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Effect of Structure on Tumor Specificity of Alicyclic α-Amino Acids[1]

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ABSTRACT

The selective affinity of [*carboxyl*-^{11}C]-1-aminocyclopentanecarboxylic acid (ACPC) for tumor tissue has led us to study the tumor-localizing characteristics of a series of alicyclic α-amino acid analogs of ACPC. The tissue distributions of [^{14}C]-1-aminocyclopropanecarboxylic acid, 1-aminocyclobutanecarboxylic acid (ACBC), 1-aminocyclohexanecarboxylic acid, 1-amino-2-methylcyclopentanecarboxylic acid, and 1-amino-3-methylcyclopentanecarboxylic acid were compared with that of ACPC in Buffalo rats bearing Morris 5123C hepatomas. ACPC and ACBC were found to have significantly higher tumor-to-nontumor concentration ratios than the other four amino acids. ACBC generally had higher tumor-to-nontumor ratios than did ACPC, significantly so for muscle, kidney, and testis and marginally so for blood. These results suggest that [*carboxyl*-^{11}C]ACBC may be a better agent than [*carboxyl*-^{11}C]ACPC for tumor imaging by positron tomography.

INTRODUCTION

In previous studies we demonstrated the potential of certain ^{11}C-carboxyl-labeled amino acids for use in the nuclear medical imaging of malignant tissue (7, 9) and the pancreas (12, 13). Because C-11 ($t_{1/2}$ = 20.4 min) decays by positron emission, these agents are suitable for high-resolution, 3-dimensional imaging with the recently developed positron computerized axial tomographic instrumentation (10, 11).

Using autoradiographic techniques with the ^{14}C-labeled compound, Berlinguet *et al.* (1) showed that there was a selective uptake of ACPC,[5] an unnatural, alicyclic amino acid, by tumor tissue. We corroborated these findings and then developed a rapid, high-temperature, high-pressure modification of the Bücherer-Strecker amino acid synthesis for production of clinically useful levels (>200 mCi) of [*carboxyl*-^{11}C]-labeled ACPC (7) and other amino acids (6). Clinical studies of [^{11}C]ACPC have been in progress in our laboratories since late 1975. The agent has shown significant potential for imaging soft tissue tumors in man (9) similar to that seen in animals (7).

This selective affinity of [^{11}C]ACPC for tumor tissue led us to investigate the effect of structural modifications on the tumor specificity of alicyclic α-amino acids.

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[5] The abbreviations used are: ACPC, 1-aminocyclopentanecarboxylic acid; ACBC, 1-aminocyclobutanecarboxylic acid; ACHC, 1-aminocyclohexanecarboxylic acid; 2-MACPC, 1-amino-2-methylcyclopentanecarboxylic acid; 3-MACPC, 1-amino-3-methylcyclopentanecarboxylic acid; ACPRC, 1-aminocyclopropanecarboxylic acid.

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MATERIALS AND METHODS

[*carboxyl*-^{14}C]ACPC (specific activity, 33.0 mCi/mmol) was obtained from New England Nuclear, Boston, Mass. ^{14}C-Carboxyl-labeled ACBC, ACHC, 2-MACPC, and 3-MACPC were synthesized by our modified Bücherer-Strecker technique (6, 7). Attempts to synthesize [*carboxyl*-^{14}C]ACPRC by this technique with cyclopropanone hydrate as a precursor were unsuccessful, apparently because of the instability of the cyclopropane ring system. However, [^{14}C]ACPRC with the label in position 1 of the cyclopropane ring was synthesized by an alternate, unambiguous technique, starting with [^{14}C]diethyl malonate and unlabeled ethylene dibromide. The method has previously been described for synthesis of unlabeled ACPRC (4, 5).

Purity of the ^{14}C-labeled amino acids was assessed by thin-layer chromatography with Eastman silica gel chromatogram sheets (13179) developed in butanol:water:acetic acid (100:10:5, v/v). Chromatographic patterns were determined either by ninhydrin development or through the use of a spark chamber (Birchover Instruments, Bancroft, United Kingdom).

