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Development of radioiodine-labeled 4-hydroxyphenylcysteamine for specific diagnosis of malignant melanoma☆

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ABSTRACT

Introduction: A specific diagnosis for melanoma is strongly desired because malignant melanoma has poor prognosis. In a previous study, although radioiodine-125-labeled 4-hydroxyphenyl-L-cysteine (^{125}I-L-PC) was found to have good substrate affinity for tyrosinase enzyme in the melanin metabolic pathway, $^{123/131}$I-L-PC had insufficient substrate affinity for tyrosinase to diagnose melanoma. In this study, we synthesized 4-hydroxyphenylcysteamine (4-PCA) and developed a novel radioiodine-125-labeled 4-hydroxyphenylcysteamine (^{125}I-PCA) to increase affinity for the melanin biosynthesis pathway.
Methods: 4-PCA was separated with 2-hydroxyphenylcysteamine (2-PCA), which is an isomer of 4-PCA, and was examined using melting point, proton nuclear magnetic resonance, mass spectrometry and elemental analysis. ^{125}I-PCA was prepared using the chloramine-T method under no-carrier added conditions. We performed biodistribution experiments using B16 melanoma-bearing mice using ^{125}I-PCA, ^{125}I-L-PC, ^{125}I-α-methyl-L-tyrosine, ^{123}I-*m*-iodobenzylguanidine and ^{67}Ga-citrate. In vitro assay was performed with B16 melanoma cells, and affinity for tyrosinase, DNA polymerase and amino acid transport was evaluated using phenylthiourea, thymidine, ouabine and L-tyrosine inhibitor. In addition, partition coefficients of ^{125}I-PCA were evaluated.
Results: In the synthesis of 4-PCA, analysis values did not differ between calculated and reported values, and 4-PCA was separated from 2-PCA at high purity. In biodistribution experiments, ^{125}I-PCA was accumulated and retained in B16 melanoma cells when compared with ^{125}I-L-PC. ^{125}I-PCA showed the highest values at 60 min after radiotracer injection in melanoma-to-muscle ratios, melanoma-to-blood ratios and melanoma-to-skin ratios. Accumulation of ^{125}I-PCA was significantly inhibited by phenylthiourea and thymidine. Partition coefficients of ^{125}I-PCA were lower than those of *N*-isopropyl-*p*-[^{123}I]iodoamphetamine and were not significantly different from ^{125}I-L-PC.
Conclusions: ^{125}I-PCA is a better substrate for tyrosinase and DNA polymerase and has higher uptake and longer retention in B16 melanoma cells when compared with ^{125}I-L-PC. Therefore, $^{123/131}$I-PCA has good potential for diagnosis for malignant melanoma.
Advance in Knowledge: ^{125}I-PCA will be a specific diagnosis tool for malignant melanoma.
Implications for Patient Care: $^{123/131}$I-PCA has good potential for the diagnosis of malignant melanoma when compared with other SPECT tracers, as well as anti-melanoma chemotherapeutic drugs.

1. Introduction

A specific diagnosis for malignant melanoma is necessary because it has such a poor prognosis. Melanoma is caused by elevation of the melanin biosynthesis pathway in melanocytes. Melanoma biosynthesis comprises conversion of tyrosine to dopa, and then to dopaquinone by tyrosinase oxidation [1].

Sulfur amine derivatives have been developed as anti-melanoma chemotherapeutic drugs and have high substrate affinity for tyrosinase, which is essential for melanin biosynthesis [2–4]. Chemotherapeutic efficacy has been reported for 4-hydroxyphenyl-L-cysteine (L-PC) [2–6] and hydroxyphenylcysteamine (PCA) [3–5,7], and their *N*-acetyl [8,9]

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and *N*-propionyl derivatives [9,10]. These compounds induce toxicity in malignant melanomas that are positive for tyrosinase. Before starting chemotherapy, positron emission tomography imaging with 2-[^{18} F] Fluoro-2-deoxy-D-glucose has recently been applied to detect metastases of melanoma [11]. However, patients cannot undergo PET at all hospitals, as PET is less versatile than SPECT. Although SPECT imaging can be used to detect metastases of melanoma, specific SPECT imaging agents for melanoma have not been developed.

