 140 keV for ^{99m}Tc and 10% asymmetric with a lower bound at 159 keV for ^{123}I. After coregistration with MRI, region-of-interest analysis was performed using predefined templates from coregistered MRI. In blocking studies, baboons were pretreated with N-methyl-2β-carbomethoxy-3β-(4-fluorophenyl)tropane (CFT, 14 mg) or raclopride (14 mg) to block DAT or D_2/D_3 binding site, respectively. **Results:** Image quality of dual-isotope studies was similar to that obtained from single-isotope studies. When one site was blocked with CFT or raclopride, the binding of the respective ligand to the other site was not affected. **Conclusion:** This is the first example that clearly demonstrates the feasibility of simultaneous imaging of both pre- and postsynaptic sites of the dopaminergic system in baboons with dual-isotope SPECT studies. With or without corrections for cross-contamination of ^{123}I into the ^{99m}Tc window, striatum-to-cerebellum ratios (target-to-nontarget) of dual-isotope experiments did not differ significantly from single-isotope experiments. This method may be a valuable and cost-effective tool for gaining comprehensive information about the dopaminergic system in one SPECT imaging session.

Key Words: dopamine transporters; dopamine D_2/D_3 receptors; SPECT imaging; dual-isotope studies

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The dopamine transporter (DAT), located on the presynaptic dopamine neuron, is the principal mechanism for regulating synaptic dopamine levels in the synaptic cleft. The majority of dopamine is taken up into the neurons by this process and is repackaged into presynaptic vesicles to be released in the next neurotransmission signal. The striatum is a dopamine-rich region and consequently has high densities of DAT (*1*). As a result of its significant role in dopamine regulation, DAT is a targeted site for various psychoactive drugs, including methylphenidate, cocaine and amphetamines (*2*). Most radiopharmaceuticals that target DAT have been labeled with positron-emitting isotopes (*3*) or the relatively expensive single-photon emitter ^{123}I (*4–6*). The growing interest in imaging studies on the dopaminergic system, particularly of the presynaptic site, DAT, and the availability of ^{99m}Tc in every clinical nuclear medicine facility have led to efforts to develop ^{99m}Tc-labeled ligands that are suitable as routine imaging agents for SPECT. The use of ^{99m}Tc-based small molecules as site-specific imaging agents has been reviewed (*7*) and several ^{99m}Tc-labeled tropanes have been reported (*8–11*). [^{99m}Tc]TRODAT-1 (technetium, [2-[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3,2,1]oct-2-yl]methyl](2-mercaptoethyl) amino-]ethyl]amino]ethanethiolato (3-)]- oxo-[1R-(exo-exo)]), a novel ^{99m}Tc-labeled tropane derivative, was initially evaluated and characterized as a potential imaging agent for DAT (*10,12*). Further pharmacological characterization of in vivo binding of [^{99m}Tc]TRODAT-1 demonstrated that the binding of this new ^{99m}Tc complex in the brain was associated with DAT (*13*).

The postsynaptic dopaminergic receptors, dopamine D_2/D_3 receptors, have been studied in vivo using SPECT with [^{123}I]iodobenzamide (IBZM) (*14*) or [^{123}I]iodobenzofuran (IBF) (*15*). Both iodinated ligands have been shown to bind reversibly with high affinity and high specificity to striatal D_2/D_3 receptors (*16*). Using [^{123}I]IBF or [^{123}I]IBZM in conjunction with SPECT, researchers have evaluated the status of the D_2/D_3 receptors in a wide variety of diseases (*14,17,18*).

It is generally recognized that imaging pre- and postsynaptic sites of the dopaminergic system is important for understanding the status of the dopaminergic system and that the images provide critical information for patient

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management. It is possible to achieve this by using ^{123}I-labeled compounds in two separate sessions. The limitation of this is that two scans must be separated by several days to allow the radioactivity of the first scan to decay. During that interval, the patient's condition and medication may have changed and the patient will most likely be in a different position on the table. Many patients, particularly psychiatric patients, may have difficulty lying still for SPECT data acquisition. Dual-isotope studies would reduce by half the time spent on the table compared with two scans performed independently, and costs would be minimized. Simultaneous assessment of pre- and postsynaptic binding sites of the dopaminergic system can facilitate the diagnosis of a disease involving the dopaminergic system. The simultaneous SPECT study can avoid the pitfalls mentioned previously, therefore, full information about the status of the DAT and D_2/D_3 receptors would be obtained in one SPECT imaging setting with images that are in accurate spatial registration to each other. The images of each energy window are in anatomic registration within 1 mm. Therefore, the influence on data analysis from placement of regions of interest (ROIs) from studies performed on different days can be eliminated. Motion artifacts can also be reduced using the dual-isotope approach. These studies can be performed in most nuclear medicine facilities with commercially available gamma cameras.

