Carbon-11-Labeled Methylated Polyamine Analogs: Uptake in Prostate and Tumor in Animal Models

Michael J. Welch, R. Edward Coleman, Maria G. Straatmann, Barbara E. Asberry, Joan L. Primeau, William R. Fair, and Michel M. Ter-Pogossian

Mallinckrodt Institute of Radiology and Washington University School of Medicine, St. Louis, Missouri

The polyamines putrescine, spermine, and spermidine were methylated by the addition of carbon-11-labeled formaldehyde followed by sodium borohydride. High labeling yields were obtained and the final products were purified by simply boiling the solution. This decomposed the excess sodium borohydride and removed the volatile impurities. The final radiochemical purity of all the methylated compounds was above 85%. All three methylated compounds accumulated in the prostates of male rats and the distribution of N-methyl-1,4-diaminobutane (the putrescine analog) was very similar to that previously obtained with tritiated putrescine. The uptake of the putrescine analog in both the prostate and in mouse tumor was slightly higher than that obtained with the other two analogs studied. Utilizing a positron transaxial tomographic scanner and the putrescine analog, we have been able to image the prostate gland of a dog.

J Nucl Med 18: 74–78, 1977

We have reported (*1*) a general method for the methylation of molecules containing a free amino group which are stable in aqueous solution at pH 8. One series of potential compounds for methylation are the polyamines putrescine [NH_2–$(CH_2)_4$–NH_2], spermine [NH_2–$(CH_2)_3$–NH–$(CH_2)_4$–NH–$(CH_2)_3$–NH_2], and spermidine [NH_2–$(CH_2)_3$–NH–$(CH_2)_4$–NH_2]. These compounds are of potential interest in tumor localization and prostate imaging. Clark and Fair (*2*), using ^{3}H-2,3-,putrescine, showed uptake of radiolabeled putrescine by the rat's ventral prostate.

The current study was undertaken for three reasons: first, to compare the in vivo behavior of a tritiated metabolite, ^{3}H-2,3-putrescine, and its methylated analog; second, to compare the in vivo behavior of analogs of putrescine, spermine, and spermidine; and third, to attempt to visualize the prostate in dogs with a methylated polyamine using the positron emission transaxial tomographic scanner (PETT) (*3,4*).

MATERIALS AND METHODS

^{11}C-Methylation of the polyamines. Methylation of the amine was carried out in the manner previously described for the methylation of proteins. Briefly, ^{11}C-labeled formaldehyde is prepared from $^{11}CO_2$ (*5*) by reduction to $^{11}CH_3OH$ and oxidation to $H^{11}CHO$ over an iron–molybdenum catalyst.

After distillation the formaldehyde solution (~2 cc) was mixed with the polyamine (0.2–2 mg) in borate buffer (0.4 *M*, pH = 7.9), and sodium borohydride (15 ± 3 μM) was added slowly over a 2-min time period. The pH is then adjusted to 7 ± 0.2 with dilute hydrochloric acid, and the solution boiled vigorously until the volume is reduced by one-half. The boiling decomposes the excess sodium borohydride and removes unreacted ^{11}C-formaldehyde and residual ^{11}C-methanol from the solution. Aliquots of the final solution were tested for sterility and pyrogenicity by conventional techniques and for radiochemical purity by liquid chromatography.

Liquid chromatography of all three methylated polyamines was carried out using a column* of ion-

Received July 6, 1976; revision accepted Aug. 30, 1976.

For reprints contact: Michael Welch, Mallinckrodt Institute of Radiology, 510 S. Kingshighway Blvd., St. Louis, MO 63110.

exchange resin (0.9 × 3.5 cm) eluted with citrate buffer. Different conditions were used for the analysis of the analog of each polyamine. For putrescine and spermine, the column was maintained at 70°C and eluted with a citrate buffer at pH 6.15 (±0.5), high ionic strength (0.7 *M*), and a flow rate of 2 ml/min. Putrescine is eluted before spermine, and their methylated analogs are eluted just after the nonmethylated compound. For spermidine, a 0.7 *M* phosphate buffer (pH 7.0) was used at room temperature and a flow rate of 2 ml/min.

