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Tumor detection with carbon-11-labelled amino acids*

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Abstract. A comparative study of tumor detection with ten ^{11}C-labeled amino acids including four newly synthesized amino acids was carried out to find the most valuable ^{11}C-labeled amino acid for the diagnosis of cancer. ^{11}C-L-methionine showed the highest uptake by the experimental rat hepatoma AH109A (2.7% administered dose/g at 20 min, tumor to blood ratio; 11.4). The second highest uptake was of ^{11}C-aminocyclopentane-carboxylic acid (ACPC). The newly synthesized ^{11}C-DL-methyl-ACPC characteristically showed higher accumulation in tumor than in liver and the tumor to liver ratio reached 3.0 at 60 min after injection. It is suggested that ^{11}C-L-methionine and ^{11}C-DL-methyl-ACPC are useful amino acids for the diagnosis of cancer using positron emission tomography.

Recent advances in the synthesis of positron emitting radiopharmaceuticals and in the technique of positron emission tomography have made possible the quantitative measurement of metabolic processes in vivo. The increase in the incorporation of amino acids into tumor tissue was well demonstrated (Busch et al. 1959). ^{13}N-labeled glutamate has been used to detect brain tumors (Reiman et al. 1982) and osteogenic sarcomas (Gelbard et al. 1979). ^{35}S-labeled-5-thio-D-glucose was reported to accumulate in hamster pancreatic tumors (Markoe et al. 1979). Some synthetic nonmetabolized amino acids, ^{11}C-α-aminoisobutyric acid (Dunzendorfer et al. 1981), ^{11}C-amino-cyclopentane-carboxylic acid (Berlinguet et al. 1962; Hayes et al. 1976), and ^{11}C-aminocyclobutane carboxylic acid (Hübner et al. 1981) were also demonstrated as potential agents for tumor detection. But differences in each agent have not yet been studied. In our present paper, the comparative study of tumor detection with five physiological and five synthetic ^{11}C-labeled amino acids, along with characterization of each agent, are described. In addition, four of the ten amino acids were newly synthesized and evaluated for the first time.

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Materials and methods

Synthesis of carbon-11-labeled amino acids

Three essential and two nonessential physiological amino acids of L-methionine (Met), DL-phenylalanine (Phe), DL-leucine (Leu), DL-phenylglycine (PGly), and DL-norleucine (NLeu), and five synthetic unphysiological amino acids, 1-aminocyclopentane-1-carboxylic acid (ACPC), DL-3-methyl-1-aminocyclopentane-1-carboxylic acid (methyl-ACPC), 1-aminocyclohexane-1-carboxylic acid (ACHC), 4-methyl-1-aminocyclohexane-1-carboxylic acid (methyl-ACHC), and DL-2-cyclohexylglycine labeled with carbon-11 were synthesized by a non-carrier-added method. ^{11}C-Met was synthesized from $^{11}CH_3I$ (Comar et al. 1976). The other nine ^{11}C-amino acids were prepared by a new no-carrier-added synthesis method. This method will be described in detail elsewhere (Iida et al. 1984), but is outlined in Fig. 1. H^{11}CN was produced by the catalytic reaction of $^{11}CH_4$ on Pt at 950° C, and it was directly bubbled into a reaction solution which contained aminosulfite. The mixture was heated for 10 min and aminonitrile was then extracted with ether. After acid hydrolysis, ^{11}C-amino acid was purified. The preparation was carried out within 60 min. For the tissue distribution study, 2–5 mCi of no-carrier-added ^{11}C-labeled amino acid was prepared.

Tissue distribution studies

Transplantable ascitic hepatoma (AH109A) cells were inoculated sc into young male Donryu rats (weighing from

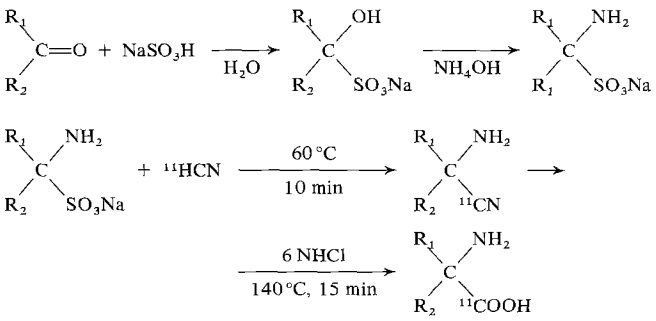

Fig. 1. The outline of a new no-carrier-added synthesis method of ^{11}C-DL-amino acids

