jnm/ PRELIMINARY NOTES

Carboxyl-Labeled ^{11}C-1-Aminocyclopentanecarboxylic Acid, a Potential Agent for Cancer Detection

Raymond L. Hayes, Lee C. Washburn, Bruce W. Wieland, Tan Tan Sun, Robert R. Turtle, and Thomas A. Butler

Oak Ridge Associated Universities and Oak Ridge National Laboratory, Oak Ridge, Tennessee

Carboxyl-labeled ^{11}C-1-aminocyclopentanecarboxylic acid (^{11}C-ACPC) has been prepared in multimillicurie amounts. The conversion of H^{11}CN to ^{11}C-ACPC ($t_{1/2}$ = 20.4 min) was accomplished by a rapid (20-min) two-step high-temperature modification of the Bücherer–Strecker amino acid synthesis technique, which should be applicable at other accelerator installations. Purification was by ion exchange techniques. Animal studies have indicated that ^{11}C-ACPC is a potential tumor-localizing agent for detecting cancer in humans by nuclear medicine scanning techniques.

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The insertion of radioisotopic labels into the intrinsic structure of compounds with high affinities for specific tissues is an ideal method of producing radiopharmaceutical localizers, since the chemical structures are not altered. The radionuclides ^{11}C, ^{13}N, and ^{15}O are all candidates for producing such agents for diagnosis by external scanning, since the decay of each is accompanied by annihilation radiation which can be used both for conventional scanning and for the recently developed positron transaxial reconstruction tomography (*1,2*). Of these three radionuclides, ^{11}C is the obvious choice as a label because of its longer half-life (20.4 min). A recent comprehensive paper by Wolf et al. (*3*) on the synthesis of short-lived radiopharmaceuticals cites the many investigators who have recognized the potential of ^{11}C for biologic applications.

The compound 1-aminocyclopentanecarboxylic acid (ACPC) was found by Berlinguet et al. (*4*) to concentrate rapidly in sarcoma-180 tumors in mice. Thus, labeled ACPC might be useful as a radiopharmaceutical agent for detecting cancer if it could be labeled with a suitable gamma-emitting nuclide without altering its in vivo behavior. It is apparent that ^{11}C-labeled ACPC would meet that need.

Before attempting to develop a method for the synthesis of ^{11}C-ACPC, we carried out corroborative tissue distribution studies on ^{14}C-ACPC in tumor-bearing animals. The results confirmed the report of Berlinguet et al. (*4*). We also substantiated the rapid tumor uptake that they had observed, an essential requirement if ^{11}C is to be used. We report here our method for the rapid synthesis and purification of carboxyl-labeled ^{11}C-ACPC, together with preliminary tissue distribution data in tumor-bearing animals.

MATERIALS AND METHODS

The transplanted mouse and rat tumor models were obtained as follows. The sources for the RFT rat tumor and the R-3259 rat giant-cell sarcoma have been previously described (*5*). The Morris rat hepatomas (5123C, 7794, 7787, and 7777) and the Novikoff hepatoma were obtained from Dr. Fred Snyder of Oak Ridge Associated Universities. Dr. Francis T. Kenney of Oak Ridge National Laboratory supplied cell cultures of the H-35 Reuber hepatoma, which we subsequently transformed into solid tumors. The B-16 mouse melanoma was obtained from Jackson Laboratory (Bar Harbor, Maine).

To produce the ^{11}C, we used the Oak Ridge National Laboratory's 86-in. cyclotron, a 22-MeV pro-

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For reprints contact: R. L. Hayes, Medical and Health Sciences Division, Oak Ridge Associated Universities, P.O. Box 117, Oak Ridge, TN 37830.

ton accelerator with a maximum internal beam current of 3 mA and a final orbit separation of 6 mm. The measured ^{11}C saturation yield for this accelerator at a beam current of 100 μA, using a standard internal-beam capsule target, is 9 Ci. Because of these high yields of ^{11}C, it is feasible to use reaction schemes involving low chemical yields or relatively long reaction times with respect to the half-life of ^{11}C. Our approach to the synthesis of ^{11}C-ACPC was through a conventional Bücherer–Strecker amino acid synthesis technique (*6*). Although amino acid production by this method normally requires many hours, alterations in reaction conditions (increased temperature and pressure) enabled us to shorten the synthesis time considerably (~20 min for both Steps 4 and 5 below). The reaction sequence in Fig. 1 shows the steps involved in our method for preparing carboxyl-labeled ^{11}C-ACPC.

