Synthesis and Biodistribution of No-Carrier-Added [1-^{11}C]Putrescine

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No-carrier-added [1-^{11}C]putrescine was synthesized in 20% radiochemical yield in a synthesis time of 50 min by the Michael addition of potassium [^{11}C]cyanide to acrylonitrile followed by reduction of the [^{11}C]dinitrile with borane-methyl sulfide complex. Biodistribution in mice at 5, 30, and 60 min showed low uptake in normal brain tissue.

J Nucl Med 26:1186–1189, 1985

The application of positron emission tomography (PET) to the study of human tumors and their response to therapy continues to be of interest, particularly in studies where valid tracer kinetic models exist and physiologic quantitation is possible (*1*). A prerequisite for such a model is the thorough understanding of the biochemistry of the tracer in the tumor and in the surrounding tissue.

Polyamines have come to be regarded as good biochemical markers for malignancy (*2–4*). Volkow et al. (*5*) reasoned that since adult brain parenchyma does not normally divide, putrescine, a polyamine and marker of cell growth and proliferation, should be taken up and metabolized solely by brain tumor. They tested the feasibility of putrescine as a PET tracer for brain tumors, using the hydrogen-3 (^{3}H) and carbon-14-(^{14}C) labeled compound, and found that the in vivo uptake into transplanted rat glioma was 35 times greater than in normal brain tissue and that metabolism to spermine by the tumor was rapid, in contrast to adjacent normal brain tissue. They concluded that putrescine labeled with carbon-11 (^{11}C) may be useful as a PET tracer for the selective metabolic imaging of brain tumor and might be used with an appropriate model as a marker for tumor growth rate.

Received Dec. 17, 1984; revision accepted Jun. 14, 1985.

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Earlier, Chaney et al. (*6*), who showed that when putrescine biosynthesis was inhibited a four-fold tumor selective enhancement in the uptake of [^{14}C]putrescine occurred, had also concluded that putrescine labeled with ^{11}C would be useful for imaging tumors.

These studies along with earlier tissue distribution and metabolism studies with [^{3}H]diamines (*7*), [^{11}C]diamines (*8*), and methylated polyamine analogs (*9*) support the contention that [1-^{11}C]putrescine may indeed be a useful tracer for quantitating the degree of malignancy in vivo and for monitoring response to radio- and chemotherapy.

We report here the synthesis of [1-^{11}C]putrescine in sufficiently high specific activity to avoid significantly perturbing the plasma concentration ($\sim 0.5\ \mu M$) (*10*) of the endogenous diamine. We also report its biodistribution in normal mice at 5, 30, and 60 min after injection.

MATERIALS AND METHODS

Materials

The acrylonitrile used was commercially available and distilled prior to use. Anhydrous methanol-hydrogen chloride was prepared by drying the methanol with $CaSO_4$, distilling it into a flask and bubbling HCl gas, which was dried by passing through concentrated sulfuric acid, into the methanol until the solution was 4–6 N by weight. Tetrahydrofuran was dried over sodium/benzophenone and distilled prior to use.

Radiochemical assay

Radiochemical and chemical purity was assayed by thin layer chromatography (TLC) on Silica Gel G by spotting [1-^{11}C]putrescine with authentic carrier material and showing that the radioactivity was coincident with the spot corresponding to authentic compound. In the TLC system of acetone:water:propionic acid (6:4:2) saturated with sodium chloride, putrescine had an R_f value of 0.33 and was visualized with ninhydrin. The radiochemical purity was also assayed by high performance liquid chromatography (HPLC) as putrescine and also as the dibenzoyl derivative of putrescine (*11*). For putrescine, a Bio-Rad Aminex HPX-72-0 column (300 mm × 7.8 mm) was used with 0.03*M* sodium hydroxide as the mobile phase. Putrescine was visualized at 220 nm. For the dibenzoyl derivative of putrescine, a reverse phase C_8 column was used with a methanol-water (52:48) mobile phase. In both cases, authentic carrier was added. The elution profile of the radioactivity was congruent with the carrier. Specific activity was determined to be >1.4 Ci/μmol by the absence of a signal on the gas chromatographic analysis (4:1 DMS-KOH s.s. 6 ft × 1/8 in., thermal conductivity detector). The analyses were performed using a calibration curve of peak area as compared to nmol concentration of seven standard putrescine solutions of 98.6 to 12.3 nmol, and it was determined that the lowest concentration of putrescine observable would be 10 nmol. The retention time of the authentic putrescine standards were 6.0 min with a helium flow rate of 50 ml/min.

