In Vivo SPECT Imaging of CNS D-2 Dopamine Receptors: Initial Studies with Iodine-123-IBZM in Humans

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Iodobenzamide (IBZM) is a D-2 dopamine receptor antagonist. In this paper the results of Phase I clinical studies of iodine-123- (^{123}I) IBZM in humans are reported. Preliminary imaging studies, both planar and single-photon emission tomography (SPECT), of no-carrier added [^{123}I]IBZM in humans show specific localization in the basal ganglia of the brain. At 2 hr after an i.v. injection, the brain uptake was 3.72% of the dose, and at 20 hr later the uptake diminished to 0.7%. Radiation dosimetry calculation indicated that the radiation dose to the brain was minimum, 0.039 rad/mCi, while the large intestine wall received the highest dose, 0.28 mrad/mCi. The radiation dosimetry and pharmacology data suggest that this agent is safe for human use.

J Nucl Med 1990; 31:573–579

Recently, in vivo imaging of the dopaminergic system in the mammalian brain has been the subject of extensive studies. Not only does this system play a role in the coordination of normal brain function, it is also a primary action site for neuroleptic drugs for treating schizophrenia and Parkinson's disease. A wealth of pharmacologic information is known about the dopamine receptor (*1–4*). On the basis of the ability of agonists and antagonists to discriminate between two different and distinct types of dopamine receptors, which are designated as D-1 and D-2, it is generally accepted that there are two dopamine receptor populations (*5–7*). These two subtypes of dopamine receptors interact with each other and affect the activity of central nervous system (CNS) dopaminergic neurons (*8–10*). Several human CNS diseases: schizophrenia, tardive dyskinesia, Parkinson's disease, and Huntington's chorea, involve changes in dopamine receptor density in the brain (*11–13*). The potential for providing important information for understanding the dopaminergic system and assisting in patient management makes in vivo imaging an attractive technique.

A variety of substituted benzamide derivatives possessing antidopaminergic properties have been reported (*14,15*). Of these, raclopride and eticlopride show specific D-2 antagonistic activity, high binding affinity (low K_d) in rat striatum tissue preparations and low nonspecific binding (Table 1). As indicated in Table 1, IBZM is a close analog of raclopride. The preliminary binding studies showed specific binding of [^{125}I]IBZM in a striatum membrane preparation of rat brain with a K_d value of 0.426 n*M*. The B_{max} of [^{125}I]IBZM in the striatum of rats was 480 fmol/mg of protein, which greatly exceeded the values for hippocampus (87 fmol/mg) and frontal cortex (47 fmol/mg). The B_{max} value is comparable to that reported in the literature using a similar procedure and [^{3}H]spiperone as the ligand (*16*). Biodistribution studies using dissection and autoradiographic techniques suggested that [^{125}I]IBZM was concentrated in rat striatum with high affinity to the D-2 receptor (*17–19*). In vivo planar imaging of the monkey showed significant brain uptake immediately after the i.v. injection of [^{123}I]IBZM (*20*). The brain uptake appeared to reach a maximum at ~10 min postinjection, and the basal ganglia region was apparent at 5 min. Since the agent is a lipid-soluble material (partition coefficient: 1-octanol/buffer = 111 and 211, at pH 7.0 and 7.4, respectively), it is likely that IBZM penetrates the blood-brain barrier by a simple diffusion mechanism. The ratios of the basal ganglia to cerebellum, and the cortex to cerebellum were 4.93 and 1.44, respectively, at 120 min postinjection (*20*).

Based on the animal data, it is predicted that this agent will be useful in imaging CNS D-2 dopamine receptors in humans. In this paper, the results from a Phase I study of [^{123}I]IBZM in humans are reported.

Received Sept. 5, 1989; revision accepted Nov. 16, 1989.