After elution of the labeled methylated analog, the eluting solution was changed to 0.3 *M* sodium hydroxide to elute any impurities still absorbed in the column.

ANIMAL STUDIES

Putrescine distribution studies. Male Sprague–Dawley rats, weighing 300–500 gm, were anesthetized with diethyl ether by inhalation and the femoral vein exposed. The labeled polyamine was then injected into the femoral vein, with the amount of radioactivity varying between 50 and 100 μCi/rat. The amount of nonmethylated polyamine injected varied from ~60 μg to 1.0 mg/rat. After about 50 min the animals were killed and tissue samples transferred directly to counting vials without washing. The samples were counted in a well scintillation counter and weighed in a Mettler balance. The carbon-11 content was recorded as amount of activity per gram of tissue, and in some cases as percent injected dose/gm tissue.

Tumor uptake studies. The methylated polyamines were injected into tumor-bearing mice (B-16 melanoma) through the tail vein. The animals were injected 11 ± 2 days after transplantation of the tumor, a timing that permits minimal observable necrosis. This tumor has previously been used in radiopharmaceutical studies (*6*). From 5 to 300 μg of polyamine was injected, the animals were killed 30 and 50 min after injection, and tissue samples were taken and counted as described above.

PETT studies. The putrescine analog (N-methyl-1,4-diaminobutane) was injected intravenously into four male dogs anesthetized with sodium pentobarbital (30 mg/kg). The dogs were placed supine in the Mallinckrodt Institute's PETT scanner (*3,4*), arranged so that the tomographic slice would pass through the center of the prostate. In all cases transmission scans (*3,4*) using a ring of ^{64}Cu were obtained before injection of the putrescine analog in order to obtain data for the attenuation correction of the positron image. In three of the dogs, the bladder was drained by a catheter between injection and imaging. In two of the dogs, an ileal–bladder urinary bypass was performed surgically prior to the radioactive study to ensure that no urinary activity interfered with the PETT scan. In two dogs diffusible tracer ($^{13}NH_3$) was injected directly into the prostate to confirm the position of the prostate in the tomogram.

RESULTS

Labeling efficiency. With the current targetry at the Washington University Cyclotron (*7*), about 50 mCi of $^{11}CO_2$ was obtained following a 10-μA·hr bombardment. An overall radiochemical yield of ~70% was obtained in the reaction to produce ^{11}C-labeled formaldehyde, this reaction taking 10–13 min. The radiochemical yield in the methylation reaction was 40–70%, and the time for this step, including purification of the final product, was 12–15 min. Final yields of 6–10 mCi of the methylated polyamine were obtained from ~50 mCi of $^{11}CO_2$, the total reaction time being 25 min. The specific activity of the methylated polyamine is uncertain, since traces of carrier CO_2 decrease the activity of the labeled formaldehyde. The procedures outlined (*1*) to reduce the level of CO_2 carrier were followed. All solutions tested proved to be sterile and pyrogen-free. Figure 1 shows a typical radiochromatographic trace obtained in the analysis of the putrescine analog, in which the labeled product is well separated from the observed impurities. Similar chromatographic traces were obtained with the analogs of spermine and spermidine. Radiochemical purities of >85% were obtained in all cases. Table 1 shows the radiochemical purity of typical samples used in the animal studies described below.

