Jpn. J. Cancer Res. 80, 1257–1260, December 1989

Kinetic Evaluation of ^{11}C-1-Aminocyclopentane Carboxylic Acid in Rabbits Bearing VX-2 Tumors Using Positron Emission Tomography

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Tumor detection with ^{11}C-1-aminocyclopentane carboxylic acid (^{11}C-ACPC) showed that this amino acid has a high affinity for malignant tumors. We studied the kinetics of intravenously injected ^{11}C-ACPC in the rabbit VX-2 tumor using positron emission tomography. Three female rabbits bearing VX-2 tumor in the thighs were used. High uptake of ^{11}C-ACPC was seen in the tumor and liver. ^{11}C-ACPC in plasma decreased rapidly after injection and its activity in the tumor increased with time. The kinetic evaluation of ACPC uptake into the tumor was performed using the unidirectional transport model. The average values of the transfer constant of ^{11}C-ACPC for VX-2 tumor were 0.030 ± 0.002 ml/min/g. This preliminary result may form the basis for a quantitative analysis of the *in vivo* distribution of ^{11}C-labeled ACPC in tumors.

Key words: ^{11}C-1-Aminocyclopentane carboxylic acid — Transfer constant — Positron emission tomography —VX-2 tumor

The unnatural amino acid 1-amino-cyclopentane-1-carboxylic acid (ACPC) has been reported to inhibit the growth of several types of tumors in experimental animals.[1] Studies on the metabolism of ACPC have shown that ACPC itself is an active inhibitor, since it is not metabolized by normal or neoplastic tissues.[2] The lack of metabolism of ACPC has been confirmed by Berlinguet *et al.*[3] and Christensen *et al.*[4] Using autoradiographic techniques with the ^{14}C-labeled compound, Berlinguet *et al.*[5] showed that there was a selective uptake of ACPC by tumor tissue. Tumor detection with ^{11}C-labeled-ACPC proved that this amino acid has a high affinity for malignant tumors.[6–8] As a consequence, ^{11}C-ACPC has been advocated as a suitable tracer for studying amino acid transport across the tumor cell membrane using positron emission tomography (PET). However, quantitative evaluation of *in vivo* ^{11}C-ACPC uptake in neoplastic lesions has not yet been reported. We therefore studied the kinetics of intravenously injected ^{11}C-ACPC in the rabbit VX-2 tumor using PET.

MATERIALS AND METHODS

The synthesis of ^{11}C-ACPC was reported elsewhere.[9] Three female Japanese white rabbits (Table I) bearing VX-2 tumor in the left thighs were used about 4 weeks after transplantation. They were fasted for 12 h prior to the experiments and anesthetized with intravenous pentobarbital sodium 25 mg/kg; further single doses of 6 mg were given as required. After transmission scanning, ^{11}C-ACPC in 3 ml of physiological saline was injected rapidly as a bolus into an ear vein. Serial scans were performed at the slice level of the tumor using a single slice PET scanner (ECAT II, EG&G Ortec, USA). Prior to administration of ^{11}C-ACPC, an indwelling catheter was placed surgically in the right femoral artery and a series of blood samples were withdrawn. The samples were centrifuged immediately, and plasma radioactivity was counted in an NaI automatic gamma counter (Model 800C, Auto gamma system, Packard Instrument Co., Inc., USA). Scans were finished 60 min after injection. The spatial resolution of the scanner was 1.2 cm FWHM (the full width at half maximum) in the high-resolution scan mode. The emission data were corrected for decay and attenuation. In the reconstructed tomographic images, regions of interest were carefully determined in order to include the pixel of maximum count in the tumor. In each rabbit, the tumor size was more than 3 cm in diameter, which corresponded to a count recovery of more than 90%.[10]

RESULTS

Figure 1 shows a tomographic image of ^{11}C-ACPC uptake in the No. 3 rabbit. ^{11}C-ACPC accumulated in the tumor. Figure 2 shows a rectilinear or planar image of the rabbit. High uptake of ^{11}C-ACPC can be seen in the tumor and liver. The time course of ^{11}C activity in plasma