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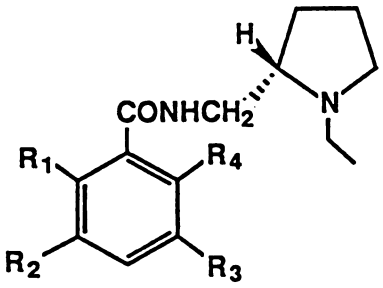
TABLE 1
Chemical Structures and In Vitro Binding Constants of Benzamides



Compound	R_1	R_2	R_3	R_4	K_d (n*M*)	Ref
Raclopride	OH	Cl	Cl	OMe	1.1	15
Eticlopride	OH	Cl	Et	OMe	0.17	15
IBZM	OH	I	H	OMe	0.43	19
BZM	OH	H	H	OMe	30.0	19
Iodopride	H	I	H	OMe	1.5	51

MATERIALS AND METHODS

Synthesis and Radiolabeling

Both unlabeled BZM and IBZM were prepared by a method described previously (*19*). The radioactive labeling of IBZM was accomplished by the hydrogen peroxide method. Hydrogen peroxide (100 μl, 3%) solution was added to a mixture of BZM (50 μl, 1 mg/ml EtOH), sodium [^{123}I]iodide (10 μl, 2–20 mCi, no-carrier added (n.c.a.), sp. act. 240,000 Ci/mmol; Nordion International Inc., Vancouver, Canada produced by Xe(p,2n) reaction, no detectable ^{124}I or ^{125}I) and pH 3 phosphate solution (0.3 ml) in a sealed vial. The reaction was allowed to proceed at 100°C for 30 min. At the end of the heating period, the reaction was terminated by addition of sodium bisulfite (0.1 ml, 50 mg/ml) and neutralized with 0.4 N sodium bicarbonate (0.5 ml). The product was extracted with ethyl acetate (3 × 1 ml). The combined organic layers were dried by passing through an anhydrous sodium sulfate column (0.2 cm × 5 cm). The organic solution was condensed under a stream of nitrogen, and the residue was dissolved in absolute ethanol (50–200 μl). The desired product, [^{123}I]IBZM, was isolated from the unreacted BZM and a small amount of unknown radioactive impurities by HPLC on a reverse-phase column (PRP-1, Hamilton, Two Rivers, WI), eluting at 0.8 ml/min with acetonitrile: ammonium phosphate (4 m*M*, pH 7.0) solvent (82:18). The fractions containing the n.c.a. product were mixed with 100 μg ascorbic acid and this mixture was evaporated to dryness. The residue was dissolved in 2–10 ml saline containing 100 μg ascorbic acid and sterilized by filtration through a 0.22-μ filter (acrodisc B, Gelman Sciences, Ann Arbor, MI). The solution was found to be sterile and free of pyrogenic material. The radiochemical purity of the n.c.a. product was >95%; the overall yield was 50%–70%. The total time required for preparation was ~2–3 hr. An improved method of preparation using peracetic acid as the oxidant was reported recently (*21*), however, the new method only expedites the preparation, but it does not affect the overall quality of the final product, [^{123}I]IBZM.

Imaging Studies

Subjects chosen for initial studies were male volunteers (a total of nine, age: 18–50, mean = 28) who had no history of prior neurologic or psychiatric disorders. Informed consent was obtained from each subject. Each volunteer underwent routine blood testing including a CBC, SMA-12, and a routine urinalysis before the study. Similar testing was also performed three days after the study to ensure there were no physiologic or pharmacologic effects from the study. An oral dose of Lugol's solution was given to each subject prior to the study to block thyroid uptake and was repeated at 24 hr.

A dose of 2–5 mCi of n.c.a. [^{123}I]IBZM was injected intravenously. Immediately after the injection, lateral images of the head were acquired (1 min/frame for 30–60 min low-energy collimator, 20% window at 150 keV). For SPECT imaging, a subject is positioned close to the center of rotation by visual inspection and by moving and rotating the camera head around so that the radius of rotation is reduced to a minimum. The computer is then set up to acquire 60 frames of 128×128 matrix images during a 360-degree rotation. Each of the images required 60 min imaging time. Standard commercial software, which was written for the computer system (MDS) connected to the SPECT camera (Siemens' dual-head Rotacam), was employed for processing the data (Fig. 1A). The data was presmoothed by 9-point-smooth technique for 5 times. Filtering as well as backprojection was performed, and subsequently, the attenuation correction was applied.

One study was conducted with a three-detector dedicated SPECT system (Triad, Trionix Research Corp., Twinsburg, OH) (Fig. 1B). For this study, a normal volunteer was injected with 4.8 mCi of n.c.a. [^{123}I]IBZM and the tomographic images were collected at 60–90 min postinjection. The tomographic image reconstruction includes presmoothing, filtering, backprojection, and attenuation correction.