There is a clear advantage to investigating DAT and D_2/D_3 receptors using dual-isotope imaging studies with [^{99m}Tc]-TRODAT-1 and [^{123}I]IBF or [^{123}I]IBZM. It has been shown that dual-isotope imaging with ^{99m}Tc and ^{123}I is feasible and information from both radionuclides can be acquired simultaneously from appropriately selected energy windows (*19–21*). The objective of this study was to evaluate this approach in vivo using [^{99m}Tc]TRODAT-1 and [^{123}I]IBF/[^{123}I]IBZM to simultaneously image DAT and D_2/D_3 receptors in healthy baboons. Additionally, the baboons were pretreated with N-methyl-2β-carbomethoxy-3β-(4-fluorophenyl)tropane (CFT; also known as WIN 35,428) or raclopride to block pre- or postsynaptic dopamine binding sites, respectively, and thereby to mimic conditions that may occur in patients displaying a loss of either DAT or D_2/D_3 receptors.

MATERIALS AND METHODS

Radiopharmaceuticals and Drugs

Preparation of [^{99m}Tc]TRODAT-1, [^{123}I]IBF and [^{123}I]IBZM was performed according to methods published previously (*12,22,23*). CFT was synthesized in our laboratory. Raclopride was purchased from Research Biochemicals (Natick, MA).

Animals

A series of 12 SPECT scans was performed on two female baboons (*Papio anubis*) under protocols approved by the local animal care committee and consistent with guidelines of the National Institutes of Health. The interval between each successive study was at least 2 wk. After fasting overnight, animals were immobilized with an intramuscular injection of Saffan® (1 mg/kg) (0.9% w/v alfaxalone, 0.3% alfadolone acetate; Pittman-Moore, Middlesex, UK). During the scanning procedure, anesthesia was maintained by intravenous (tibial vein) infusion of a solution of Saffan® (2.4 mg/mL) at a rate of 45 mL/h. Passive inhalation of oxygen (1.5 L/min) was also maintained to keep the oxygen saturation >95%. An additional intravenous line in a superficial brachial vein was used for hydration (0.9% physiological saline solution, 5 mL/kg/h), as well as for ligand administration. A cylindrical polycarbonate positioning device equipped with a customized foam head-holder enabled reproducible placement of the animal in the SPECT camera. The core body temperature was maintained using a circulating warm water pad maintained at 37°C. A single-bolus injection of [^{99m}Tc]TRODAT-1 and [^{123}I]IBF (or [^{123}I]IBZM) was administered immediately before each scan with a mean bolus injection of 729.64 ± 45.88 MBq (19.72 ± 1.24 mCi) [^{99m}Tc]TRODAT-1 and 183.89 ± 19.61 MBq (4.97 ± 0.53 mCi) [^{123}I]IBF (or [^{123}I]IBZM). A total of eight blocking studies were performed; four with the additional injection of raclopride (1 mg/kg) and another four with the additional injection of CFT (1 mg/kg). These compounds were injected 5 min before the administration of the radiopharmaceuticals.