Rat and mouse distribution studies. The distribution of ^{11}C-methylated putrescine in Sprague–Dawley rats, 40 min after intravenous injection, is shown in Table 2. We include in parentheses, for comparison, the data of Clark and Fair (*2*), who injected tri-

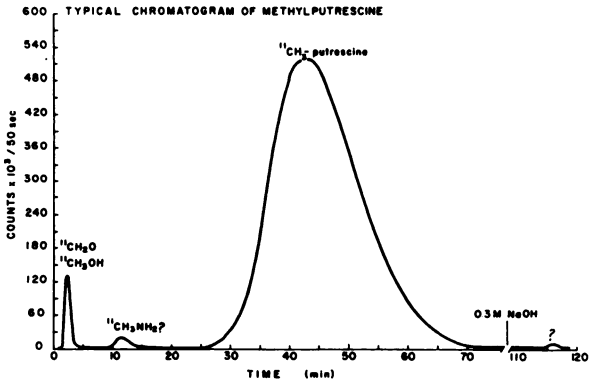

FIG. 1. Typical chromatogram of methylated putrescine. Analysis was performed on 3.5 × 0.9-cm column of ion-exchange resin operated at 70°C eluted with 0.7 M citrate buffer (pH 6.1) at flow rate of 2 ml/min.

TABLE 1. PURITY OF TYPICAL METHYLATED POLYAMINE PREPARATIONS

Agent	% Formaldehyde/methanol	% $^{11}CH_3NH_2$	% Methylated polyamine	% Eluted with NaOH
Putrescine	1.4	0.6	97.8	0.1
	6.3	0.6	92.5	0.6
Spermine	9.3	1.6	89.1	—
	10.1	0.6	89.4	—
Spermidine	7.0	0.1	91.2	1.7
	9.0	—	89.4	1.6

TABLE 2. UPTAKE OF METHYLATED PUTRESCINE IN RAT ORGANS 40 MIN AFTER INTRAVENOUS INJECTION* (n = 11)

Tissue	% Dose per gram tissue		Ratio of organ to muscle (abdominal wall)	
Abdominal wall muscle	0.10 ± 0.2	(0.11)	1.0	
Ventral prostate	0.88 ± 0.5	(0.91)	8.8 ± 5	(8.3)
Dorsal prostate	1.0 ± 0.7	(0.36)	10.1 ± 7	(3.3)
Pancreas	0.29 ± 0.11	(1.16)	2.9 ± 1.1	(10.5)
Liver	0.63 ± 0.15	(0.31)	6.3 ± 1.5	(2.8)
Kidney	0.47 ± 0.006	(0.52)	4.7 ± 0.6	(5.3)
Whole blood	0.072 ± 0.004	(0.11)	0.72 ± 0.04	(1)

* Figures in parentheses are for ^{3}H-2,3-putrescine 1 hr after injection (from Ref. 2).

tiated putrescine and sampled 1 hr later. Table 3 shows a comparison of the ratios between various organs and the muscle of the abdominal wall for the analogs of the three polyamines studied. Similar ratios were obtained at 30 min and at 1 hr for all organs except the kidney, whose ratio dropped during this period.

Table 4 shows tumor-to-organ ratios of the three analogs studied at 30 and 50 min in the tumor-bearing mice (tumor weight, ~1 gm). In many of the animals some of the sample was lost during the injection, which prevented quantitative determination of % injected dose/gm. Statistically there is no difference between the data obtained at 30 and 50 min.

PETT studies. The putrescine analog was chosen for the prostatic imaging studies because it exhibited the lowest kidney activity at 40 min (Table 3). With this compound, therefore, the problem of bladder background may be minimized. In all five dogs, a single source of activity was observed in the region of the prostate. In the dogs in which a tracer was injected into the prostate, the position of the prostate is confirmed. Figure 2 shows the typical PETT scans of an animal with a urinary bypass. The two scans had the animal in the same position on the PETT table. The transmission image records the cross-sectional distribution of attenuation coefficients at the level of the prostate, with the hind legs spread laterally, the dorsal surface down, and the ventral up. The area of the prostate is clearly visualized in the radionuclide image. The corrected counts observed in the image are directly proportional to the activity in that area, and the counts can be obtained as a numerical printout. With this feature the tomograph offers quantitative data (*8*). From the numerical counts over different areas of the scan, the ratio of activity in the prostate to that in the surrounding muscle is calculated to be ~6.5.