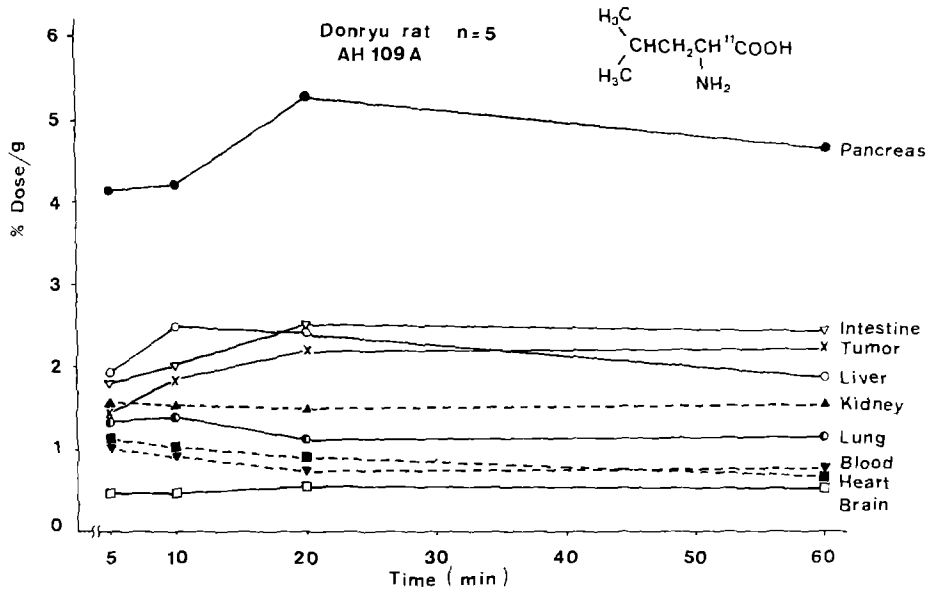

Fig. 2. The time-activity curves of ^{11}C-leucine. ^{11}C-Leu accumulated in the pancreas with high concentration and its peak (5.3% dose/g) appeared 20 min after injection. Donryu rats and ascitic hepatoma AH109A were used. Mean of 5 animals

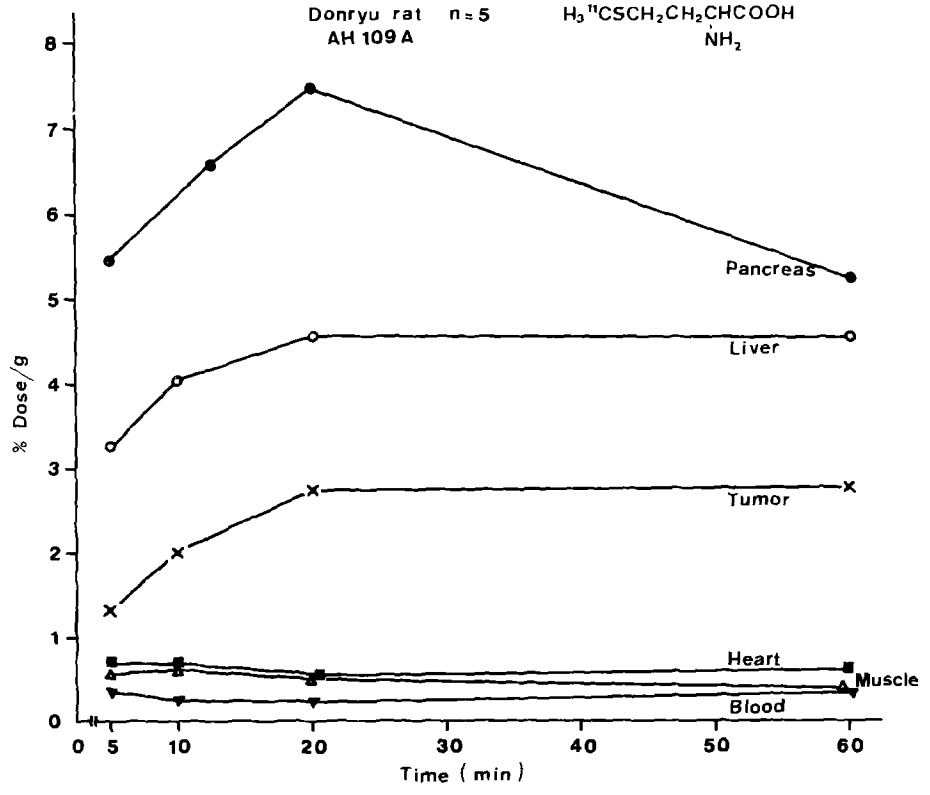

Fig. 3. The time-activity curves of ^{11}C-methionine in Donryu rats. Pancreas activity was the highest (7.5% dose/g) at 20 min after injection. Tumor and liver activities also reached their maximum at 20 min after injection. Although tumor activity was lower than those of the liver and pancreas, the tumor was clearly distinguished from the other tissues