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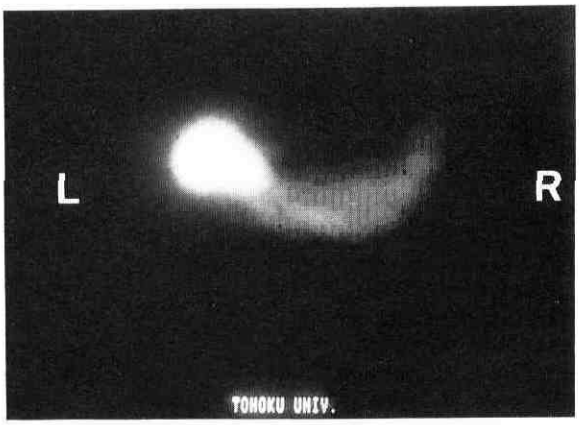

Fig. 1. Tomographic image of rabbit VX-2 tumor using ^{11}C-ACPC 60 min after injection. The tumor in the left thigh showed high uptake of ^{11}C-ACPC.

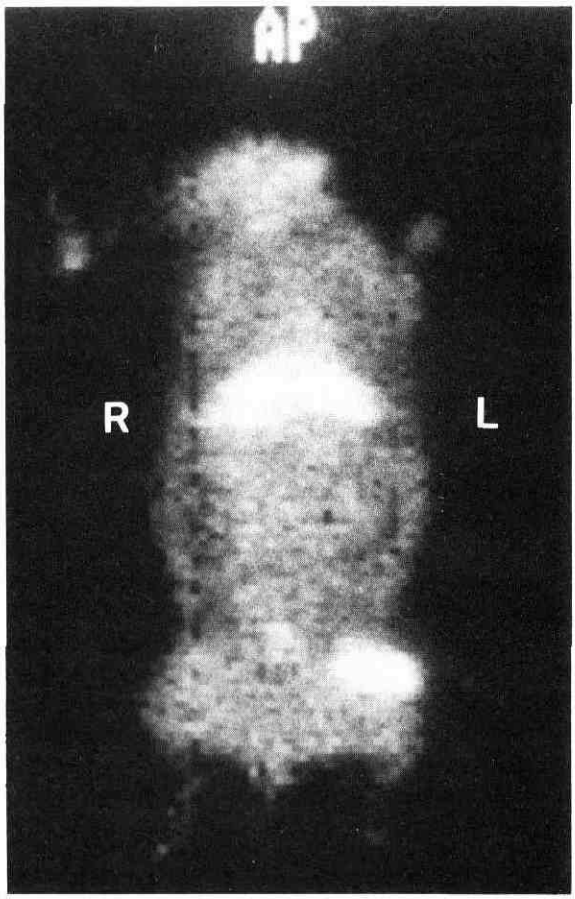

Fig. 2. Rectilinear scan of a female rabbit in the supine position bearing transplanted VX-2 tumor in the left thigh. The scan was carried out after the sequential scans of the tumor. High uptake of ^{11}C-ACPC can be seen in the tumor and liver.

and tumor is presented in Fig. 3. The activity data in plasma were converted to pixel data of PET. ^{11}C-ACPC in plasma decreased rapidly after injection and its activity in the tumor increased with time. Tumor radioactivity exceeded that of plasma within 5 min of ^{11}C-ACPC administration. For technical reasons, the measured radioactivity was not corrected for residual ^{11}C-ACPC within the vascular compartment of tumor tissue.

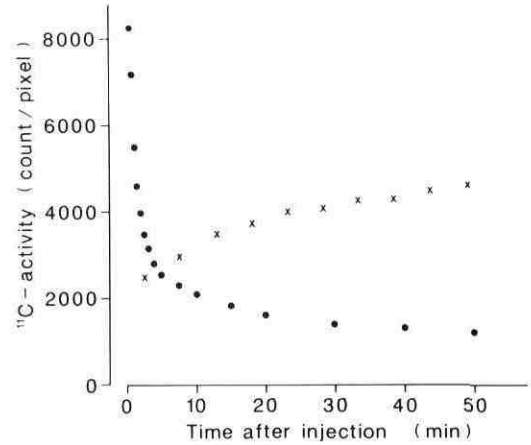

Fig. 3. Time course of ^{11}C activity in arterial plasma (filled circles) and VX-2 tumor (crosses) after bolus intravenous administration of 2 mCi of ^{11}C-ACPC in the rabbit.

