A Kit Formulation for Preparation of Iodine-123-IBZM: A New CNS D-2 Dopamine Receptor Imaging Agent

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A method for the preparation of iodine-123IBZM, a central nervous system D-2 imaging agent, is reported. By using a rapid filtration technique to remove the unreacted iodide, the preparation can be completed in less than 20 min (overall yield >60%). The product, with high purity (≥95%) and specific activity, is suitable for human use.

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IBZM (S)-(−)-3-iodo-2-hydroxy-6-methoxy-N-[(1-ethyl-2- pyrrodinyl)methyl] benzamide) belongs to a group of structurally related benzamides which display significant antidopaminergic activity (*1*). The iodine-125- IBZM has been used as a probe for the study of D-2 receptors (Kd = 0.4 n*M* in rat striatum tissue preparation) (*2–4*), particularly for the detergent-solubilized D-2 receptors (*5*). The results from the in vivo and in vitro data all indicated that IBZM binds specifically to the central nervous system (CNS) dopamine D-2 receptor with high affinity and stereospecificity. The [^{123}I]IBZM is a highly selective CNS D-2 dopamine receptor ligand useful for SPECT imaging studies in normal subjects (*6,7*) and is potentially useful for patients with various neurologic disorders.

Preparation of the no-carrier-added (NCA) [^{123}I] IBZM has been achieved by an oxidative iodination of BZM (the uniodinated starting material) with sodium [^{123}I]iodide and peracetic acid as an oxidant (*8*). The final product (NCA [^{123}I]IBZM) is obtained through several steps of organic extraction and high-performance liquid chromatography (HPLC) purification. The multistep synthesis and purification procedure requires at least 2 hr. Using this procedure, a limited Phase 1 clinical trial has been accomplished (*6*). In order to optimize this procedure for the routine nuclear medicine clinic use, a faster and easier method for preparing [^{123}I]IBZM is preferred. In this paper, we report a simplified and expeditious procedure for the preparation of [^{123}I]IBZM based on a kit formulation for routine clinical use. For convenience, the products prepared by using HPLC separation and by using a kit separation are designated as [^{125}I] or [^{123}I]IBZM-NCA and IBZM-KIT, respectively.

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MATERIALS AND METHODS

Reagents

The uniodinated starting material, BZM(S)-(−)-2-hydroxy-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide) and nonradioactive IBZM were prepared by the method described previously (*2*). Sodium [^{125}I]iodide was obtained from NEN/Dupont in a NCA form (specific activity 17 Ci/mg; 2200 Ci/mmol). Sodium [^{123}I]iodide was obtained from Atomic Energy of Canada Ltd. (specific activity 2.4 × 10^5 Ci/mmol). Peracetic acid (32 wt % solution in dilute acetic acid) was obtained from Aldrich Chemicals, (St. Louis, MO) and diluted from stock solution with distilled water before use. The Accubond-C4 (butyl) columns (100 mg/ml) were obtained from Bodman Chemicals, Aston, PA. The columns were prewashed with 1 ml of absolute ethanol followed by 2 ml of distilled water before use. Ethyl acetate and acetonitrile were of HPLC grade and purchased from J.T. Baker. All other chemicals were of reagent grade and purchased commercially.

Radiolabeling

NCA Preparation (IBZM-NCA). The [^{125}I] and [^{123}I]IBZM were prepared by the method reported previously (*8*). Briefly, 50 μg of the starting material, BZM, was reacted with radioactive iodide in the presence of ammonium acetate and peracetic acid as the oxidant. After quenching and neutralization, the reaction mixture was extracted with ethyl acetate. The final product, after purification with HPLC, was designated as IBZM-NCA.

Kit Formulation (IBZM-KIT). The method was developed as follows: a small amount of BZM (4 μg) dissolved in 20 μl absolute ethanol was mixed with 100 μl of 0.5 *M* ammonium acetate, pH 4.0, and 20 μl of radioactive sodium ([^{123}I] or [^{125}I]) iodide. The oxidative iodination was initiated by the addition of 50 μl of diluted peracetic acid (0.3% wt from stock solution prepared freshly every day) and the mixture was allowed to proceed at room temperature for 5 min. After quenching with 100 μl of sodium bisulfite (300 mg/ml), the reaction was

neutralized by addition of 0.7 ml of saturated sodium bicarbonate solution. To remove the unreacted radioactive iodide, the reaction mixture was passed through a prewashed accubond-C4 column and washed with distilled water (2 ml). The desired product, radioactive IBZM, was eluted from the column with absolute ethanol (1 ml) and its radiochemical purity was analyzed on HPLC (PRP-1 column, acetonitrile/5.0 m*M* dimethylglutaric acid buffer pH 7.0, 90:10) as described previously (*8*). The IBZM obtained through this method was designated as IBZM-KIT.