120 to 140 g). Five rats were used for each data point. When the tumor grew to about 1 g, the animals were fasted for 24 h and 100 μCi ^{11}C-labeled amino acid was injected iv through the tail vein. The animals were killed by cervical dislocation at 5, 10, 20, and 60 min after injection. Organs and tissue samples were excised, blotted to remove adhering blood, weighed, and counted in a well-type NaI (Tl) scintillation counter (Autogamma 800C, Packard) and the results were corrected for decay. Data was expressed as the percentage of administered dose per gram of tissue (PAD, % dose/g). Ratios for tumor to blood, tumor to muscle, and tumor to liver were then calculated.

Results

Two series of experiments were carried out. First, three essential and two nonessential physiological amino acids were studied. The other series consisted of studies of five synthetic unphysiological amino acids.

In the first series, three essential amino acids, ^{11}C-Met, ^{11}C-Phe, and ^{11}C-Leu, and two nonessential ^{11}C-PGly and ^{11}C-NLeu were administered to the tumor-bearing rats and then their tumor uptake and other tissue distributions were studied. Figure 2 shows the time-activity curves of ^{11}C-Leu. ^{11}C-Leu accumulated in the pancreas at a high concentra-

Table 1. Tissue distribution of ^{11}C-amino acids

	% Administered dose/g of tissue[a]				
	Tumor[b]	Pancreas	Tumor to blood	Tumor to muscle	Tumor to liver
^{11}C-Methionine	2.74±0.36[c]	7.48±1.13[c]	11.40	4.70	0.60
^{11}C-Leucine	2.20±0.21	5.25±1.19	2.85	2.39	0.89
^{11}C-Norleucine	1.56±0.13	2.42±0.21	2.01	1.88	1.55
^{11}C-Phenylalanine	1.42±0.25	5.43±1.19	3.32	2.67	0.77
^{11}C-Phenylglycine	1.40±0.31	2.99±0.25	2.08	2.36	1.82

[a] 20 min after iv injection, Donryu rats $n=5$, Mean ±SD
[b] Experimental rat hepatoma (AH109A)
[c] $P<0.02$ (Student's t-test), compared with ^{11}C-leucine

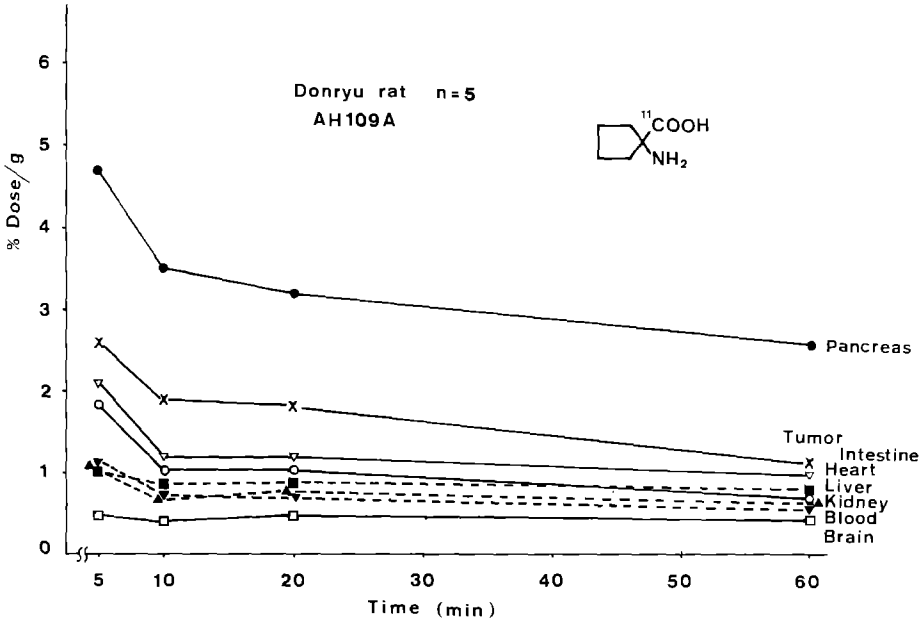

Fig. 4. The time-activity curves of ^{11}C-ACPC. ACPC was shown to accumulate in the tumor and pancreas