[^{11}C]Putrescine

Carbon-11-labeled hydrogen cyanide was produced according to previously published method (*12*) and trapped in 0.2 ml of a 1% potassium hydroxide solution. Acrylonitrile (0.5 ml) and tetrahydrofuran (0.5 ml) were added and the solution was stirred and heated at 65°C for 5 min. The solution was evaporated to dryness under vacuum and ethanol (2 × 0.25 ml) was added and removed in vacuo to remove the residual acrylonitrile and water. The residue was taken up in 1.0 ml of dry tetrahydrofuran and passed through a drying tube containing anhydrous potassium carbonate and potassium hydroxide pellets into a flask fitted with a reflux condenser. The addition vessel was rinsed with 1.0 ml of dry tetrahydrofuran and this solution was also passed through the drying tube into the reflux vessel. A slow stream of nitrogen was bubbled through the mixture and 1.5 ml of a 2*M* borane-methyl sulfide solution in tetrahydrofuran* was added. The solution was stirred and refluxed at 140°C for 10 min. The solution was cooled and 0.5 ml of an anhydrous methanol-hydrochloric acid solution was slowly added. The mixture was evaporated to dryness under vacuum. A small amount of white residue was present, and this was taken up in 1.0 ml of a 0.03*M* sodium hydroxide solution and transferred to a syringe fitted with a 0.45 μ filter (Rainin). The reaction vessel was rinsed with 0.5 ml of the 0.03*M* sodium hydroxide solution and this was also transferred to the syringe. The reaction mixture was filtered and the filter was washed with 0.5 ml of the sodium hydroxide solution. The filtrate was injected onto a Bio-Rad Aminex HPX-72-0 HPLC column (300 mm × 7.8 mm) using a 0.03*M* sodium hydroxide solution as the mobile phase. [1-^{11}C]putrescine had a retention time of ~10 min with a flow rate of 1.5 ml/min. The diamine was collected over a period of 2.0 min and was contained in a volume of 3.0 ml. The diamine was made isotonic by the addition of sterile solutions of 1*M* hydrogen chloride (0.55 ml) and 1*M* sodium bicarbonate (0.70 ml). The solution was filtered (sterile 0.22 μ*M* millipore) into a sterile injection vial. The filter was washed with 0.5 ml of a sterile saline solution which was also collected in the injection vial. The reaction time was ~50 min and the radiochemical yield was 20% at end of bombardment (EOB) based on starting activity of hydrogen [^{11}C]cyanide produced at the end of cyclotron bombardment. In a typical experiment using a 25 microamp beam for 1 min, 20.7 mCi of hydrogen [^{11}C]cyanide was produced and 754 μCi of [1-^{11}C]putrescine was obtained of a reaction time of 50 min. Radiochemical purity was assayed by thin-layer chromatography and found to be >97.0% purity with ^{11}C-labeled 3-aminobutyronitrile as the minor impurity.

Tissue distribution studies

Male Swiss albino mice (BNL strain, 20–32 g) were used in this study. [1-^{11}C]putrescine (0.05–0.20 mCi/mouse of specifc activity >1.4 Ci/μmol; 36–140 pmol/mouse) was injected by tail vein into mice and the animals were killed by cervical dislocation at the desired time interval (5, 30, and 60 min). The various organs were rapidly removed, blotted free of blood, and placed in preweighed counting vials and the vials were sealed. Tissue samples as well as injection standards were counted in a Packard automated sodium iodide well counter. Both percent injected dose per gram of tissue and percent injected dose per organ were determined from the decay corrected activity.