Radiation Dosimetry

Biodistribution in normal volunteers was measured using a conjugated counting technique (*22*). Transmission measurements are made by positioning a 1-cm thick 20-mCi technetium-99m (^{99m}Tc) area source (50 × 50 cm) behind each organ and counting. Organs to be measured are lungs, liver, spleen, and brain. At 2 and 18 hr postinjection, spot images of the head, chest, and abdominal areas were acquired. The organ activities will be calculated according to the equation below. Following background subtraction, the equation will be solved on a pixel-by-pixel basis and the results summed over the organ area. Correction will be made for the difference in transmission between ^{123}I and ^{99m}Tc.

$$A = \left[\frac{{}^{I}A\,{}^{I}P}{e^{-\mu x}}\right]^{1/2} \frac{f}{C},$$

where

- A = organ activity (fraction of administered dose)
- C = activity in total dose (c/min)
- ^{I}A = anterior view counts (c/min)
- ^{I}P = posterior view counts (c/min)
- μ = effective linear attenuation coefficient through the patient (cm^{-1})
- x = patient thickness in region of organ (cm)
- f = correction for organ thickness.

Data from five normal volunteers were collected. The biodistribution data were used in conjunction with the MIR-

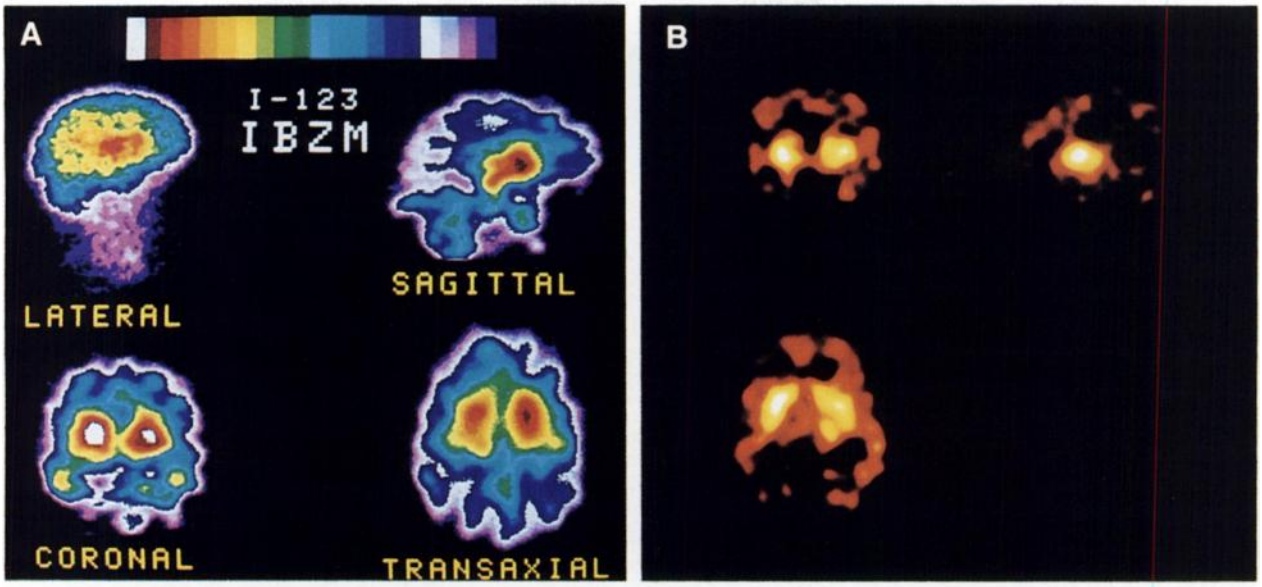


FIGURE 1
Planar and SPECT images of one normal volunteer after i.v. injection of [^{123}I]IBZM: planar right lateral view (A, upper left), SPECT imaging; saggital view; coronal view; transaxial view at the canto-meatal line. These images clearly demonstrate that the agent concentrated in the basal ganglia of a living human brain. Panel A (60–120 min postinjection) obtained by Rotacam (Siemens, Inc.). Panel B (60–90 min postinjection) obtained by Triad (Trionix Research Laboratories Inc.).