Targetry (Step 1). The H_3BO_3, applied to a multifin molybdenum target support, is resistance-heated in vacuum to produce molten B_2O_3, which coats the fins and fills a series of narrow slits across them. The loaded support is installed in a standard aluminum capsule assembly (*7*) for internal-beam bombardment in the cyclotron. Surface tension due to the slits in the molybdenum target support keeps the B_2O_3 in a configuration designed to approach "thick-target geometry." During bombardment with 150 μA of 22-MeV protons, the B_2O_3 melts and ^{11}C is produced from the $^{11}B(p,n)^{11}C$ reaction. The ^{11}C combines with oxygen and diffuses into the helium sweep as $^{11}CO_2$ and ^{11}CO (Step 1). Release of labeled gas is enhanced by the liquefaction of B_2O_3. The target assembly is connected to an instrumented gas-flow loop located in a high-level remote-control hot cell adjacent to the cyclotron.

Production of $^{11}CH_4$ and $H^{11}CN$ (Steps 2 and 3). Both $^{11}CH_4$ and $H^{11}CN$ are produced by a modification of the catalytic methods reported by Bánfi et al. (*8*). The helium stream from the cyclotron target's gas loop is mixed with a hydrogen stream and fed through a quartz tube containing nickel on kieselguhr (Alfa Products, Beverly, Mass.) held at 370°C. The output gas from this conversion (containing $^{11}CH_4$) is passed through a $CaSO_4$/NaOH trap (to remove water and any unconverted $^{11}CO_2$) and mixed with a stream of anhydrous NH_3. This mixture is then passed through another quartz tube containing platinum on alumina (Alfa Products) heated to 950°C.

Production of ^{11}C-ACPC (Steps 4 and 5). Before starting a ^{11}C-ACPC production run, a stainless-steel pressure vessel with an internal volume of 3 ml is charged with a mixture of 33.3 mg cyclopentanone, 72 mg $(NH_4)_2CO_3$, 6.7 mg NH_4Cl, and 8.2 mg KCN. The gas stream from Step 3, containing the $H^{11}CN$, is bubbled into a gas absorption column containing 1 ml of 0.005 *N* NaOH. This solution is introduced into the charged reaction vessel, and the closed vessel is then rapidly heated to 210°C, maintained at that temperature for 10 min, and then rapidly cooled. One milliliter of 6.25 *N* NaOH is introduced and the vessel is again heated at 210°C for another 10 min.

Purification of ^{11}C-ACPC. After Step 5 the cooled reaction mixture is filtered and loaded directly onto a 1.5 × 5 cm AG 1-X2 100–200-mesh anion-exchange bed (Bio-Rad Laboratories, Rockville Center, N.Y.) in the hydroxide form. Anionic components, including the ^{11}C-ACPC, are retained by the column whereas cationic and nonionic components pass through. After washing repeatedly with distilled water, the column is eluted with 1 *N* HCl. This eluate is then loaded onto a 1.0 × 15 cm AG 50W-X2 50–100-mesh cation-exchange bed in the hydrogen form (Bio-Rad Laboratories). Cationic material (^{11}C-ACPC) is retained while anionic material passes through the column. After being washed repeatedly with distilled water, the column is slowly eluted with 0.2 *N* NaOH; an ion chamber monitors the elution process in order to minimize the volume of eluate containing the product (~10 ml). The

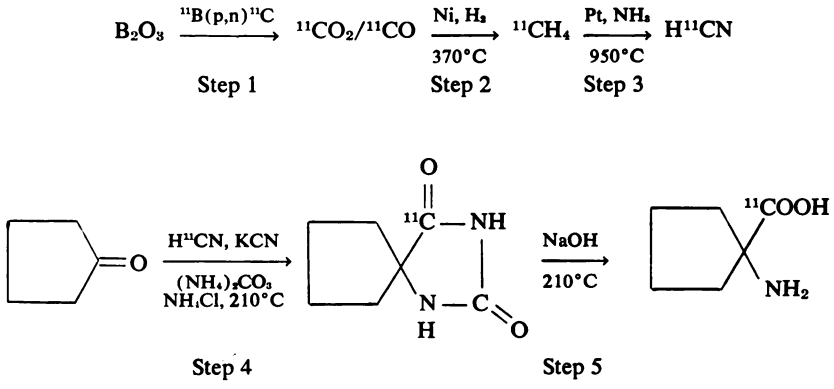

FIG. 1. Reaction sequence used in preparation of carboxyl-labeled ^{11}C-ACPC.

purified ^{11}C-ACPC solution can then be adjusted to physiologic pH with HCl and sterilized by microfiltration for investigational use.

The purity of the ^{11}C-ACPC product has been assessed by thin-layer chromatography using Eastman silica gel chromatogram sheets (13179) developed in butanol–water–acetic acid (100:10:5 v/v). When required, ^{14}C chromatogram patterns were viewed with the aid of a spark chamber (Birchover Instruments, Bancroft, U.K.). The ^{14}C-ACPC was obtained from New England Nuclear Corp. (Boston, Mass.).