In Vivo Study

Male Sprague-Dawley rats (225–300 g) which were allowed access to food and water ad libitum were used for all of the studies. The total brain uptake and the regional brain distribution of [^{125}I]IBZM in rats were obtained after an i.v. injection of 0.2 ml saline containing 1–2 μCi of [^{125}I]IBZM-NCA or [^{125}I]IBZM-KIT. The rats were sacrificed at various time points postinjection by cardiac excision under ether anesthesia. After dissecting, weighing and counting samples from different brain regions (cortex, striatum, hippocampus and cerebellum), % dose/g of samples was calculated by comparing the sample counts with the counts of the diluted initial dose. The ratio of uptake in each region was obtained by dividing % dose/g of each region with that of the cerebellum.

In Vitro Binding Study

Rat striatal tissue homogenates were prepared as described previously (*2*). The binding assays were performed by incubating 50 μl of the striatal membrane preparation containing 40–60 μg protein with increasing amounts of [^{125}I]IBZM-NCA or [^{125}I]IBZM-KIT (for saturation analysis) or appropriate amounts of labeled ligands and nonradioactive IBZM (for displacement study) in a total volume of 0.2 ml of the assay buffer (50 m*M* Tris buffer, pH 7.4, 120 m*M* NaCl, 5 m*M* KCl, 2 m*M* $CaCl_2$, and 1 m*M* $MgCl_2$). The incubation and the following procedure were carried out as described previously (*2*). The nonspecific binding was determined in the presence of 10 μM of spiperone and the data points were analyzed using the iterative, nonlinear least-square's curve-fitting program, LIGAND (*9*).

Ultraviolet Determination of BZM

The amount of BZM present in the final product, radiolabeled IBZM, was determined by measuring the absorbance at 308 nm (the characteristic wavelength for BZM) using a spectrophotometer (Beckman DU-7). The measurement was done with an adequate sample for the blank (the blank also went through the same process as the samples except BZM was eliminated in the oxidative iodination). From the calibration curve generated with various amounts of BZM (2–16 μg), the amount of BZM in the samples (radiolabeled IBZM) was thus calculated.

RESULTS

By using a smaller amount of the starting material, BZM, 4 μg in this method for preparation of IBZM-KIT as compared to the 50 μg used in the preparation of IBZM-NCA, the labeling yield remained consistently high (70%–80%). This method eliminates the solvent extraction and the HPLC purification steps, and the simplified procedure can be completed in less than 20 min. The radiochemical purity of the final product, IBZM-KIT, analyzed on HPLC, shown in Figure 1, is high (95%–98%, n = 10) and the HPLC profiles are consistent with those using the previous method. The amount of uniodinated BZM present with the radioactive IBZM product was determined by UV measurement and calculated from the BZM calibration curve (Fig. 2). The value was found to be 3.14 ± 0.16 μg (n = 5).

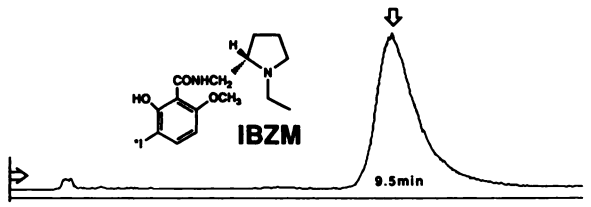

FIGURE 1
HPLC of kit-formulated [^{123}I]IBZM (gamma detector) with reverse-phase column (PRP-1), solvent system of acetonitrile:dimethyl glutaric acid (5 m*M*, pH 7.0)/90:10, flow rate = 1.0 ml/min.

IBZM prepared with this new method (IBZM-KIT) was compared to the IBZM-NCA prepared by HPLC purification using the in vivo biodistribution and in vitro binding studies. Table 1 shows the brain uptake in rats with [^{125}I]IBZM prepared from the two methods. Similar values were obtained with IBZM-KIT and IBZM-NCA at 30 and 60 min postinjection. The striatum/cerebellum ratios in rat brain were 2.86, 4.18 and 2.67, 4.61 for [^{125}I]IBZM-NCA and [^{125}I]IBZM-KIT at 30 and 60 min, respectively (Table 2). Furthermore, comparable Kd values (0.31 n*M*, 0.24 n*M* for NCA and KIT) were obtained using the in vitro binding assay in rat striatum membrane preparation (Table 3). The displacement studies with nonradioactive IBZM also gave similar Ki values (0.29 n*M* for NCA and 0.27 n*M* for KIT). Since the Ki values are almost identical to the

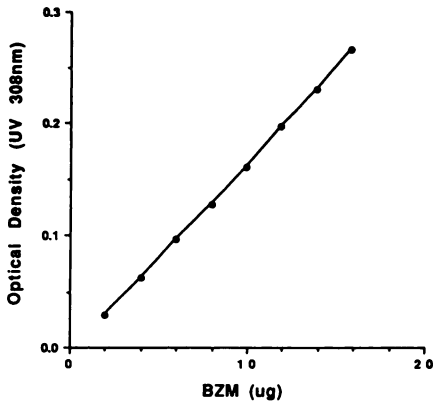

FIGURE 2
Calibration curve for determination of amount of BZM in the final product. The absorption at 308 nm (characteristic wavelength for BZM) was chosen for the measurement.

