TUMOR ACCUMULATION OF D-SELENOMETHIONINE-⁷⁵Se IN TUMOR-BEARING MICE

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The radioactivity distribution of ⁷⁵Se-labeled D-selenomethionine (D-SeMet-⁷⁵Se) was investigated in tumor-bearing mice in order to develop a new radioactive diagnostic agent. D-SeMet-⁷⁵Se was enzymatically prepared from commercially available L-selenomethionine-⁷⁵Se (L-SeMet-⁷⁵Se) by using amino acid racemase and immobilized L-amino acid oxidase. No difference between D- and L-SeMet-⁷⁵Se was found in the excretion rate into urine and feces in normal mice within 48 h after administration. The *in vivo* uptake of D-SeMet-⁷⁵Se in both Ehrlich solid tumor and sarcoma-180 solid tumor was several times higher than that of L-SeMet-⁷⁵Se but the uptake of D- and L-SeMet-⁷⁵Se were similar in the pancreas. These results indicated that D-SeMet-⁷⁵Se might be useful as a tumor-imaging agent.

In vitro experiments on Ehrlich ascites tumor cells showed that D-SeMet-⁷⁵Se was incorporated into the tumor cells by a Na⁺-dependent active transport system similar to L-SeMet-⁷⁵Se and that a transport mechanism specific for D-SeMet-⁷⁵Se might be present in tumor cells in addition to a transport system common to both D- and L-forms.

Keywords — D-selenomethionine-⁷⁵Se; L-selenomethionine-⁷⁵Se; radiopharmaceutical; Ehrlich solid tumor; sarcoma-180 solid tumor; tumor-imaging agent

INTRODUCTION

In order to find new diagnostic radiopharmaceuticals, we investigated the distribution of ¹⁴C-labeled D- and L-amino acids, *e.g.*, alanine, leucine, methionine and tryptophan in tumor-bearing animals.¹⁾ We found that the uptake of these ¹⁴C-labeled D-amino acids in the tumor was several times higher than the uptake of the corresponding L-isomer, while there was no such preference uptake of D-amino acids in pancreas. These results suggest that some radiolabeled D-amino acids may be useful as tumor-imaging agents. D-Amino acid may have another advantage as a diagnostic agent. Since D-amino acids are poorly utilized for protein synthesis, the biological half-lives of radioactive D-forms may be shorter than that of the L-forms resulting in lower radiation exposure.

When a D-amino acid is found to be useful for an *in vivo* radioactive diagnostic agent, its labeling with a positron emitter (*e.g.* ¹¹C and ¹³N) produced by cyclotron is favorable because of the short half-life and no change in chemical properties of the amino acid. Actually, DL-phenylalanine-¹¹C,²⁾ DL-leucine-¹¹C³⁾ DL-valine-¹¹C,^{3,4)} methionine-¹¹C⁵⁾ and DL-tryptophan-¹¹C,^{3,6)} have been reported as pan-

creas-imaging agents and α-aminocyclopentane carboxylic acid-¹¹C,⁷⁾ α-aminocyclobutane carboxylic acid-¹¹C⁸⁾ and DL-methionine-¹¹C,⁹⁾ as tumor-imaging agents. However, a cyclotron, at present, is not readily available. Therefore, we were interested in ⁷⁵Se-labeled D-selenomethionine (D-SeMet-⁷⁵Se) of which the L-form has been used as a pancreas imaging agent. The uptake of D-SeMet-⁷⁵Se has been found to be several times higher than that of the L-isomer in tumors, showing that D-SeMet-⁷⁵Se may be suitable for diagnosis of some types of tumors.

MATERIALS AND METHODS

Chemicals — ⁷⁵Se-Labeled L-selenomethionine was purchased from New England Nuclear Research Products, U.S.A. D- and L-Amino acid oxidases were the products of Boehringer Mannheim Yamanouchi, Tokyo, Japan. Immobilized D- and L-amino acid oxidases using cyanogen bromide-activated Sepharose 4B (Sigma Chemical Co., U.S.A.) were prepared according to the methods of Casey *et al.*¹⁰⁾ and Digenis *et al.*,¹¹⁾ respectively. Amino acid racemase¹²⁾ was kindly provided by Dr. Soda, Kyoto University, Japan. Other chemicals were of analytical grade.