Image Acquisition and Processing

For SPECT image acquisition, we used a triple-head gamma camera equipped with ultra-high-resolution fanbeam collimators (Picker Prism 3000S, Picker International, Cleveland, OH). The acquisition parameters comprised a rotational radius of 14 cm, a 15% energy window centered on 140 keV and a 10% asymmetric energy window with a lower bound at 159 keV, 120 projection angles over 360° and a 128 × 128 matrix with a pixel width of 2.11 mm in the projection domain. Data collection started 20 min after injection. Three 10-min scans (15 s/step) followed by two 40-min scans (60 s/step) were obtained over a total of 210 min. The projection images were reconstructed by filtered backprojection. Then a three-dimensional, count-rate dependent postprocessing filter (Wiener filter, Picker International) with a modulation transformer function specific to both ^{99m}Tc and ^{123}I was applied. For uniform attenuation correction, Chang's first-order method was used. MR images were acquired on a 1.5-T instrument (General Electric Medical Systems, Milwaukee, WI) using a repetition time of 3000 ms and echo times of 30 and 80 ms with 5-mm spacing that produces 0.97 × 0.97 × 1 mm voxels. Slices were interleaved so that there was no interslice gap. The MR images were resized and resliced in planes parallel to the one containing the anterior and posterior commissures. ROIs for the nucleus caudate and the putamen were drawn directly onto the MR image inside the outer edge of each structure to minimize the effects of volume averaging. Coregistration of the SPECT scans and the MRI was achieved using a customized software package developed to rotate and translate each SPECT scan with respect to a fixed MRI dataset. Fusion images of the SPECT and MR images were generated to visually validate the accuracy of the coregistration in the sagittal, coronal and transverse planes. To assess specific tracer uptake in the striatum, we used the ROI template created with the MRI. Mean specific uptake in the striatum region (STR) was calculated by subtracting the mean counts per pixel in the cerebellum as background (BG) from the mean counts per pixel in the STR and dividing the result by the mean counts per pixel in the BG, (STR − BG)/BG. Uptake values were expressed as corrected values on the basis of the results of the phantom studies for cross contamination. The ^{99m}Tc scans were corrected for down contamination of ^{123}I by subtracting 14.9% of the ^{123}I counts. ^{123}I scans were corrected by

subtracting 0.54% of the ^{99m}Tc counts. For statistical analyses, analysis of variance (ANOVA), regression analyses (least-squares method) and the Student t test were used. Monoexponential or linear fitting was applied to all data. Data were considered to be statistically significant with a P value of <0.05. Results were compared with single-isotope SPECT scans of [^{99m}Tc]TRODAT-1, which were acquired previously.

RESULTS

A total of 12 dual-isotope SPECT studies were performed: 4 control studies, 4 studies with pretreatment of CFT and 4 studies with pretreatment of raclopride. Preliminary studies indicated that no statistically significant difference was observed for [^{99m}Tc]TRODAT-1 in both dual-isotope and single-isotope scans (data not given). The average specific uptake values for [^{99m}Tc]TRODAT-1 at 210 min after injection were 2.18 ± 0.11. The slope of the uptake curves did not show any significant differences between single- and dual-isotope experiments, and the uptake ratios correlated well (0.95). Figure 1 displays typical SPECT scans of [^{123}I]IBF and [^{99m}Tc]TRODAT-1 at 170–210 min postinjection and the coregistered MR images. Similar scans of dual-isotope studies with [^{123}I]IBZM and [^{99m}Tc]TRODAT-1 were observed (data not given). Both SPECT scans show normal uptake and homogeneous binding of the radiopharmaceutical to the binding site of the baboon striatum. Specific uptake of [^{123}I]IBF was calculated as 9.59 ± 0.77 and of [^{123}I]IBZM as 2.91 ± 0.19. Under pretreatment with raclopride (1 mg/kg), a specific blocker for D_2/D_3 receptors, the specific binding of [^{123}I]IBF in the nucleus caudate-putamen area of the baboons was dramatically diminished (Fig. 2A), while the simultaneously acquired scan of [^{99m}Tc]-TRODAT-1 remained unchanged (Fig. 2B). Similar results were observed for [^{123}I]IBZM scans under pretreatment with raclopride (data not given). The semiquantitative analysis revealed a comparable specific uptake value for [^{99m}Tc]-TRODAT-1 (reflecting DAT uptake) in normal and raclopride-treated baboon studies (Fig. 3A), whereas the specific uptake for [^{123}I]IBF or [^{123}I]IBZM, unlike the untreated ratios, increased only slightly over time, resulting in a markedly diminished specific binding of 1.02 ± 0.03 and 0.68 ± 0.05 for [^{123}I]IBF and [^{123}I]IBZM, respectively (Fig. 3B), in raclopride-treated baboons. The results of pretreatment with CFT are shown in Figure 4 for the presynaptic receptor ^{99m}Tc-labeled compound and for the postsynaptic ^{123}I-labeled compounds (IBF and IBZM, respectively). Because of the high nonspecific binding of [^{99m}Tc]TRODAT-1 compared with [^{123}I]IBF, the blockade of the DAT binding sites does not reveal an "empty shell" image like the one of [^{123}I]IBF in a raclopride-pretreated baboon. However, the SPECT images of the ^{99m}Tc window revealed a remarkable reduction of specific uptake of [^{99m}Tc]TRODAT-1 with a specific uptake value of 0.32 ± 0.06 for the crosstalk corrected scans at 210 min postinjection (Fig. 5). The specific uptake ratio curve of CFT-treated studies increases only slightly over time, suggesting the blockade of specific binding of [^{99m}Tc]TRODAT-1 to DAT. This result is in concordance with the effect of CFT pretreatment on [^{99m}Tc]-TRODAT-1 binding to DAT in rats (*24*). The binding of [^{123}I]IBF (or [^{123}I]IBZM) to D_2/D_3 receptors in CFT-pretreated baboons, as shown in Figure 5B, however, does not significantly differ from the scans without any pretreatment.