DOSE program (Oak Ridge National Laboratory, Oak Ridge, TN) to determine the radiation dosimetry.

Metabolism Studies

Venous blood samples (~3 ml each, at 2 min, 10 min, 30 min and 120 min) were collected in heparinized tubes at different time points after injection for the determination of the pharmacokinetics and the evaluation of metabolite(s) in the blood. Urine samples were obtained from some subjects, and the total radioactivity and total metabolite(s) in the urine were examined.

Total radioactivity in blood and urine were determined using a Beckman gamma counter (Model 4000, Waldwick, NJ). Total activity in the blood was calculated assuming that it is 7% of total body weight.

The plasma samples (1–1.5 ml) were separated from the formed elements by centrifugation and then they were extracted three times with an equal volume of ethyl acetate in the presence of a small amount of carrier, IBZM (100 μg). The combined ethyl acetate extracts were then evaporated; the resulting residue was dissolved in a small volume of EtOH and injected into high performance liquid chromatography (HPLC) (PRP-1 column) in a flow rate of 1 ml/min with an isocratic solvent (82% acetonitrile:18% 5 m*M* 3,3′-dimethylglutarate, pH 7.0). One milliliter fractions were collected during the chromatography and counted for radioactivity. The percentage of unmetabolized [^{123}I]IBZM in the blood can, thus, be calculated from the percentage of ethyl acetate extraction and the percentage of IBZM in the total HPLC profile. These values also were corrected by a recovery coefficient (determined by addition of [^{123}I]IBZM to whole blood and identical centrifugation, extraction, and chromatographic analysis).

Protein Binding

The binding of [^{125}I]IBZM to human plasma proteins was determined by equilibrium dialysis. Human plasma (0.3 ml, pooled) and 0.3 ml PBS containing radioactive material (~0.025 μCi) were separated by a dialysis membrane. The dialysis cells were rotated in a water bath at 37°C for 18 hr. At the end of incubation, aliquots from both sides were weighed and counted. The percentage of protein binding was determined by calculating the radioactivity concentration ratio of buffer to serum, multiplied by 100. To determine possible membrane binding, the membrane was counted at the end of experimentation. Less than 5% of the original activity was found on the membrane.

RESULTS

Imaging of D-2 Dopamine Receptors in the Living Human Brain with [^{123}I]IBZM

Immediately after injection, significant uptake in the brain was observed in the planar images of the subjects examined (Fig. 2). The summed planar images (lateral view, 45–60 min postinjection) clearly demonstrated that the agent was concentrated in the basal ganglia (Fig. 1A). The SPECT images [from data acquired between 60–120 min and 60–90 min for Fig. 1A (Siemens' Rotacam) and 1B (Triad), respectively] of the head confirmed that the agent localized in the basal ganglia region. Although some uptake was noted in other structures (cerebral cortex and cerebellum), the basal ganglia clearly appeared to be the primary site of concentration. Also, dynamic data indicated that significant washout took place from the sites of nonspecific binding (cortex and cerebellum).

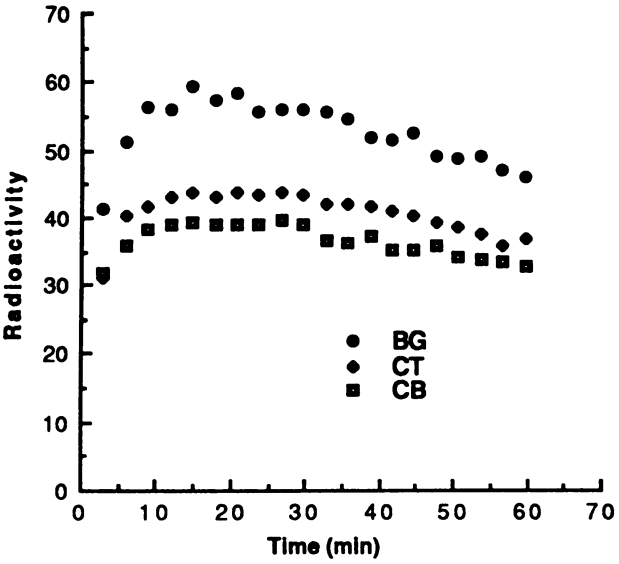


FIGURE 2
A representative uptake and retention curve of [^{123}I]IBZM in normal human brain. BG: basal ganglia, CT: cortex, CB: cerebellum.