DISCUSSION

With the methylation techniques, good yields of all the methylated polyamines were obtained. As is seen in Table 2, the distribution of the ^{11}C-methylated putrescine at 40 min is similar to that obtained by Clark and Fair (*2*) with tritiated putrescine at 1 hr. Examination of the data with tritiated compounds (Clark and Fair) shows that, with the exception of the kidney, the tissue values are very similar from 30 min to 1 hr, so a comparison of their data with the results of this study appears valid. The major differences are that more methyl analog was taken up in the dorsal prostate and liver and less in the pancreas than was the tritiated compound.

In our study the amount of unlabeled polyamine varied between 5 and 100 times that used by Clark and Fair and, if the unlabeled amines act as a carrier for the methylated analogs, no carrier effect was observed. It is well documented, however, that pancreatic uptake of amino acids decreases as the specific activity of the administered compound decreases (*9*). A possible explanation for the difference in pancreas

TABLE 3. UPTAKE AT 40 MIN OF THE METHYLATED ANALOGS OF THE THREE POLYAMINES IN THE MALE RAT

Tissue	Ratio of organ to muscle (abdominal wall)		
	Putrescine (n = 11)	Spermine (n = 8)	Spermidine (n = 6)
Ventral prostate	8.8 ± 5	6.2 ± 3.1	9.06 ± 4.2
Dorsal prostate	10.1 ± 7	3.6 ± 1.7	6.7 ± 2.5
Pancreas	2.9 ± 1.1	2.7 ± 1.4	5.3 ± 1.8
Liver	6.3 ± 1.5	9.1 ± 4.0	13.5 ± 3.2
Kidney	4.7 ± 0.6	43.5 ± 17	54.5 ± 15
Whole blood	0.72 ± 0.04	0.62 ± 0.27	2.25 ± 0.7

TABLE 4. TUMOR-TO-ORGAN RATIOS OF METHYLATED POLYAMINES IN TUMOR-BEARING MICE

	Putrescine		Spermine		Spermidine	
Organ	30 min*	50 min†	30 min*	50 min†	30 min*	50 min†
Blood	9.55 ± 2.6	8.0 ±2.0	5.85 ± 2.3	4.2 ± 1.8	2.9 ± 1.6	1.8 ± 0.6
Muscle	4.14 ± 0.8	6.6 ± 3.0	5.3 ± 2.2	5.0 ± 2.1	2.9 ± 1.7	1.8 ± 0.4
Pancreas	1.6 ± 0.3	1.4 ± 0.3	1.8 ± 0.7	1.9 ± 0.6	0.9 ± 0.4	0.7 ± 0.2
Liver	0.46 ± 0.14	0.4 ± 0.14	0.35 ± 0.16	0.33 ± 0.13	0.34 ± 0.2	0.2 ± 0.04
Kidney	0.9 ± 0.3	1.1 ± 0.3	0.12 ± 0.04	0.12 ± 0.04	0.27 ± 0.14	0.16 ± 0.013

* n = 6
† n = 4

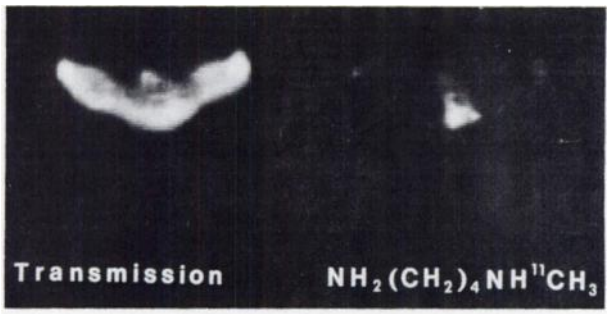

FIG. 2. Typical PETT transmission and positron images of normal dog prostate. Subject is supine, legs spread apart with urinary bypass.