tion and the peak (5.3% dose/g) appeared 20 min after injection. Tumor uptake increased gradually and also reached a peak (2.2% dose/g) 20 min after injection, but the tumor activity was lower than that in the liver and intestine. Figure 3 shows the time-activity curves of ^{11}C-Met. Pancreatic activity was highest 20 min after injection, and was 7.5% dose/g. Tumor and liver activities also reached their maximum at 20 min, and their mean activities were 2.7 and 4.6 respectively. Although the tumor activity was lower than those of the liver and pancreas, the tumor was clearly distinguished from the other tissues.

Table 1 summarizes the tissue distribution of all five ^{11}C-amino acids in the first series. Tumor uptake of ^{11}C-Met was the highest of all, followed by ^{11}C-Leu, ^{11}C-NLeu, ^{11}C-Phe, and ^{11}C-PGly. Compared with three essential amino acids, two nonessential amino acids, ^{11}C-NLeu and ^{11}C-PGly, characteristically showed lower uptake in the liver. Therefore, the tumor to liver ratio of these nonessential amino acids became higher than the essential amino acids.

In the second series, tumor and tissue distribution of five synthetic unphysiological amino acids, ^{11}C-ACPC, ^{11}C-methyl-ACPC, ^{11}C-ACHC, ^{11}C-methyl-ACHC, and ^{11}C-DL-cyclohexylglycine, were studied.

Figure 4 shows the time-activity curves of ^{11}C-ACPC. ACPC was shown to accumulate in the tumor and pancreas. The time-activity curves decreased to a plateau at 20 min after injection.

Figure 5 shows the time-activity curves of ^{11}C-methyl-ACPC. The radioactivity in the tumor and pancreas was higher than in the other tissues. The time-activity curve of the pancreas showed fluctuation which may be due to experimental variation.

Table 2 summarizes the tissue distribution of five synthetic unphysiological amino acids. Tumor uptake of ^{11}C-ACPC was the highest of all, followed by ^{11}C-methyl-ACPC, ^{11}C-cyclohexylglycine, ^{11}C-ACHC, and ^{11}C-methyl-ACHC. Blood clearance of ^{11}C-methyl-ACPC was faster than that of ^{11}C-ACPC, and liver uptake of ^{11}C-methyl-ACPC was lower than that of ^{11}C-ACPC. Therefore, the tumor to blood, tumor to muscle, and tumor to liver ratios of ^{11}C-methyl-ACPC were all higher than that of ^{11}C-ACPC.

In conclusion, ^{11}C-Met exhibited the highest uptake by the tumor, liver, and pancreas of the five physiological amino acid. ^{11}C-methyl-ACPC had the strongest specific tumor detecting ability among the five unphysiological amino acids.

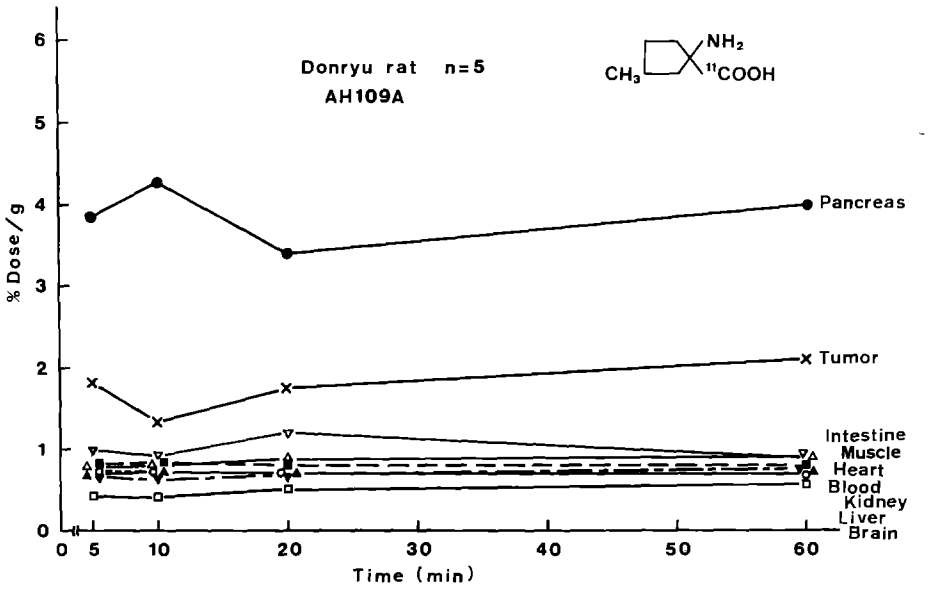

Fig. 5. The time-activity curves of ^{11}C-methyl-ACPC. Radioactivity of tumor and pancreas was higher than the other tissues