Plasma Activity and Urine Excretion

Blood samples obtained from normal volunteers showed a rapid blood clearance (Fig. 3). At 5 min after the i.v. injection, the blood activity dropped to <1% of the dose. The radioactivity in the plasma can be extracted by ethyl acetate to separate the organic soluble and the nonorganic soluble component. The data of one subject, presented in Figure 3, clearly indicate that the organic extractable fraction remained constant from a few minutes after injection up to 1 hr postinjection. The nonextractable fraction, presumably metabolite (most likely free iodide), increased with time. This result suggests that the in vivo metabolism of IBZM is rapid and that a large portion of the metabolite is water soluble. The urine excretion pattern also confirmed the rapid clearance. Urine samples were collected from two subjects and both showed over 50% urine excretion per 24 hr (67% and 52%). In vitro plasma protein binding experiments suggest that IBZM is bound tightly to plasma proteins (12.3% and 7.8% unbound using two different plasma samples). This pattern of metabolism is found to be the same in all of the subjects.

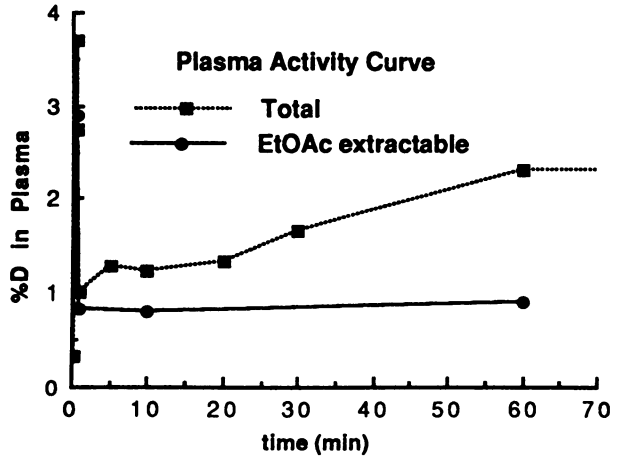


FIGURE 3
Plasma activity versus time curve of one normal subject. Apparently, a significant amount of the activity is due to the nonextractable component (most likely free iodide).

Possible Metabolites in Plasma-HPLC Results

When the organic extractable fractions of each blood sample were subjected to HPLC analysis, the results indicated that there was at least one metabolite which eluted faster from the reverse phase column (Fig. 4). This metabolite peak was observable at 10 min postinjection. At 134 min postinjection, the majority of the organic extractable activity was associated with the metabolite peak. This metabolic pattern was uniformly true for all of the subjects studied in this project, but the relative rate of metabolism was slightly different among the subjects.

Radiation Dosimetry

The biodistribution of IBZM in 5 normal volunteers was measured using a conjugate counting technique (Table 2) (*22*). There was high uptake in brain (3.72%) as well as lung (8.12%) at 2 hr postinjection. The majority of the activity appeared in the biliary system and eventually passed through the small intestine. A large portion of the radioactivity was disassociated from the IBZM molecule in free iodide form, resulting from rapid urine clearance (Kung, unpublished data). Rapid excretion in urine is very beneficial for lowering the radiation dose to the total body. Effective blockage of thyroid by Lugol's solution decreased the radiation dose to this organ. Based on the biodistribution data and MIRDOSE calculation, the radiation dosimetry is summarized in Table 3. The urinary bladder wall, large intestine wall, and spleen were the organs that received the highest radiation dose. Neither the blood-forming organs nor the lens of the eye received any significant radiation dose. The radiation dose in Table 3 suggests that it is reasonably safe to use a 10-mCi dose of n.c.a. [^{123}I]IBZM in humans.

Pharmacology and Safety

None of the normal volunteers showed signs of pharmacologic effects, and no change was observed in the blood chemistry and urinalysis before and after the injection of this agent. Based on the human data coupled with the toxicology tests reported previously (*23*), it appears that [^{123}I]IBZM is a safe radiopharmaceutical for human use.