RESULTS AND DISCUSSION

Cyclotron production of $^{11}CO_2/^{11}CO$ at saturation, using a beam current of 150 μA and the targetry described above, is approximately 4 Ci. The conversion of $^{11}CO_2/^{11}CO$ through $^{11}CH_4$ to $H^{11}CN$ is 90% efficient. The combined yield in Steps 4 and 5 is approximately 40%, with the synthesis and purification steps requiring about 1 hr. Our overall batch yield of ^{11}C-ACPC for investigational use is approximately 100 mCi for a 40-min bombardment.

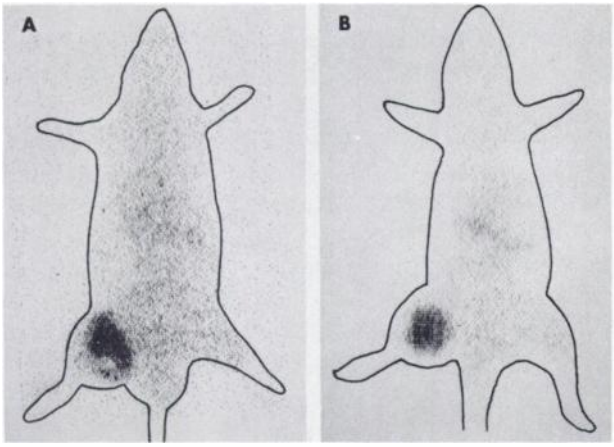

FIG. 2. (A) Rectilinear scan of male Buffalo rat bearing transplanted Morris 5123C hepatoma (12 gm) in left thigh. Rat was killed 30 min after receiving 750 μCi of ^{11}C-ACPC by intravenous injection. Tumor shows necrotic area of decreased density. (B) Similar ^{11}C-ACPC scan of male ACI rat bearing solid transplanted H-35 Reuber hepatoma. Both scans were carried out with 88-hole 6.5-in. focusing collimator with focal length 3 in.

TABLE 1. EARLY (30-MIN) TISSUE DISTRIBUTION OF LABELED ACPC IN MALE BUFFALO RATS BEARING MORRIS 5123C HEPATOMAS

Tissue	Percent administered dose per gm	
	^{14}C-ACPC*	^{11}C-ACPC†
Liver	0.46	0.46
Spleen	0.54	0.71
Kidney	0.65	0.49
Muscle	0.47	0.41
Marrow	0.61	0.67
Small intestine	0.79	0.54
Pancreas	3.1	2.2
Hepatoma	2.3	3.1

* Average of six animals.
† From animal shown in Fig. 2A (see text and legend).

In developmental studies before actually producing the ^{11}C-ACPC, carbon, hydrogen, and nitrogen determinations and infrared spectrometry were used to show that the synthesis and purification processes intended for use in the incorporation of ^{11}C into tests of ^{11}C-ACPC using standard USP techniques gave negative results, and no radiolytic decomposition of ^{11}C-ACPC has appeared in the runs made to date.

A test sample of ^{11}C-ACPC (from a run spiked with $K^{14}CN$ prior to synthesis) gave a thin-layer chromatographic pattern similar to that of ^{14}C-ACPC and gave no indication of any impurities when the radiochromatograms were viewed with a spark chamber. Ninhydrin development of the chromatograms also indicated the presence of only a single component.

The tissue distribution of ^{11}C-ACPC in tumor-bearing animals was the same as that obtained with ^{14}C-ACPC. Figure 2A shows a scan obtained 30 min after injecting ^{11}C-ACPC intravenously into a rat bearing a 5123C Morris hepatoma, and Fig. 2B shows a similar scan of a rat bearing an H-35 Reuber hepatoma. Table 1 shows the tissue distribution of ^{11}C-ACPC in the animal shown in Fig. 2A, based on a ^{14}C spike introduced in the production of the ^{11}C-ACPC used for that scan. The table also includes tissue distribution data obtained under the same conditions in 5123C hepatoma rats given ^{14}C-ACPC. Similar results were obtained in preliminary ^{11}C-ACPC scanning studies in a number of other animal tumor systems: Morris 7794 and 7787 hepatomas, RFT rat tumor, B-16 mouse melanoma, Novikoff hepatoma, and R-3259 giant-cell sarcoma. The Morris 7777 hepatoma, on the other hand, showed no preferential uptake of ^{11}C-ACPC.

Loss of ^{11}C activity by in vivo decarboxylation of ^{11}C-ACPC does not appear to pose a problem in its use (*6*). Although the data obtained in rats (Table 1) and mice (*4*) suggest that ^{11}C-ACPC has potential as a pancreas-visualizing agent in humans, poor uptake results obtained by others in the rabbit, dog, and monkey (*9,10*) indicate that the concentration of ACPC in the rodent pancreas may be species-specific.

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