Male Buffalo rats bearing Morris 5123C hepatomas (obtained originally from Dr. Fred Snyder of our laboratory) were used at 6 to 8 weeks after transplantation. All animals in each comparative study were from the same transplantation group, and each experimental group consisted of 4 animals. The ^{14}C-labeled amino acid solutions were diluted with 0.9% NaCl solution and injected via the tail vein. In studies of the effect of ring size, 0.02 mmol of amino acid (ACPRC, ACBC, ACPC, and ACHC) per kg was injected. In studies of the effect of a methyl substituent, 0.088 mg of ACPC, 2-MACPC, and 3-MACPC per kg were administered. [Studies with both [^{14}C]ACPC and [^{14}C]ACBC have shown that the level of carrier amino acid is unimportant in the range of 0.1 to 5.0 mg/kg (L. C. Washburn, T. T. Sun, and R. L. Hayes, unpublished observations).] The animals were given 5 to 40 μCi of ^{14}C-labeled amino acid per kg of body weight. At 30 min postinjection the rats were killed by exsanguination after light ether anesthesia. Weighed tissue samples were dissolved in NCS tissue solubilizer (Amersham/Searle Corporation, Arlington Heights, Ill.) and assayed by liquid scintillation counting.

A 30-min time interval between administration of the ^{14}C-labeled amino acids and sacrifice of the animals was chosen because previous studies with both [^{14}C]ACPC and [^{14}C]ACBC had shown that the blood clearance and tissue uptake of these 2 amino acids was essentially complete by 30 min postinjection (L. C. Washburn, T. T. Sun, and R. L. Hayes, unpublished observations). Also, because of the short half-life of carbon 11 any potential application of ^{11}C-labeled alicyclic α-amino acids for positron tomographic tumor detection will require early imaging. A 30-min post-

injection period was, therefore, selected as the appropriate time interval for our investigation.

RESULTS

Thin-layer chromatograms of each of these ^{14}C-labeled amino acids showed a single spot when visualized either by ninhydrin development or by use of a spark chamber. A typical spark chamber photograph (Fig. 1) shows that the R_F of these alicyclic α-amino acids (in the solvent system used) increased as the size of the alicyclic ring system increased.

To assess the effect of ring size on the tissue distribution of alicyclic α-amino acids, we compared the tissue distributions of ^{14}C-labeled ACPRC, ACBC, ACPC, and ACHC at 30 min postinjection in rats bearing Morris 5123C hepatomas (Table 1). The agents with 4- and 5-membered alicyclic ring systems (ACBC and ACPC) showed significantly greater tumor specificities than did ACPRC and ACHC, the analogous compounds with 3- and 6-membered rings. Comparison of the tissue distributions of ACPC and ACBC indicated that ACBC generally had higher tumor-to-nontumor concentration ratios than did ACPC. However, these differences were significant ($p < 0.05$) only for kidney, muscle, and testis and had borderline significance ($p = 0.05$ to 0.10) for blood. On the other hand, for marrow the difference had a borderline significance ($p = 0.05$ to 0.10) in favor of ACPC.

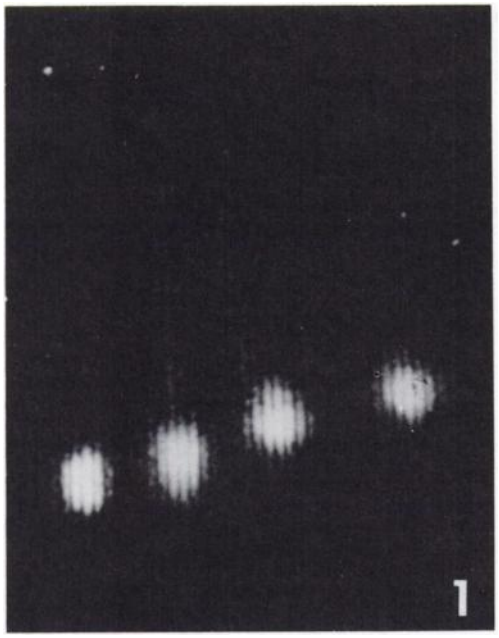

Fig. 1. Spark chamber photograph of a thin-layer chromatogram of (*left to right*) ^{14}C-labeled ACPRC, ACBC, ACPC, and ACHC.

To ascertain the effect of ring substituents on the tissue distribution of alicyclic α-amino acids, we studied the tissue distributions of ^{14}C-labeled ACPC, 2-MACPC, and 3-MACPC, again at 30 min postinjection in rats bearing Morris 5123C hepatomas (Table 2). The presence of a methyl group at either position 2 or 3 of ACPC obviously produced a significant decrease in the tumor-to-nontumor concentration ratios for all tissues. On the other hand, 2-MACPC and 3-MACPC had similar distribution patterns with only a few exceptions. 2-MACPC showed a remarkably greater affinity for kidney, whereas 3-MACPC had a higher accumulation in muscle. Liver and blood concentrations for the 2 agents were also significantly different ($p < 0.05$) although less pronounced.