DISCUSSION

Basic blood chemistry and vital sign measurements before and after the i.v. injection indicate that there

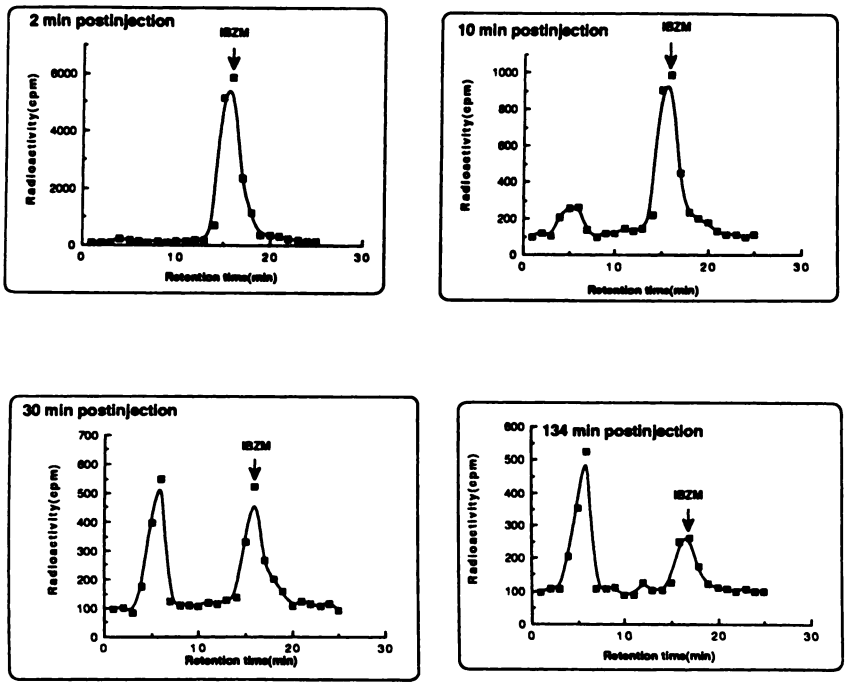


FIGURE 4
Representative HPLC profiles of the organic-extractable fraction at different time points show at least one major metabolite of IBZM in plasma (one hydrophilic peak with a shorter retention time). The exact chemical nature of the polar metabolite(s) is unknown at this time.

were no observable pharmacologic effects. Assuming that [^{123}I]IBZM is carrier-free, the theoretical specific activity is 2.4×10^5 Ci/mmol. At a dose of 10 mCi, a total of 42 pmole of IBZM is injected. If the brain uptake is 5% of the dose, then ~2 pmole is in the whole brain. In the basal ganglia, the D-2 dopamine receptor concentration is ~15 pmole/ml (*24,25*). Assuming the total volume of the basal ganglia is 20 ml, then the total receptor number is 300 pmole. Therefore, the D-2 dopamine receptor occupancy after an i.v. injection of [^{123}I]IBZM is one in one hundred and fifty (1/150). It is unlikely that the agent will be pharmacologically active at this low dosage, which is confirmed by the fact that toxicology study in animals (*23*) showed no pharmacologic effect at levels 10,000 times greater than the clinical doses and that no observable change in vital signs and blood chemistry was found in any of the subjects. Based on biodistribution data of normal volunteers, obtained by a conjugate counting technique, the radiation dose for this agent in humans suggests that it is safe at a dose up to 10 mCi. The intestine wall, spleen, and bladder wall appear to be the critical organs. The major metabolite of this agent in blood was a water soluble material, probably free iodide. Since the water soluble fraction most likely will not cross the blood brain barrier, it will not be a contributing factor for brain imaging. There was a lipid-soluble metabolite in plasma, which increased with time. The chemical identity of this has not been elucidated.

TABLE 2
Organ Distribution of [^{123}I]IBZM in Five Normal Volunteers

	2 hr (% dose)*	20 hr (% dose)†
Brain	3.72 ± 1.16	0.74 ± 0.28
Gall Bl cont.	3.76 ± 1.62	2.87 ± 0.62
Small Intestine	14.20 ± 4.1	19.50 ± 7.6
Liver	9.58 ± 8.0	5.82 ± 6.6
Lung	8.12 ± 2.72	2.08 ± 1.09
Spleen	2.82 ± 1.17	1.50 ± 0.28
Uri. Bl. cont.	9.0 ± 2.65	11.73 ± 6.11
Remainder	75	25

* Estimated from brain dose.
† Dynamic bladder model/4.8 hr void.