RESULTS AND DISCUSSION

Although [1-^{11}C]putrescine has been prepared by the method shown in Eq. 1 and shown to have high uptake in tumors (*8*), the described method used carrier-added sodium cyanide (0.205–0.401 mmol) in a slight molar excess to the bromonitrile producing labeled polyamines with a specific activity which was too low to be acceptable for human studies (*13*).

$$K[^{11}CN] + BrCH_2CH_2CN \xrightarrow[225°]{THF}$$

$$N[^{11}C]CH_2CH_2CN$$

1

$$\xrightarrow[THF]{BH_3} H_2N[^{11}C]H_2CH_2CH_2CH_2NH_2 \quad (1)$$

Since no-carrier-added [1-^{11}C]putrescine was required for human studies, the initial synthetic strategy involved reinvestigation of the above reaction under conditions where the alkyl halide (β-chloropropinonitrile or β-bromopropionitrile) was used in a large molar excess relative to cyanide to simulate reaction conditions if NCA [^{11}C]cyanide were used. The reaction was carried out in dimethyl sulfoxide at temperatures up to 160° and under these conditions **1** was produced in ~2.0% yield as determined by gas chromatography. The formation of **1** from the reaction of 1,2-dichloroethane again with a molar excess of sodium cyanide has been reported (*14*). These results demonstrated that potassium cyanide must be present in a slight molar excess relative to the halonitrile to produce the desired dinitrile. Therefore the above methods were not suitable for producing the no-carrier-added diamine.

One possible rationalization for the inability to form the dinitrile (**1**) in the absence of an excess of NaCN is that the first step of the reaction may involve elimination of HX (X = Cl or Br) from the alkyl halide to form acrylonitrile followed by Michael addition of hydrogen cyanide to form **1** (*15*). If this is the case, in the NCA situation or when the alkyl halide is used in large excess relative to cyanide, then cyanide may be consumed in the removal of HX to form acrylonitrile. This speculation suggested that the carbon-carbon bond formation by the Michael addition followed by reduction as shown in

$$K[^{11}C]N + CH_2{=}CHCN \xrightarrow[THF]{1\%\ KOH}$$

2

$$N[^{11}C]CH_2CH_2CN$$

$$\xrightarrow[THF]{BMS} H_2N[^{11}C]H_2CH_2CH_2CH_2NH_2. \quad (2)$$

Equation 2 should be investigated. Using this method the desired dinitrile **1** was prepared in ~70% yield by the Michael addition of NCA K[^{11}C]N to acrylonitrile **2**. The remaining activity was present as unreacted potassium cyanide. The addition reaction was carried out under a variety of concentrations and temperatures, as shown in Table 1, to optimize the yield and synthesis time. A satisfactory yield was obtained after heating

TABLE 1
Yield of [^{11}C]Succinonitrile from the Reaction of Acrylonitrile and Potassium [^{11}C]Cyanide

Temperature (°C)	Time (min)	Acrylonitrile (ml)	Tetrahydrofuran (ml)	1% KOH Solution (ml)	% Yield*
65	5	0.50	0.50	0.20	73.9
65	5	0.10	0.20	0.10	57.0
65	5	0.02	0.20	0.04	38.6
65	5	0.02	0.20	0.10	23.7
65	5	0.03	0.30	0.015	40.3
65	5	0.05	0.30	0.025	48.8
48	10	1.40	0.50	0.20	62.8
48	5	1.40	0.50	0.20	64.6
48	10	0.50	0.50	0.20	88.2
48	5	0.50	0.50	0.20	44.0
48	10	0.20	0.20	0.20	34.1
48	10	0.20	0.40	0.20	41.6

* Yield from EOB from hydrogen [^{11}C]cyanide produced. Analyzed by gas chromatography and compared to carrier succinonitrile.

0.5 ml of acrylonitrile, 0.5 ml of tetrahydrofuran, and 0.2 ml of a 1% potassium hydroxide solution at 65°C for 5 min.