DISCUSSION

The dual-isotope SPECT imaging performed in this study in baboons provided similar information and image quality as the corresponding single-isotope studies. We also used the additional pretreatment of drugs that are known to selectively block one binding site to mimic the loss of this site with the other site remaining intact. The blocking studies with CFT or raclopride, specific for DAT or dopamine D_2/D_3 receptors, respectively, indicated that there is no effect on the radiopharmaceutical binding to the sites that were not blocked. These findings are in concordance with the experimental phantom studies (data not given) and may represent

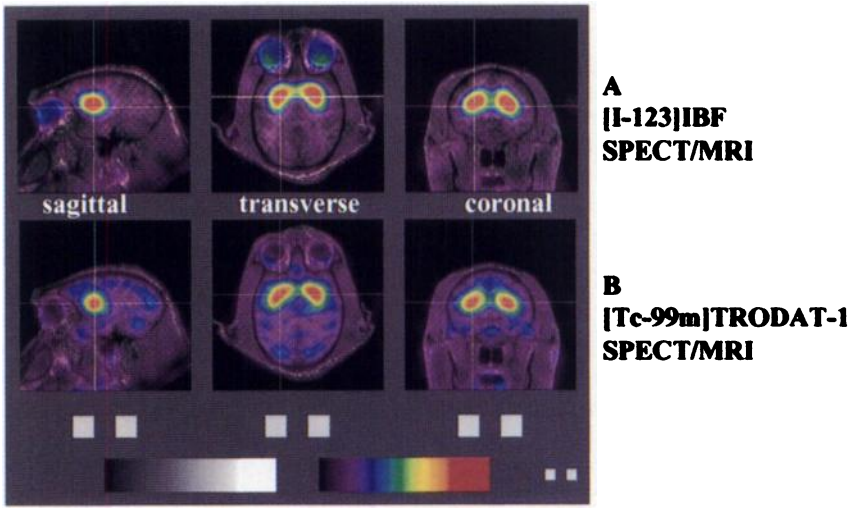

FIGURE 1. Dual-isotope SPECT scan with [^{99m}Tc]TRODAT-1 and [^{123}I]IBF 170–210 min postinjection. (A) [^{123}I]IBF coregistered to T1-weighted MR image in sagittal, transverse and coronal views displays normal binding of ligand to dopamine D_2/D_3 receptors. Specific binding of 9.6 is demonstrated. (B) [^{99m}Tc]-TRODAT-1 coregistered to T1-weighted MR image in sagittal, transverse and coronal views reveals regular binding of radiopharmaceutical to DAT. Specific binding is 2.2 for crosstalk corrected image.