TABLE 1
Brain Uptake (%dose/organ) in Rats after i.v. Injection of [^{125}I]IBZM (Average of Three Rats and Range)

Compound	30 min	60 min
[^{125}I]IBZM-NCA	1.84 (1.70–2.00)	1.20 (1.13–1.31)
[^{125}I]IBZM-KIT	1.73 (1.52–2.12)	0.98 (0.96–0.99)

TABLE 3
In Vitro Binding Constants of [^{125}I]IBZM Using Rat Striatal Membrane Preparation

Method	Kd (n*M* ± s.e.m.)*	Ki (n*M* ± s.e.m.)†
IBZM-NCA	0.31 ± 0.06	0.29 ± 0.05
IBZM-KIT	0.24 ± 0.05	0.27 ± 0.04

Each value represents the mean ± s.e.m. of 3–5 determinations.

* Kd values were determined from Scatchard analysis of saturation isotherms.

† 0.20–0.30 n*M* [^{125}I]IBZM-KIT or [^{125}I]IBZM-NCA was incubated in the presence of nonradioactive IBZM in 7–11 concentrations.

Kd values, it is reasonable to assume that both preparations have very similar specific activity and are probably carrier-free (reaching the theoretical value—the specific activity of radioactive iodine, i.e., 2200 Ci/mmol for ^{125}I and 240,000 Ci/mmol for ^{123}I, respectively).

DISCUSSION

In order to image D-2 dopamine receptors in the brain with iodinated ligands, it is essential to use selective D-2 ligands with high specific activity. In the basal ganglia of normal human brain, there are about 300 p*M* (15 p*M* in about 20 ml of basal ganglia) of D-2 receptors (*10–12*). One major objective of this paper is to demonstrate that it is possible to prepare radioactive labeled [^{123}I] or [^{125}I]IBZM with high specific activity using a kit formulation. Since the isotopic exchange labeling generally will not give a product with a high specific activity, direct oxidative iodination of BZM is the method of choice for preparing IBZM with high specific activity.

Using the previously reported procedure, [^{123}I] or [^{125}I]IBZM-NCA can readily be prepared. The procedure started with 50 μg of uniodinated BZM. After the oxidative iodination step, the unreacted starting material and polar radioactive impurities were removed by extraction with organic solvents followed by a HPLC purification. The desired [^{123}I] or [^{125}I]IBZM-NCA could thus be obtained (yield ~50%, >95% purity). The major disadvantages of this procedure are: (a) the time required for completing the steps is excessively long (~2 hr) and (b) an adequate HPLC setup is needed for purification of the product. Both of these may prevent the widespread application of this agent in regular nuclear medicine clinics.

In order to eliminate the time-consuming steps, such as organic extraction and HPLC purification, for the preparation of NCA radioactive IBZM (*8*), the iodination conditions were optimized. The amount of starting material, buffer, and oxidant as well as the reaction time have been systematically investigated. The removal of unreacted radioactive iodide and polar side products can be easily achieved by passing the reaction mixture through a small Accubond-C4 column and the desired radioactive IBZM product can be recovered by eluting the column with ethanol. One major difference between IBZM-NCA and IBZM-KIT preparation is the presence of a small amount of uniodinated BZM in the latter preparation. Since the difference in binding affinity for D-2 receptors between IBZM and BZM is high (50 versus 1) (*2*), the small amount of uniodinated BZM together with the radioactive IBZM product will not affect the binding or imaging studies of the labeled IBZM. Further studies confirm this assumption. The results for the in vivo biodistribution and the in vitro binding study indicated that the IBZM-KIT, even in the presence of small amount of BZM, behaves similarly to the IBZM-NCA. Tests on sterility and pyrogenicity suggested that as expected, the kit preparation produced a sterile and pyrogen-free [^{123}I]IBZM, suitable for human use.

Recently, several new iodinated benzamides with higher affinity than IBZM were reported (*13,14*). The newer benzamides are labeled by an iododestannylation reaction. Since the tri-n-butyltin compounds and the products, iodinated benzamides, show a large difference

TABLE 2
Regional Brain Ratio in Rats after i.v. Injection of [^{125}I]IBZM (Average of Three Rats and Range)

	[^{125}I]IBZM-NCA		[^{125}I]IBZM-KIT	
	30 min	60 min	30 min	60 min
ST/CB	2.86 (2.67–3.03)	4.18 (4.11–4.20)	2.67 (2.49–2.81)	4.61 (4.20–5.01)
HP/CB	1.51 (1.44–1.59)	1.74 (1.72–1.76)	1.48 (1.40–1.58)	1.79 (1.75–1.83)
CX/CB	1.80 (1.63–1.94)	1.76 (1.72–1.83)	1.64 (1.51–1.80)	1.70 (1.66–1.73)

ST = striatum; HP = hippocampus; CX = cortex; and CB = cerebellum

in their lipid-solubility, the type of kit formulation reported for IBZM preparation may also be applicable for these newer benzamides.

In conclusion, a method for the preparation of [^{123}I] IBZM is reported. By eliminating the time-consuming steps of organic extraction and HPLC purification, the kit preparation can be completed in less than 20 min. The studies suggest that the IBZM prepared by the kit formulation is of high specific activity and is suitable for routine clinical use.

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