Preparation of D-SeMet-⁷⁵Se — D-SeMet-⁷⁵Se was prepared from L-SeMet-⁷⁵Se according to the procedure reported by Goto *et al.*¹³⁾ who described the enzymatic synthesis of non-radioactive D-SeMet. A mixture of L-SeMet-⁷⁵Se (200 μ Ci, 0.4 ml, 5.47 Ci/mmol), 0.7 ml of 0.286 M potassium phosphate buffer (pH 8.0), 0.4 ml of 0.05 mM pyridoxal 5-phosphate and 0.2 ml of amino acid racemase (1 U) was incubated at 37 °C for 30 min, heated for 5 min in a boiling bath, filtered through Millex[®]-GS (Milipore Corp. 0.22 μ m, i.d. 25 mm) and freeze-dried. The residue dissolved in 20 ml of 0.1 M tetrasodium pyrophosphate buffer (pH 8.3) was gently incubated with Sepharose 4B-immobilized L-amino acid oxidase at 37 °C for 40 min with passage of oxygen. The immobilized enzyme was removed from the reaction mixture by use of a sintered glass filter. The filtrate was acidified with 1 ml of HCl and the mixture was added to a column of Dowex 50W-X2 (1.5 $\times$ 15 cm). The column was washed with 100 ml of distilled water and then eluted with 0.2 N NaOH (1 ml/tube). The radioactive peak fractions were combined, neutralized with HCl and freeze-dried. The yield of D-SeMet-⁷⁵Se was 10.7%. The chemical purity of the D-form was higher than 80% which was determined with immobilized D-amino acid oxidase. The specific activity was 5.47 Ci/mmol which was equal to that of L-form.

Tumor Cell Suspension — Cell suspensions of Ehrlich ascites and sarcoma-180 tumor were prepared from dd/Y male mice inoculated i.p. with the respective tumor cells 7 d before harvesting.

Tumor-Bearing Mice — Mice (dd/Y) bearing Ehrlich solid tumor or sarcoma-180 solid tumor were obtained by subcutaneous injection of 0.2 ml of the appropriate cell suspension (10^8 cells/ml in physiological saline) 7 d before use.

In Vivo Experiment — 1) Excretion of D- or L-SeMet-⁷⁵Se into Urine and Feces in Mice: Five mice per group were intravenously injected with D- or L-SeMet-⁷⁵Se (2×10^5 cpm/0.2 ml saline/25 g body weight, 5.47 Ci/mmol). The urine and feces were separately collected at various time intervals and radioactivity was measured by an auto well gamma counter (Beckmann 5500).

2) Distribution of D- or L-SeMet-⁷⁵Se in Tumor-Bearing Mice: A group of five tumor-bearing mice injected intravenously with D- or L-

SeMet-⁷⁵Se (10^5 cpm/0.2 ml saline/25 g body weight, 5.47 Ci/mmol) was sacrificed by inhalation of ether gas 30 and 60 min after the administration. Solid tumor, blood and other organs were excised and weighed. After addition of 2 ml of 5% trichloroacetic acid (TCA), these tissues were separated into the TCA-soluble and -insoluble fractions as described previously.^{1a)} The radioactivity of each fraction was counted by a gamma counter.

In Vitro Experiment — Ehrlich ascites tumor cell suspension (1.5 ml) in 0.64 mM CaCl₂-containing Krebs-Ringer phosphate buffer, pH 7.2, was incubated with D- or L-SeMet-⁷⁵Se (1.25×10^4 cpm) and with other reagents (ouabain, 2,4-dinitrophenol, D- or L-methionine). After a given time, the cells were washed three times with the same buffer and, after addition of 2 ml of 5% TCA, TCA-soluble and -insoluble fractions were obtained as described previously.¹⁴⁾ The radioactivity was counted by a gamma counter.