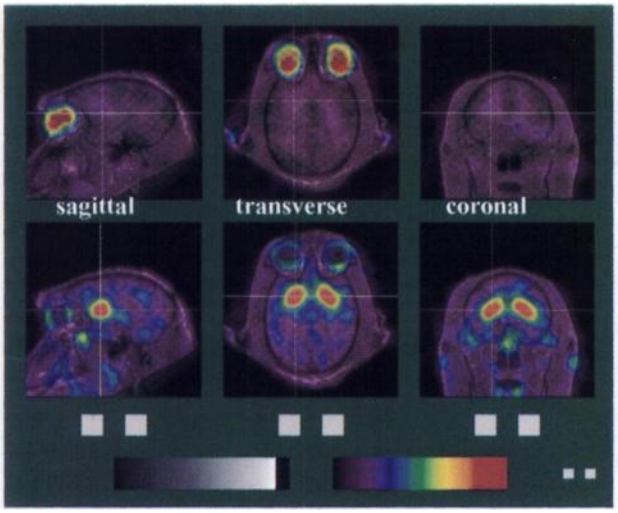

FIGURE 2. Dual-isotope SPECT scan with [^{99m}Tc]TRODAT-1 and [^{123}I]IBF 170–210 min postinjection. Baboon was pretreated with raclopride (1 mg/kg) 5 min before injection of radiopharmaceuticals. (A) [^{123}I]IBF coregistered to T1-weighted MR image in sagittal, transverse and coronal views displays marked reduction of specific binding of ligand to dopamine D_2/D_3 receptors. There is almost no uptake visible in striatal area. Scan reveals specific binding of 1.02. (B) [^{99m}Tc]-TRODAT-1 coregistered to T1-weighted MR image in sagittal, transverse and coronal views shows regular binding of radiopharmaceutical to DAT as seen in Figure 1B. Specific binding is 2.18 for crosstalk corrected image.

conditions that may appear in patients suffering from various diseases.

There is considerable interest in using in vivo imaging techniques to study the dopaminergic system in the human brain. Mozley et al. (*25*) found a strong correlation between DAT density and age. A close relationship between striatal [^{123}I]N-(3′-iodopropen-2′-yl)-2β-carbomethoxy-3β-(4-chlorophenyl)tropane (IPT) or [^{123}I]β-(1R)-2β-carbomethoxy-3β-(4-iodophenyl)tropane (CIT) binding and clinical features of patients suffering from Parkinson's disease was observed, suggesting that this method can be used to objectively follow the course and progression of the disease (*5,26*). The potential of imaging the DAT will be further enhanced and broadened with the availability of a ^{99m}Tc-labeled ligand,