TABLE 3
Radiation Dose Estimates for [^{123}I]IBZM*

Organ	rad/mCi
Brain	0.039
Gallbladder wall	0.15
Lower large intestine wall	0.28
Small intestine	0.12
Upper large intestine wall	0.25
Liver	0.12
Lungs	0.09
Ovaries	0.083
Red marrow	0.038
Bone surfaces	0.059
Spleen	0.22
Testes	0.031
Urinary bladder wall	0.21
Total body	0.042

* Based on data gathered in five volunteers. Assumed distribution and retention: gallbladder receives activity leaving liver; voids once every 6 hr; 15% to small intestine, follows GI tract kinetics as in ICRP 30.
Remainder 75%, T_b = 24 hr; 10%, T_b = infinite.

Imaging studies of the CNS D-2 dopamine receptor with PET in humans with [^{11}C]raclopride (labeled at the N-ethyl group) have been reported (*25–29*). A high ratio of specific striatal to nonspecific cerebellar binding in living human brain was observed. Using an equilibrium model and Scatchard plots, the affinity constant (K_d = 7.1 n*M*, B_{max} = 15 pmole/ml) in the living human brain was measured by PET (*25–29*). The values for the dopamine D-2 receptor density were comparable to those determined earlier by using a different imaging agent: N-[^{11}C]-methylspiperone (K_d = 0.097 n*M*, B_{max} = 16.6 pmole/g) (*24,30*). In addition, several new fluorine-18-labeled compounds including spiperone itself, N-methyl spiperone (*31*), and N-fluoroalkyl-spiperones (*32–37*) have been reported.

Several potential SPECT CNS D-2 dopamine receptor imaging agents, based on iodinated spiperone derivatives, have been reported (*38–40*). Preliminary studies of an iodinated 2′-iodo-spiperone (2′-ISP) (*38*) indicate that the spiperone analog displays excellent D-2 specificity (K_d = 0.25 n*M*, rat striatum) and in vivo stability as compared to 4-iodo-spiperone reported earlier (*41*). An N-iodovinyl derivative of spiperone was recently reported (*42,43*). Initial studies have shown [^{123}I]iodolisuride, a D-2 antagonist, to possess good potential as a useful imaging agent for SPECT (*44–46*). Several new iodobenzamide derivatives have been reported. They appear to show higher target-to-nontarget ratios; however, no human studies have been reported yet (*47–51*).

Preliminary imaging results from the [^{123}I]IBZM human study suggest that this agent localizes in the basal ganglia and to a lesser extent in the cortex and the cerebellum. SPECT imaging displays specific regional uptake in the basal ganglia. The image quality from the 3-detector, dedicated SPECT system is far superior to that obtained with the older rotating camera system. Providing both faster scanning time and excellent image quality, the dedicated system is preferred for imaging CNS D-2 dopamine receptors with [^{123}I]IBZM. Higher sensitivity and resolution of SPECT imaging is very important for agents, such as [^{123}I]IBZM, which display dynamic changes in regional concentration in brain. One major issue which remains unanswered is the quantitation of D-2 dopamine receptors based on the SPECT images. A kinetic model of the uptake and retention of the agent in the basal ganglia region ($T_{½}$ ~ 1–2 hr) is needed in order to obtain quantitative information on receptor density. Since single-head SPECT requires a 30–60-min data acquisition time, it is unlikely that more than one data point can be obtained. Dynamic scanning with dedicated SPECT, such as Triad, may provide the kinetic data necessary for quantitating receptor density in brain. With newer and faster SPECT equipment being introduced and additional research efforts being done on attenuation and scatter correction, the full potential of D-2 dopamine receptor quantification in nuclear medicine clinics may be realized.

ACKNOWLEDGMENTS

The authors thank Dr. Harvey Ziessnan for helpful participation, Mr. Michael Stabin for his advice in MIRDOSE calculation, and Mrs. F. Chapman and Ms. H. Cullen for their assistance in preparing this manuscript. The technical assistance of Kim Sorgentoni and Beth Harkness is appreciated.

This project is supported by NIH grant NS-24538.

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