RESULTS

Excretion Rate of D- and L-SeMet-⁷⁵Se into Urine and Feces in Mice

Since D-amino acid itself is poorly utilized for protein synthesis, the D-form is expected to have a shorter biological half-life compared with that of the L-form. However, as shown in Fig. 1, there was no difference between D- and L-SeMet-⁷⁵Se in the excretion rate into urine and feces in

FIG. 1. Excretion of D- or L-SeMet-⁷⁵Se into Urine and Feces in Mice

Experiment was carried out as described in text. ○, L-SeMet-⁷⁵Se; ●, D-SeMet-⁷⁵Se. Each point represents mean $\pm$ S.D. from five mice.

FIG. 2. Distribution of D- or L-SeMet-⁷⁵Se in Tumor and Organs of Mice Bearing Ehrlich Solid Tumor (A) and Sarcoma-180 Solid Tumor (B)

Experiment was carried out as described in text. Upper bar, 30 min after injection; lower bar, 60 min after injection; , TCA-soluble fraction; , TCA-insoluble fraction. Each bar represents mean $\pm$ S.D. from five mice.

normal mice; approximately 30% of the dose of both amino acids was excreted 48 h after administration. The excretion into urine was higher than that into feces for both amino acids.

Distribution of D- and L-SeMet-⁷⁵Se in Tumor-Bearing Mice

Blau and Manske have reported that several amino acids tested showed a high localization in the pancreas 1 h after administration.¹⁵⁾ We have also described the maximal uptake of ¹⁴C-labeled D- and L-leucine in tumor and organs in mice bearing Ehrlich ascites tumor cells was observed within 2 h after injection.^{1a)} Clinically, pancreas scanning using L-SeMet-⁷⁵Se is started 30 min after injection. Therefore, the distribution of D- and L-SeMet-⁷⁵Se in Ehrlich solid

tumor- and sarcoma-180 solid tumor-bearing mice was determined 30 and 60 min after administration (Fig. 2). D-SeMet-⁷⁵Se showed its highest uptake in the pancreas, as did L-SeMet-⁷⁵Se. With respect to tumor, the uptake of D-SeMet-⁷⁵Se was several times higher than that of the L-isomer in both types of tumor cells 60 min after administration. This higher uptake of the D-form was not seen in other organs, except muscle. The higher uptake of D-SeMet-⁷⁵Se in tumor cells was attributed to its higher uptake in the TCA-soluble fraction. In most organs, the radioactivity of the D-form in the TCA-insoluble fraction was relatively high compared with that of some other ¹⁴C-labeled D-amino acids (alanine, leucine and tryptophan).¹⁾

TABLE I. Inhibition of D- and L-SeMet-⁷⁵Se Uptake by Metabolic Inhibitors in Ehrlich Ascites Tumor Cells after Incubation for 30 min

Inhibitor	Conc. (mM)	Inhibition (%)	
		D-SeMet- ⁷⁵ Se	L-SeMet- ⁷⁵ Se
Ouabain	0.1	33.3 $\pm$ 3.0	11.7 $\pm$ 1.6
	1.0	56.4 $\pm$ 2.2	27.1 $\pm$ 2.4
2,4-Dinitrophenol	0.1	69.7 $\pm$ 1.5	51.6 $\pm$ 5.4
	1.0	70.0 $\pm$ 2.0	64.1 $\pm$ 1.4

TABLE II. Distribution Ratio of D- and L-SeMet-⁷⁵Se in Ehrlich Ascites Tumor Cells

	Distribution ratio ^{a)}	
	1 min	30 min
D-SeMet- ⁷⁵ Se	3.57 ± 0.11	6.62 ± 0.50
L-SeMet- ⁷⁵ Se	4.44 ± 0.08	9.37 ± 0.16

a) Distribution ratio of intracellular fluid to extracellular fluid (cpm/ml) was estimated as described previously.¹⁴⁾