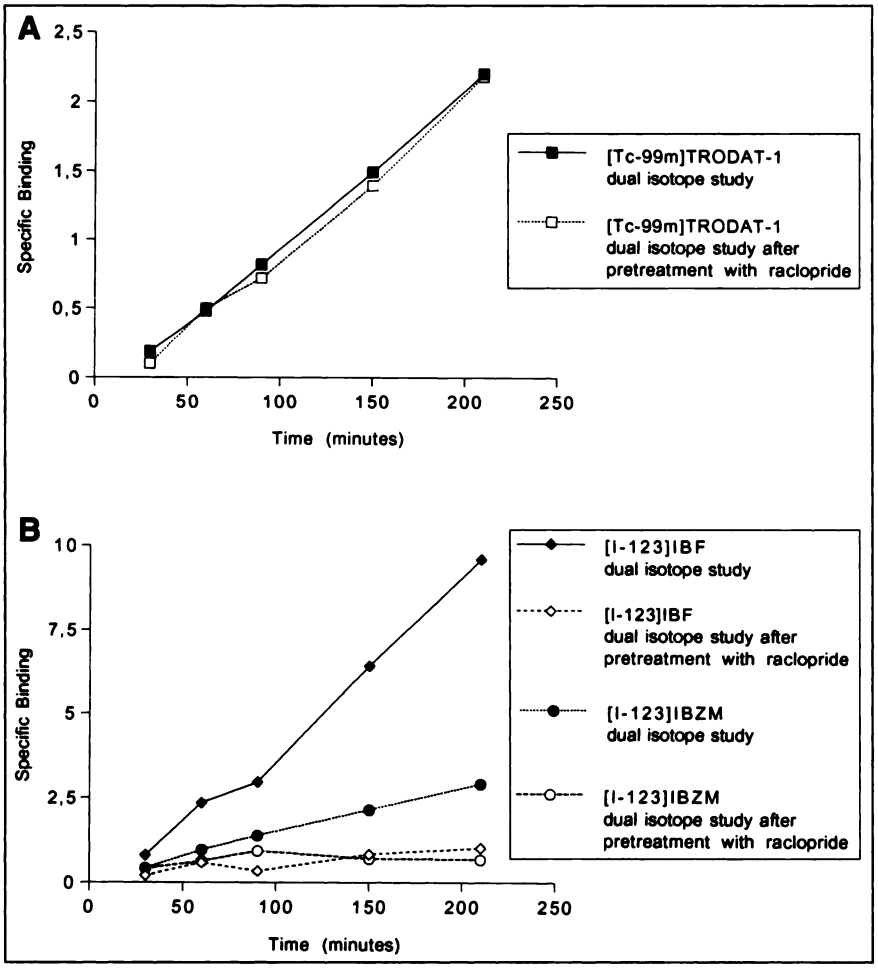

FIGURE 3. Dual-isotope SPECT studies with [^{99m}Tc]TRODAT-1 and [^{123}I]IBF (or [^{123}I]IBZM). Baboons were pretreated with raclopride (1 mg/kg) 5 min before injection of radiopharmaceuticals. (A) Specific binding is displayed for dual-isotope [^{99m}Tc]-TRODAT-1 SPECT scans corrected for crosstalk for control baboons and baboons that were pretreated with raclopride. Data for blocking with raclopride does not significantly differ from data obtained without blocking. (B) Specific binding is displayed for dual-isotope SPECT scans of [^{123}I]IBF and [^{123}I]IBZM in baboons with and without pretreatment with raclopride. Binding of [^{123}I]IBF (or [^{123}I]IBZM) to dopamine D_2/D_3 receptors is markedly diminished.

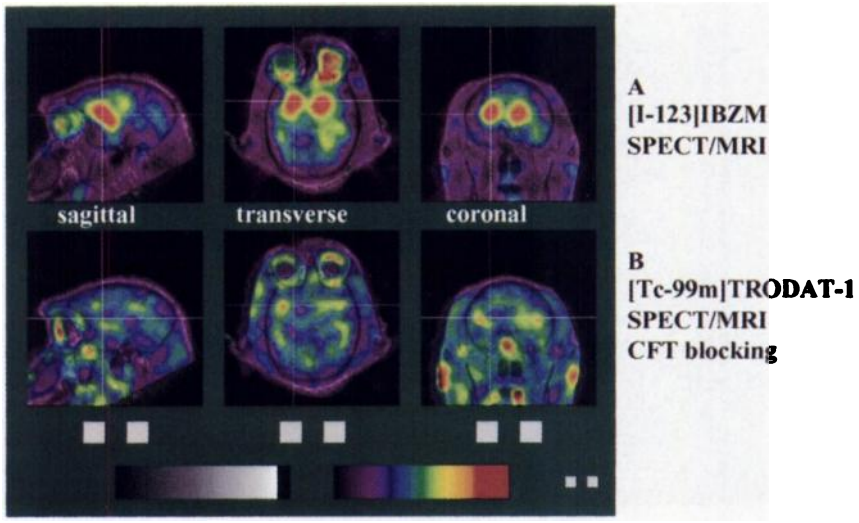

FIGURE 4. Dual-isotope SPECT scan with [^{99m}Tc]TRODAT-1 and [^{123}I]IBZM 170–210 min postinjection. Baboon was pretreated with CFT (1 mg/kg) 5 min before injection of radiopharmaceuticals. (A) [^{123}I]IBZM coregistered to T1-weighted MR image in sagittal, transverse and coronal views displays normal and homogeneous specific binding of ligand to dopamine D_2/D_3 receptors. Scan reveals specific binding of 2.93. (B) [^{99m}Tc-]TRODAT-1 coregistered to T1-weighted MR image in sagittal, transverse and coronal views shows heterogeneous and markedly reduced binding of radiopharmaceutical to DAT. Note relatively high nonspecific background uptake, which is virtually indistinguishable from any uptake in striatum area. Specific binding is 0.32 for crosstalk corrected image.

which may readily be used in every nuclear medicine facility. There are numerous clinical advantages of [^{99m}Tc]-labeled ligands compared with [^{123}I]-based central nervous system receptor imaging compounds. Most important, ^{99m}Tc is the radionuclide of choice for nuclear medicine because it is readily available, is relatively inexpensive and gives lower radiation exposure compared with ^{123}I. It has been established by in vivo and in vitro studies that [^{99m}Tc]TRODAT-1 reliably assesses the DAT status of healthy baboons and humans and yields high-contrast SPECT imaging at 2–3 h