Table 2. Tissue distribution of ^{11}C-amino acids

	% Administered dose/g of tissue[a]					
	Time[b]	Tumor[c]	Pancreas	Tumor to blood	Tumor to muscle	Tumor to liver
^{11}C-ACPC	5 min	2.32±0.33[d]	4.69±1.33	2.06	2.17	1.25
^{11}C-methyl ACPC	5 min	1.84±0.29[e]	3.87±0.35	2.73	2.35	2.45
	60 min	2.11±0.03[d]	4.01±0.40	2.89	2.29	3.04
^{11}C-ACHC	20 min	1.32±0.11	2.96±0.83	1.21	2.40	1.06
^{11}C-methyl ACHC	20 min	1.02±0.13	1.72±0.31	1.14	1.89	1.42
^{11}C-DL-2 Cyclohexyl glycine	10 min	1.41±0.14	4.70±0.61	1.82	1.91	1.71

[a] Donryu rats $n=5$, Mean ±SD
[b] Time after injection
[c] Experimental rat hepatoma (AH109A)
[d] $P<0.001$ (Student's t-test), compared with ACHC, methyl-ACHC, and DL-2 cyclohexyl glycine
[e] $P<0.001$ compared with ACHC, methyl-ACHC, and $P<0.02$ compared with DL-2 cyclohexyl glycine

Discussion

The purpose of the present study was to find the most valuable ^{11}C-labeled amino acid for tumor detection. Through studying tissue distribution of ten amino acids, and comparing each time-activity curve, ^{11}C-Met showed the highest uptake by the experimental rat hepatoma AH109A than other nine agents, the second was ^{11}C-ACPC, and ^{11}C-methyl-ACPC showed the highest contrast in the liver.

The mechanisms of synthetic amino acid accumulation in the tumor seemed to be different from those of physiological amino acids. Since ^{11}C-ACPC was not metabolized nor incorporated into protein (Berlinguet et al. 1962), it was suggested that the increased tumor uptake of this agent reflected the pathological permeability of tumor capillaries and increased tumor cell activity of amino acid transport.

The same mechanisms are suggested by the tumor uptake of the four new synthetic amino acids, ^{11}C-methyl-ACPC, ^{11}C-ACHC, ^{11}C-methyl-ACHC, and ^{11}C-cyclohexylglycine. Relatively slow clearance from the blood pool and low concentration in the liver of these four amino acids seems to support the hypothesis. When it is compared with other amino acids, ^{11}C-methyl-ACPC may offer an advantage because the high tumor to liver ratio will diminish the interference of the liver in the scintigraphy of abdominal tumors.

On the other hand, one of the important routes of physiological amino acids is incorporation into protein synthesis. It was reported that human pancreatic tumor did not accumulate ^{11}C-L-Met (Syrota et al. 1982), but no other human tumors have been studied yet. From our experimental data and previous reports of human distribution (Syrota et al. 1979), it seems possible to detect human tumors of brain, lung, mediastinum, peritoneal cavity, and extremities. Physiologic tumor-localizing agents may provide an unique approach to the analysis of tumor metabolism in vivo. If an amino acid is taken up actively in a tumor tissue, the extent to which an amino acid accumulates in a tumor may be correlated with its metabolic requirements. An important part of the utilization of amino acids by the tumors seems to be the biosynthesis of nuclear protein (Busch et al. 1959).

Quantitative measurements of amino acid uptakes into the tumor with PET will give a good metabolic parameter

for tumor proliferation. Further studies will be pursued to develop a model system for objectively measuring response of tumors to therapy based on metabolic parameters rather than standard radiographic or subjective clinical evaluations.

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Note added to proof

Clinical study was recently reported by: Kubota K, Ito M, Fukuda H, Abe Y, Ito K, Fujiwara T, Yoshioka S, Hatazawa J, Matsuzawa T, Iwata R, Watanuki S, Ishiwata K, Ido T (1983) Cancer diagnosis with positron computed tomography and carbon-11-labelled-L-methionine. Lancet 2:1192–1193