In Vitro Experiment

To determine whether the high distribution of D-SeMet-⁷⁵Se in tumor cells *in vivo* was closely related to its membrane transport mechanism, the assay was done on Ehrlich ascites tumor cells *in vitro*. As shown in Table I, the incorporation of D-SeMet-⁷⁵Se was inhibited, as well as the L-form, by two metabolic inhibitors, ouabain (inhibitor of Na⁺, K⁺-adenosine triphosphatase) and 2,4-dinitrophenol (uncoupler of oxidative phosphorylation). The inhibition was mainly caused by a decrease of radioactivity incorporated into the TCA-soluble fraction which reflected the intracellular free amino acid pool, suggesting the participation of an energy dependent membrane transport. Furthermore, whether D-SeMet-⁷⁵Se is actively transported into the cells was investigated by the determination of the distribution ratio¹⁴⁾ of the intracellular fluid to extracellular fluid. Table II shows that the ratios of D- and L-SeMet-⁷⁵Se 1 min and 30 min after incubation were much higher than 1.0, although the value of D-SeMet was lower than that of the L-form, contrary to *in vivo* uptake.

Since animal serum contains much larger quantities of endogenous L-methionine (26–49 μM in human¹⁶⁾ and approximately 45 μM in rat¹⁷⁾) than that of D- or L-SeMet-⁷⁵Se administered (about 0.64 μM), the uptake of D- or L-SeMet-⁷⁵Se, especially the L-form, may be com-

petitively inhibited by L-methionine. Therefore, the effect of non-radioactive L-methionine on the uptake of D- or L-SeMet-⁷⁵Se in Ehrlich ascites tumor cells was studied. As shown in Table III, the uptake of L-SeMet-⁷⁵Se was inhibited about 23% in the presence of 0.1 mM L-methionine while D-SeMet-⁷⁵Se was not, although, at a high concentration (1.0 mM), both ⁷⁵Se-labeled amino acids were inhibited nearly to the same extent. The inhibitory effect on the uptake of both the forms of SeMet by D-methionine was also observed.

DISCUSSION

L-SeMet-⁷⁵Se used clinically as a pancreas-imaging agent has some disadvantages, such as long physiological and biological half-lives, and high energy, resulting in a high radiation exposure to a patient. Lathrap *et al.*¹⁸⁾ had reported that 13.1% of the injected L-SeMet-⁷⁵Se has a biological half-life of 0.55 d, 44.3% has that of 46 d, and 41.9% has that of 220 d, based on total body retention and excretion measurement of L-SeMet-⁷⁵Se in humans. A shorter biological half-life was expected in D-SeMet-⁷⁵Se because of its poor utilization for protein synthesis. However, the present study showed that the excretion rate of D-SeMet-⁷⁵Se was not different from that of the L-form, at least within 48 h after administration. Therefore, D-SeMet-⁷⁵Se is not superior to

TABLE III. Effect of D- or L-Methionine on D- or L-SeMet-⁷⁵Se Uptake in Ehrlich Ascites Tumor Cells 2 min after Incubation

Amino acid	Conc. (mM)	Inhibition (%)	
		D-SeMet- ⁷⁵ Se	L-SeMet- ⁷⁵ Se
L-Methionine	0.1	— 6.5 ± 0.1	23.1 ± 0.1
	1.0	50.1 ± 0.4	40.4 ± 0.1
D-Methionine	0.1	13.4 ± 0.2	7.6 ± 0.1
	1.0	47.2 ± 0.2	50.9 ± 0.1

TABLE VI. Tumor-to-Tissue Ratio of D- and L-SeMet-⁷⁵Se in Tumor-Bearing Mice 60 min after Administration