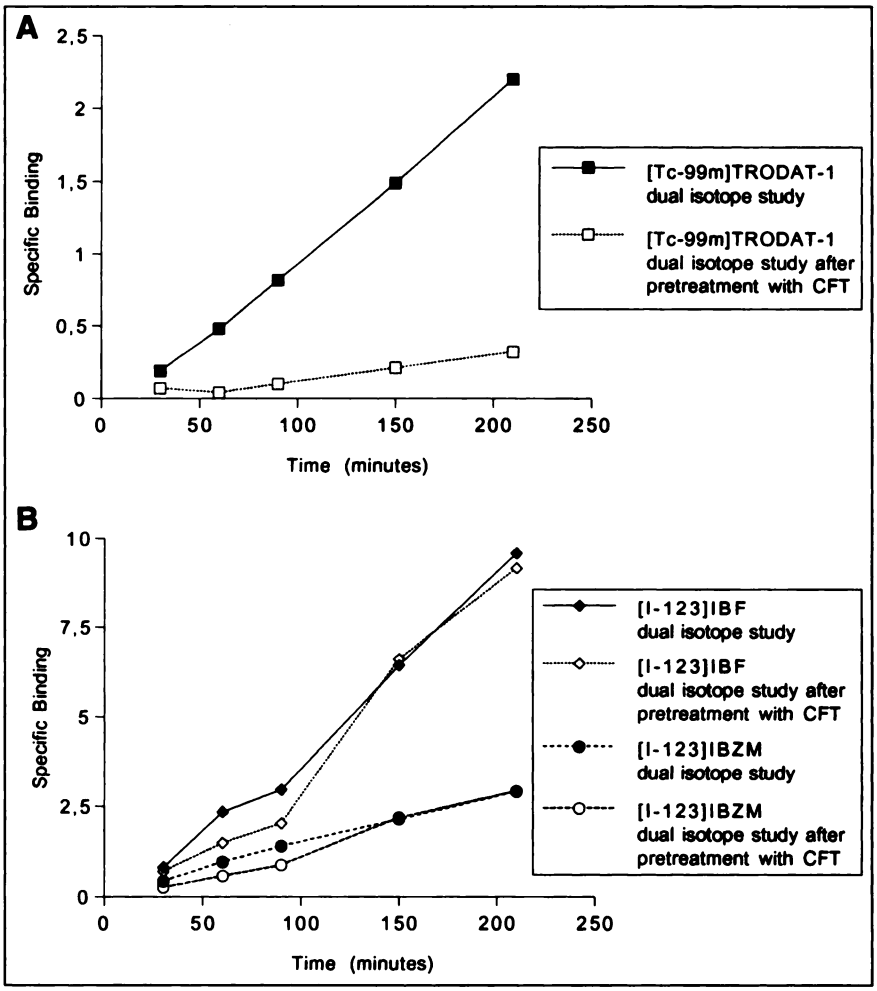

FIGURE 5. Dual-isotope SPECT studies with [^{99m}Tc]TRODAT-1 and [^{123}I]IBF (or [^{123}I]IBZM). Baboons were pretreated with CFT (1 mg/kg) 5 min before injection of radiopharmaceuticals. (A) Specific binding is displayed for the dual-isotope [^{99m}Tc]-TRODAT-1 SPECT scans corrected for crosstalk with and without pretreatment with CFT. Uptake is significantly reduced compared with SPECT scans in untreated baboons. (B) Specific binding is displayed for dual-isotope SPECT scans of [^{123}I]IBF and [^{123}I]IBZM with and without pretreatment with CFT. CFT pretreatment of baboons did not affect binding of [^{123}I]IBF and [^{123}I]IBZM.

after injection (*10,13*). The recently completed phase I study of [^{99m}Tc]TRODAT-1 demonstrated that this ligand is safe and suitable for human imaging (*27*).

Studies of the dopamine D_2/D_3 receptors with [^{123}I]IBZM SPECT imaging have been performed since 1988. There is a wealth of information on its clinical applications based on more than 150 articles published in the literature. Currently, [^{123}I]IBZM is commercially available in Europe, and [^{123}I]IBF is undergoing phase III clinical trial in Japan. Because of their potential clinical application in the future, we chose these two D_2/D_3 receptor imaging agents for this study. It was reported that the most effective use of [^{123}I]IBZM SPECT studies is to differentiate the idiopathic and nonidiopathic parkinsonian syndrome (*14*). The occupancy of dopamine D_2/D_3 receptors in schizophrenic patients under treatment with neuroleptics and its relationship with extrapyramidal side effects has been described in several studies (*17,18,28*). The coexistence of both Parkinson's disease and schizophrenia has been reported. The comorbidity makes the differential diagnosis difficult. These patients are usually treated with neuroleptics that might cause the parkinsonian syndrome by binding to the D_2/D_3 receptor (*18,29–31*). Simultaneous assessment of both sites of the dopaminergic system will significantly simplify the correct diagnosis. Furthermore, the involvement of the dopaminergic system in many other diseases associated with movement disorders has not yet been fully explored. It has been reported that in patients suffering from Huntington's disease the function of the dopaminergic system might be compromised (*32*). Other movement disorders, such as amyotrophic lateral sclerosis, multiple sclerosis, multisystem atrophy, Wilson's disease and progressive supranuclear palsy, have been evaluated for alteration of either the dopamine transporter or D_2/D_3 receptor system (*33,34*). The simultaneous dual-isotope imaging technique proposed in this article may provide an even more powerful tool for improving the diagnosis of these complex disorders.