DISCUSSION

[*carboxyl*-^{14}C]ACPC was shown by Berlinguet *et al.* (1) some years ago to concentrate rapidly in tumor tissue. This amino acid has also been shown to be highly reactive with amino acid Transport Systems A and L (3). However, just why ACPC should show a special avidity for cancers is not known. Alterations in the plasma membranes of tumor cells as proposed by Holley (8) could be responsible.

We have observed a dramatic increase in tumor specificity with decreasing ring size in the homologous series ACHC through ACBC (6- through 4-membered ring systems). On the basis of these results one might project that ACPRC (3-membered ring) would show an even greater avidity for tumor tissue than does ACBC. That we did not observe this behavior was possibly the result of a significant

Table 1

Effect of ring size on the tissue distribution of ^{14}C-labeled alicyclic α-amino acids (0.02 mmol/kg) in male Buffalo rats bearing Morris 5123C hepatomas (30 min postinjection)

Tissue	ACPRC	ACBC	ACPC	ACHC
	% administered dose/g[a]			
Tumor	1.20 ± 0.04[b]	4.64 ± 0.49	3.58 ± 0.17	0.93 ± 0.01
	Tumor-to-tissue concentration ratio			
Liver	3.24 ± 0.18	9.33 ± 1.05	7.82 ± 0.62	2.12 ± 0.04
Spleen	2.29 ± 0.22	6.25 ± 1.01	7.05 ± 0.53	2.32 ± 0.56
Kidney	0.28 ± 0.04	6.50 ± 0.66	2.69 ± 0.16	1.87 ± 0.05
Lung	2.53 ± 0.08	7.11 ± 0.69	7.74 ± 0.46	1.86 ± 0.04
Muscle	3.82 ± 0.13	16.47 ± 1.96	9.24 ± 0.42	2.08 ± 0.04
Marrow	2.15 ± 0.15	4.48 ± 0.41	5.70 ± 0.25	1.69 ± 0.02
Blood	2.98 ± 0.11	11.27 ± 1.16	8.64 ± 0.43	2.01 ± 0.02
Pancreas	0.44 ± 0.02	1.05 ± 0.09	1.22 ± 0.11	0.25 ± 0.01
Small intestine	2.06 ± 0.10	4.15 ± 0.62	4.44 ± 0.35	1.38 ± 0.10
Testis	6.47 ± 0.37	20.41 ± 2.04	11.08 ± 0.75	2.41 ± 0.04

[a] Normalized to body weight of 250 g.
[b] Average of 4 animals ± S.E.

Table 2

Effect of methyl substituent on the tissue distribution of ^{14}C-labeled ACPC, 2-MACPC, and 3-MACPC (0.088 mg/kg) in male Buffalo rats bearing Morris 5123C hepatomas (30 min postinjection)

Tissue	ACPC	2-MACPC	3-MACPC
	% administered dose/g[a]		
Tumor	2.89 ± 0.17[b]	1.18 ± 0.03	1.28 ± 0.06
	Tumor-to-tissue concentration ratio		
Liver	6.72 ± 0.44	2.33 ± 0.06	3.00 ± 0.17
Spleen	6.16 ± 0.37	2.66 ± 0.08	2.52 ± 0.08
Kidney	3.82 ± 0.13	0.34 ± 0.02	1.77 ± 0.07
Lung	7.13 ± 0.51	2.67 ± 0.43	2.72 ± 0.17
Muscle	7.43 ± 0.46	4.10 ± 0.05	2.34 ± 0.12
Marrow	5.80 ± 0.60	2.58 ± 0.34	2.60 ± 0.12
Blood	7.67 ± 0.32	3.37 ± 0.07	2.98 ± 0.06
Pancreas	0.94 ± 0.02	0.42 ± 0.03	0.38 ± 0.04
Small intestine	3.56 ± 0.43	2.20 ± 0.22	2.04 ± 0.03

[a] Normalized to body weight of 250 g.
[b] Average of 4 animals ± S.E.

ring strain and consequent instability associated with this ring system. This effect may account for the fact that we were unable to synthesize ACPRC by our modified Bücherer-Strecker technique, which was readily applicable to the synthesis of other alicyclic α-amino acids (6). The superiority of ACPC over 2-MACPC and 3-MACPC is in agreement with the projections of Christensen (2), who found in *in vitro* studies of intracellular amino acid concentration by Ehrlich ascites carcinoma cells that the degree of concentration decreased with an increase in the number of carbon atoms in an aliphatic hydrocarbon side chain.

These results point to the potential superiority of [^{11}C]ACBC over [^{11}C]ACPC as an agent for positron tomographic tumor imaging. A clinical study of [^{11}C]ACBC as a tumor-imaging agent, in comparison with both [^{11}C]ACPC and ^{67}Ga-citrate, is now in progress at our institution.

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