	Ehrlich solid tumor		Sarcoma-180 solid tumor	
	L-SeMet- ⁷⁵ Se	D-SeMet- ⁷⁵ Se	L-SeMet- ⁷⁵ Se	D-SeMet- ⁷⁵ Se
Tumor	2.51 ± 0.56	5.32 ± 1.36	2.70 ± 0.31	8.38 ± 1.19
	% dose/g ^{a,b)}			
	Tumor-to-tissue ratio ^{a)}			
Brain	3.14 ± 0.78	4.59 ± 1.22	2.90 ± 0.50	6.70 ± 1.69
Pancreas	0.11 ± 0.03	0.20 ± 0.06	0.13 ± 0.02	0.42 ± 0.14
Liver	0.25 ± 0.06	0.49 ± 0.15	0.27 ± 0.04	1.06 ± 0.31
Spleen	0.69 ± 0.17	1.30 ± 0.38	0.55 ± 0.09	1.90 ± 0.70
Kidney	0.19 ± 0.04	0.31 ± 0.09	0.21 ± 0.03	0.48 ± 0.17
Stomach	1.41 ± 0.40	2.84 ± 0.93	1.65 ± 0.33	5.34 ± 1.41
Duodenum	0.40 ± 0.10	0.69 ± 0.22	0.56 ± 0.10	1.95 ± 0.47
Muscle	2.92 ± 0.81	2.89 ± 1.10	2.52 ± 0.48	5.27 ± 1.64
Lung	0.85 ± 0.20	2.25 ± 0.61	1.24 ± 0.16	4.84 ± 1.39
Heart	1.61 ± 0.37	2.42 ± 0.75	2.31 ± 0.36	5.94 ± 1.63
Testis	2.76 ± 0.73	4.22 ± 1.31	3.51 ± 0.44	8.14 ± 2.43
Blood	0.85 ± 0.19	2.13 ± 0.72	0.72 ± 0.11	3.69 ± 0.82

a) Mean ± S.D. of five animals. b) Normalized to body weight of 25 g.

L-SeMet-⁷⁵Se as a pancreas-imaging agent although the D-form as well as the L-form was taken up to a high extent in the pancreas. The relatively high incorporation of D-SeMet-⁷⁵Se into the TCA-insoluble fraction seems to be an important factor for its slow excretion. This unexpected high incorporation suggests the utilization of the D-isomer in protein synthesis *via* some metabolic pathways, because almost the same pattern was observed on the uptake for D-methionine[3,4-¹⁴C].^{1c)}

The uptake of D-SeMet-⁷⁵Se was several times greater than the uptake of the L-isomer 60 min after injection into both types of tumor cells *in vivo*, resulting in the higher tumor-to-organ ratios of D-SeMet-⁷⁵Se uptake in most of organs than the L-isomer (Table IV). Herrera *et al.*¹⁹⁾ reported that a group of malignant lymphoma exhibited a degree of uptake of L-SeMet-⁷⁵Se which permitted their demonstration by means of photoscan. Therefore, D-SeMet-⁷⁵Se may be more suitable for the diagnosis of some kinds of tumors, especially malignant lymphomas, than the L-isomer.

An *in vitro* experiment using Ehrlich ascites tumor cells showed that the inhibition of D- and L-SeMet-⁷⁵Se uptake occurred mainly in TCA-soluble fractions by two metabolic inhibitors (ouabain and 2,4-dinitrophenol). Furthermore,

their distribution ratios of the intracellular fluid to the extracellular fraction were higher than 1.0. These results suggest that Na⁺-dependent active transport mechanism is concerned in the incorporation of both D- and L-SeMet-⁷⁵Se into tumor cells which is in agreement with that of ¹⁴C-labeled D- and L-leucines.^{13,20)} Contrary to the *in vivo* result, the *in vitro* intracellular uptake of the D-form was lower than that of the L-form, although there was a lesser stereospecificity than that of D- and L-form uptake of ¹⁴C-labeled alanine and valine *in vitro* reported by Oxender.²¹⁾ The difference of uptake of D- and L-SeMet-⁷⁵Se between *in vivo* and *in vitro* may be partially due to the effect of endogenous L-methionine which is present in serum. As indicated in Table III, the uptake of L-SeMet-⁷⁵Se was competitively inhibited by L-methionine at 0.1 mM which is similar to the physiological concentration in serum, while that of the D-form was not altered by the same concentration of L-methionine. If the higher uptake of D-SeMet-⁷⁵Se in tumor cells is due only to the competitive inhibition by the endogenous L-methionine, such high uptake should also be observed in other organs. But, this was not the case. As shown in Table III, the inhibition of the uptake of D-SeMet-⁷⁵Se was greater than that of the L-form in the presence of 0.1 mM D-

methionine in tumor cells. Therefore, tumor cells may have a transport mechanism specific for D-SeMet-⁷⁵Se, in addition to a transport system common to both D- and L-forms which is present in normal and tumor cells. This point is an interesting subject which must be clarified in the future.

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