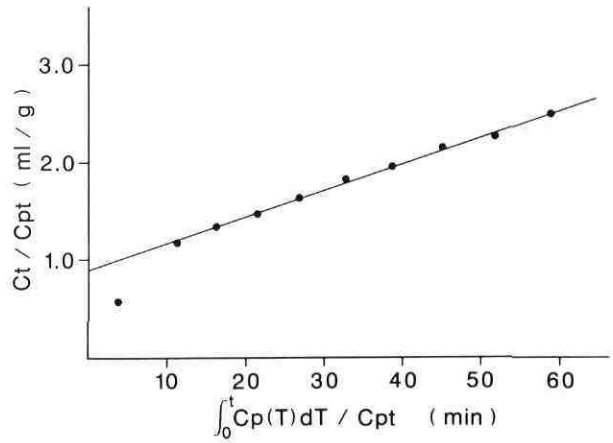

Fig. 4. Graphical analysis of the tumor in the rabbit over a time course of 3 to 60 min. The y-axis represents the ratio of tumor ^{11}C-ACPC activity [Ct] and plasma arterial radioactivity [Cpt] evaluated at time t; the x-axis represents the time integral of plasma arterial radioactivity [Cp(T)] from 0 to T, divided by plasma radioactivity [Cpt]. The solid line represents the best linear fit by a least-squares analysis of the PET data points from 20 to 60 min. The correlation coefficient was 0.99; slope = 0.027; intercept = 0.89.

The kinetic evaluation of ACPC uptake into the tumor was attempted using the unidirectional transport model developed by Patlak *et al.*[11] and Blasberg *et al.*[12] The method involves plotting the ratio of the total tissue activity at time t [Ct] and the concentration of a test substance in the plasma [Cpt] versus the time integral of the plasma activity at time t divided by Cpt. The slope of such a plot, if linear, is equal to the blood-to-tumor transfer constant (Ki).

Figure 4 illustrates this approach applied to the radioactivity data of the VX-2 tumor. The original imaging and blood sample times differed and interpolated values of Cpt were used in the calculation. A linear relationship was established from 20 min after injection to 60 min, which indicated that the transport was unidirectional.

Approximating the slope with a least-squares analysis yielded a value of Ki (0.027 ml/g/min) and showed that 15 min was needed for ^{11}C-ACPC to achieve a steady state between blood and its rapidly exchangeable distribution volume. The transfer constant (Ki) and the intercept of the slope (Vi) obtained in other experiments are given in Table I.

Table I. Graphically Determined ACPC Transfer Constant (Ki) and Intercept of the Slope (Vi) of VX-2 Tumor

Rabbit No.	Body weight (kg)	Dose (mCi)	Ki (ml/g/min)	Vi (ml/g)
1	3.2	2.1	0.033	0.71
2	3.2	1.5	0.031	0.97
3	5.4	4.5	0.027	0.89
		mean	0.030	0.86

DISCUSSION

Two nonmetabolizable amino acids, ACPC and α-aminoisobutyric acid (AIB) have been extensively utilized in transport studies of various tissues and cells. AIB appears to be transported across cellular membranes mainly by the small neutral amino acid carrier, or A, system and to a lesser extent by the alanine-serine-cysteine, or ASC, system, whereas ACPC seems to be transferred primarily by the large neutral amino acid carrier, or L, system.[13–15] Accordingly, AIB serves as a model amino acid for the A-system and ACPC for the L-system.

As reported earlier,[16] we observed a high affinity of ^{11}C-ACPC for VX-2 tumors. Although no prior values of transfer constant (Ki) of ^{11}C-ACPC have been published for rabbit VX-2 tumor, our average value of Ki (0.030 ml/min/g) is consistent with Ki values in Walker 256 tumors of 0.024–0.10 ml/min/g for AIB.[17]

At the present time, limitations of spatial resolution with PET scanners make it unlikely that PET would contribute to the early diagnosis of neoplasms. However, the use of physiologic tumor-localizing agents may provide a unique approach to quantitative *in vivo* analysis of tumor metabolism. If ^{11}C-ACPC is taken up actively in a tumor tissue, the extent to which ^{11}C-ACPC accumulates in a tumor may be correlated with its metabolic requirements. PET studies with ^{11}C-ACPC could be a useful research tool for evaluation of the efficacy of chemotherapy or radiation. The preliminary results presented here may provide the basis for the quantitative analysis of the *in vivo* distribution of ^{11}C-labeled ACPC in tumors.

ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Health and Welfare, Japan, and by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

(Received July 31, 1989/Accepted October 18, 1989)

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