The reduction of the dinitrile was carried out with borane-methyl sulfide complex in ~ 75% yield. Other radioactive products observed were 3-aminopropiononitrile and a polymeric residue from the reduction of the dinitrile. Borane-methyl sulfide complex (BMS) was used as the reducing agent and gave a consistently higher yield of putrescine than did borane because the stability of the BMS solution at the high temperature needed to carry out the complete reduction of **1** (*16*).

As expected, 3-amino-1-propanol **3**, was also observed as a product from the competing Michael addition of water to **2** as shown in Eq. 3. However,

$$H_2O + CH_2{=}CH{-}CN \xrightarrow[THF]{1\%\ KOH} HOCH_2CH_2CN$$

2

$$\xrightarrow[THF]{BMS} HOCH_2CH_2CH_2NH_2 \quad (3)$$

3

3 is effectively separated from the [1-^{11}C]putrescine by HPLC. The solution containing the [1-^{11}C]putrescine after separation by HPLC was analyzed by gas chromatography and there was no mass associated with the product or solution. Control experiments indicated that concentrations of putrescine greater than 10 nmol would be observed.

Table 2 shows the results of the biodistribution of [1-^{11}C]putrescine in mice at 5-min, 30-min, and 60-min intervals. The accumulation of activity is less in the brain than that for other tissues. Most of the [1-

TABLE 2
Tissue Distribution (% Dose/Organ)* of NCA [1-^{11}C]Putrescine in Mice at 5, 30, and 60 Min (n = 6 Mice/Time Point)

Organ	Sacrifice time (min)		
	5 min	30 min	60 min
Blood†	1.37 ± 0.18	0.82 ± 0.11	0.76 ± 0.46
Brain	0.12 ± 0.01	0.15 ± 0.01	0.13 ± 0.01
Heart	0.19 ± 0.04	0.15 ± 0.02	0.12 ± 0.01
Lungs	0.57 ± 0.08	0.48 ± 0.08	0.43 ± 0.08
Liver	5.96 ± 1.2	4.85 ± 0.75	3.70 ± 0.50
Spleen	0.57 ± 0.35	0.56 ± 0.25	0.48 ± 0.16
Kidney	10.5 ± 2.0	2.63 ± 0.36	1.27 ± 0.21
Small intestine	13.7 ± 5.0	7.61 ± 2.00	4.40 ± 1.66

* Each value represents the average ± s.d. of six mice.
† % dose/g.

^{11}C]putrescine was taken up by the liver, kidneys, and small intestine. The biodistribution of NCA [1-^{11}C]putrescine is similar to the biodistribution in rats using carrier-added [1-^{11}C]putrescine (*8*) and in mice using [^{14}C] and [^{3}H]putrescine (*5*). Although the chemical form of the ^{11}C activity in brain was not determined in these studies, previous studies in tumor bearing animals using [^{3}H]putrescine showed rapid metabolism to [^{3}H]spermine along with the production of significant amounts of [^{3}H]water (*5*). In the case of [1-^{11}C]putrescine, predominant metabolities would be expected to be labeled products of polyamine metabolism and [^{11}C]carbon dioxide based on previous studies with [^{14}C]putrescine in rats (*17*).

In summary, NCA [1-^{11}C]putrescine has been prepared by the Michael addition of NCA potassium [^{11}C]cyanide to acrylonitrile followed by the subsequent reduction of the ^{11}C-labeled dinitrile. The [1-^{11}C]putrescine produced in this manner is of a suitable purity and specific activity for use in humans in PET studies of cerebral malignancy. The use of [1-^{11}C]putrescine in the quantitation of tissue proliferation using PET will require the development of a tracer kinetic model as well as other measurable variables, such as the time course and chemical form of ^{11}C in the arterial plasma, in addition to PET measurements of radioactivity concentration.

ACKOWLEDGMENTS

This research was carried out at Brookhaven National Laboratory under contract DE-AC02-76CH00016 with the U.S. Department of Energy and supported by its Office of Health and Environmental Research and was also supported by NIH Grants NS-15638 and NS-15380. The authors acknowledge Robert MacGregor for some of the initial experimental work and for his advice in preparing this manuscript, and Dave Alexoff for his assistance.

FOOTNOTE

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