data is this variability in specific activity, although an alternative argument can be made that the pancreas and not the prostate uptake is sensitive to the methyl group in the putrescine analog. We note that whereas the rat pancreas contains relatively large amounts of polyamines, the human pancreas does not (*10*). Thus, even if an analog of higher specific activity concentrated in the rat pancreas, it probably would not be useful as a human pancreas agent. It has been observed (*2,11,12*) that the biosynthesis of the polyamines involves the formation of spermine and spermidine through putrescine. If the metabolism of the putrescine analog releases small aliphatic amines, they might be expected to concentrate in the liver, as does ammonia and probably methylamine (*13*), which explains our high liver uptake, which was not observed by Clark and Fair.

We conclude that the methylated putrescine is a good analog for putrescine. Although it is possible to label putrescine with carbon-11 by the reaction of $H^{11}CN$ with 1,2-dibromoethane followed by reduction, the methylation technique used by us is simple and fast, the product is easy to purify, and the specific activity is high and depends upon the specific activity of the ^{11}C-formaldehyde that can be obtained. The methylation technique can be applied to any amine, and a stereospecific amine can be methylated with retention of its stereospecificity. The methylation technique therefore appears to have potential for labeling analogs of any compound containing an >NH and $-NH_2$ group. If the compound is not stable in basic solution, the reduction can be carried out in acid solution using sodium cyanoborohydride rather than sodium borohydride (*14,15*).

Of the three methylated polyamines, the putrescine analog was chosen for further studies for several reasons. First, although all three analogs accumulate in the prostate (and in the tumor model studied), the putrescine analog does appear to accumulate more than the other two amines studied in both models (Tables 3 and 4). Second, the kidney activity is much lower at 40 min with the putrescine analog (Table 3). It may therefore be easier to reduce the bladder activity in the case of the putrescine analog than with the other two analogs. Finally, while spermine has been shown to be somewhat nephrotoxic, spermidine is less toxic, and putrescine is nontoxic (*16*).

The uptake of polyamines in tumors was studied because certain tumors are known to contain large amounts of polyamines (*17,18*), possibly because putrescine transport is greatly increased in cells starting to proliferate (*19*). This needs further study. It has been postulated (*20,21*) that plasma and serum levels of polyamines may be useful in assessing effects of chemotherapy. Russell et al. (*20*) postulate that spermidine can serve as an index of tumor-cell kill, while putrescine appears to reflect the proliferative behavior or growth factor of the tumor; and, further, that polyamines may provide important new information on tumor cytokinetic parameters. It is possible that our carbon-11-labeled analogs, together with the PETT scanner, may be used to quantitate polyamine uptake in a specific tumor site at various stages of therapy. Russell's model (*21*) predicts high intracellular levels of putrescine in tumor cells during tumor growth and that this level will drop drastically when the cells are killed.

Figure 2 shows that the feasibility of using the methylated putrescine to visualize the prostate area

in a normal dog. The prostate-to-muscle ratios obtained from the quantitative tomographic image are very similar to those obtained in the rat model. The prostate can easily be visualized if the bladder is emptied prior to scanning. The relative uptake of the polyamines in prostate tumors compared to normal prostate must still be studied before the compound could be used to study prostate tumors.

CONCLUSION

We have established the following:

1. The in vivo behavior of tritiated putrescine and its carbon-11-labeled analog are very similar in the male rat.
2. The analogs of all three of the polyamines putrescine, spermine, and spermidine accumulate in the rat prostate and in the mouse melanoma studied. The putrescine analog appears most promising for further studies.
3. Using the PETT scanner, it is possible to visualize the dog prostate with the putrescine analog.

ACKNOWLEDGMENTS

This work was supported by U.S. PHS Grant 5 PO1 HL13851. The assistance of the staff of the Washington University Medical School Cyclotron and of Robert Feldhaus is gratefully acknowledged.

FOOTNOTE

* Durrum D.C.-1A.