In general, SPECT imaging with [^{99m}Tc]TRODAT-1 gives lower target-to-nontarget ratios (2–3) than ^{123}I tropane derivates (10–15 for β-CIT and IPT). This may be due to its lower binding affinity to DAT and a faster in vivo metabolism (*35*). In addition, the down contamination of ^{123}I into the ^{99m}Tc window may further contribute to the high background activity. It is important to note that the slope of the uptake and retention curves with [^{99m}Tc]TRODAT-1 did not differ significantly between single- and dual-isotope studies. The in vivo studies in primates further confirm the results obtained from phantom studies. The cross contamination of ^{123}I into the ^{99m}Tc windows is consistent over a wide range of activities and therefore does not change relative uptake ratios significantly. The target-to-background ratios of the uncorrected images have shown a trend to be slightly higher compared with the corrected data due to the contamination of the other isotope. However, this difference was not statistically significant ($P < 0.05$). The loss of one binding site (mimicked by drug pretreatment) can be imaged accurately in the presence of unchanged binding of the other sites in the striatum. The crosstalk corrections in each energy window did not change the outcome measure significantly.

To our knowledge, there is no previously published study on simultaneous dual-isotope imaging of the dopaminergic system. However, there are reports of studies using ^{99m}Tc- and ^{123}I-labeled radiopharmaceuticals on cardiac imaging (*36*) and brain blood-flow imaging (*19–21,37*). There is a major difference between the blood-flow studies and the specific binding site imaging presented in this article. The distribution mechanisms of both agents used for the blood-flow studies are similar. Generally, they are nonspecific simple diffusion mechanisms. The ligands used in this study, however, target different sites, pre- and postsynaptic dopaminergic binding sites, which are functionally distinct and separated by the synaptic cleft about a few microns apart. Thus, subtle changes in physiological conditions of one binding site may or may not directly influence the other binding site. The binding sites for [^{99m}Tc]TRODAT-1 and [^{123}I]IBF (or [^{123}I]IBZM) in the dopaminergic system are functionally different, even though their localization in the striatum is identical. Partial volume effects in SPECT imaging are of importance, but it is expected that the effects on ^{123}I and ^{99m}Tc are similar. The net effects on the specific binding ratios are not dramatically different between these two isotopes. In contrast, the differences in blood-flow images, for example, during monitoring cognitive activation or measuring minor increases in regional cerebral blood flow after stimulation, may be more subtle and not as characteristic and anatomically well defined as in SPECT scans of the striatum (*19*).

In this study, no effort was made to obtain quantitative kinetic information. The blocking studies were performed using a relatively high blocking dose: raclopride (1 mg/kg) and CFT (1 mg/kg) for D_2/D_3 receptors and DAT sites, respectively. One should view the data in this article as preliminary proof of principle in using simultaneous dual-isotope SPECT to study pre- and postsynaptic dopamine binding sites. A more detailed kinetic study, including a full evaluation of input function and in vivo metabolites, will be necessary to validate the quantitative aspect for the binding site measurements. In addition, there will be shades of gray (*38*) on changes of binding sites in normal and disease states; therefore, a careful consideration of detection sensitivity of this imaging technique is required. It is encouraging that both of these issues have been addressed previously using single-isotope SPECT imaging technique for D_2/D_3 receptors and DAT binding sites. At tracer levels, both of the ligands used for measuring pre- and postsynaptic dopamine binding sites behave independently without interfering with each other. Therefore, it is likely that the kinetic modeling of both ligands will follow a similar model as that reported for single-isotope studies. A dual-isotope kinetic modeling study in vivo will be evaluated and published in the future.

CONCLUSION

We have demonstrated dual-isotope imaging of the dopaminergic system using [^{99m}Tc]TRODAT-1 and [^{123}I]IBF (or [^{123}I]IBZM) in nonhuman primates. Simultaneous imaging of both pre- and postsynaptic binding sites of the dopaminergic system is feasible and produces images of comparable quality as single-isotope scans. To our knowledge, this is the first example of dual-isotope SPECT imaging of the DATs and dopamine D_2/D_3 receptors in the living brain.

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