REFERENCES

1. STRAATMANN MG, WELCH MJ: A general method for labeling proteins with ^{11}C. *J Nucl Med* 16: 425–428, 1975

2. CLARK RB, FAIR WR: The selective in vivo incorporation and metabolism of radioactive putrescine in the adult male rat. *J Nucl Med* 16: 337–342, 1975

3. TER-POGOSSIAN MM, PHELPS ME, HOFFMAN EJ, et al.: A positron emission transaxial tomograph for nuclear imaging (PETT). *Radiology* 114: 89–98, 1975

4. HOFFMAN EJ, PHELPS ME, MULLANI NA, et al.: Design and performance characteristics of a whole body positron transaxial tomograph. *J Nucl Med* 17: 493–502, 1976

5. WELCH MJ, TER-POGOSSIAN MM: Preparation of short half-lived radioactive gases for medical studies. *Radiat Res* 36: 580–587, 1968

6. HAYES RL, WASHBURN LC, WIELAND BW, et al.: Carboxyl-labeled ^{11}C-1-aminocyclopentanecarboxylic acid. A potential agent for cancer detection. *J Nucl Med* 17: 748–751, 1976

7. WIELAND BW: *Development and Evaluation of Facilities for the Efficient Production of Compounds Labeled with Carbon-11 and Oxygen-15 at the Washington University Cyclotron.* Ph.D. Thesis, Ohio State University, 1973

8. TER-POGOSSIAN MM, WEISS ES, COLEMAN RE, et al.: Computed tomography of the heart. *Am J Roentgenol Radium Ther Nucl Med* 127: 79–90, 1976

9. ATKINS HL, CHRISTMAN DR, FOWLER JS, et al.: Organic radiopharmaceuticals labeled with isotopes of short half-life. V. ^{18}F-labeled 5- and 6-fluorotryptophan. *J Nucl Med* 13: 713–719, 1972

10. TABOR H, TABOR CW: Spermidine, spermine and related amines. *Pharmacol Rev* 16: 245–300, 1964

11. PEGG AE: Biosynthesis of putrescine and polyamines in mammalian tissues. *Ann NY Acad Sci* 171: 977–987, 1970

12. JANNE J: Studies on the biosynthetic pathway of polyamines in rat liver. *Acta Physiol Scand (Suppl)* 300: 1–71, 1967

13. CARTER CC, LIFTON JF, WELCH MJ: Organ uptake and blood pH and concentration effects of ammonia in dogs determined with ammonia labeled with 10-minute half-lived nitrogen-13. *Neurology* 23: 204–213, 1973

14. BORCH RF, BERNSTEIN MD, DURST HD: The cyanohydridoborate anion as a selective reducing agent. *J Am Chem Soc* 93: 2897–2904, 1971

15. BORCH RF, HASSID AI: A new method for the methylation of amines. *J Org Chem* 37: 1673–1674, 1972

16. TABOR CW, ROSENTHAL SM: Pharmacology of spermine and spermidine. Some effects on animal and bacteria. *J Pharmacol Exp Ther* 116: 139–155, 1956

17. RUSSELL DH, LEVY CC: Polyamine accumulation and biosynthesis in a mouse L1210 leukemia. *Cancer Res* 31: 248–251, 1971

18. BACHRACH U, BEN-JOSEPH M: Tumor cell polyamines and polyamine derivatives. In *Polyamines in Normal and Neplastic Growth*, Russell DH, ed. New York, Raven Press, 1973, pp 15–26

19. POHJANPELTO P: Putrescine transport is greatly increased in human fibroblasts initiated to proliferate. *J Cell Biol* 68: 512–520, 1976

20. RUSSELL DH, DURIE BGM, SALMON SE: Polyamines as predicators of success and failure in cancer chemotherapy. *Lancet* 2: 797–799, 1975

21. RUSSELL DH, RUSSELL SD: Relative usefulness of measuring polyamines in serum, plasma and urine as biochemical markers of cancer. *Clin Chem* 21: 860–863, 1975