We previously synthesized radioiodine-125-labeled L-PC (^{125}I-L-PC) in order to specifically diagnose melanoma [12]. Although ^{125}I-L-PC showed some affinity for tyrosinase, there was insufficient uptake into melanoma for diagnosis with $^{123/131}$I-L-PC. We therefore developed a novel radioiodine-125-labeled 4-PCA (^{125}I-PCA) (Fig. 1) to increase the specific uptake into melanoma, as compared with ^{125}I-L-PC.

2. Materials and methods

2.1. Synthesis of PCA

We followed the PCA synthesis method of Miura et al. [5]. A mixture of 28.2 g (300 mmol) of phenol (Nacalai Tesque, Kyoto, Japan) and 16.9 g (75 mmol) of cystamine dihydrochloride (Wako, Osaka, Japan) in 500 ml 47% HBr was refluxed for 2 h in an oil bath. The orange solution was evaporated to dryness in a rotary evaporator. The residue was dissolved in approximately 100 ml of water, and the solution was adjusted to pH 8.4 with 28% ammonia (Nacalai Tesque), and was then stirred in a refrigerator for one week. Pale pink needles of pure 2-PCA (1.1% yield) were obtained. The mother liquor of the pH 8.4 mixture was adjusted to a pH of approximately 10 to give colorless needles of pure 4-PCA (8.7% yield). The 4-PCA and 2-PCA were analyzed by thin-layer chromatography (TLC) (Merck Millipore, Billerica, MA) with each sample spotted at 1 μl and methanol/acetate (100/1) used as developing solvent, and was detected by ultraviolet absorption at 254 nm. The structure of 4-PCA was confirmed by determination of proton nuclear magnetic resonance (^{1}H-NMR, JEOL JNM-ECS400; Jeol Resonance Inc., Tokyo, Japan), electrospray ionization-mass spectrometry (MS, Shimadzu LCMS-2010A EV; Shimadzu, Kyoko, Japan) and elemental analysis (PerkinElmer 2400; PerkinElmer, Massachusetts). ^{1}H-NMR data (CD_3OD, 400 MHz), δ 2.69–2.72 (t, J = 4.8 Hz, 2H, -CH_2-NH_2), 2.83–2.86 (t, J = 5.8 Hz, 2H, -S-CH_2-), 6.71–6.74 (d, J = 8.8 Hz, 2H, aromatic), 7.26–7.29 (d, J = 9.2 Hz, 2H, aromatic); MS, found MH(+) 170 and calculated MH(+) 170; elemental analysis, calculated for $C_8H_{11}NOS$: C, 56.78, H, 6.55, N, 8.28, S, 18.94 and found for C, 56.41, H, 6.50, N, 8.41, S, 19.03 and melting point 116-119 °C. These data were compared with previously reported values [2,5].

2.2. ^{125}I- and ^{127}I-labeled 4-PCA

^{125}I-PCA was prepared by the chloramine-T method under no-carrier added conditions [12]. Briefly, 0.33 mM chloramine-T (Nacalai Tesque) at a concentration of 1.0×10^{-8} mol in 0.05 M phosphate buffer (pH 6.2) was added to a mixture of 4-PCA (0.1 μ mol) in 0.5 mM phosphoric acid (pH 6.2) and no-carrier added 37 MBq Na^{125}I (Perkin Elmer, Waltham, MA) in 0.4 M phosphate buffer (pH 8.5). Five minutes later, 10 nmol of sodium metabisulfite in 0.05 M phosphate buffer (pH 6.2) was added to stop the reaction. Labeling efficiency was examined by TLC with a solvent system of methanol/acetic acid (100/1). ^{125}I-PCA was purified by high performance liquid chromatography (HPLC) using a C18-MS-II column (Nacalai Tesque) with 85% methanol and 15% acetic acid as eluent at a flow rate of 0.5 ml/min. The structure of I-PCA was confirmed by NaI-127 (Nacalai Tesque) labeled PCA (^{127}I-PCA). ^{127}I-PCA was also synthesized using the same labeling method and HPLC method as for ^{125}I-PCA. Briefly, 0.33 mM chloramine-T (Nacalai Tesque) in 0.05 M phosphate buffer (pH 6.2) was added to a mixture of 10 μmol 4-PCA in 0.5 mM phosphoric acid (pH 6.2) and 0.33 mmol Na^{127}I in 0.4 M phosphate buffer (pH 8.5). One hour later, 10 nmol of sodium metabisulfite in 0.05 M phosphate buffer (pH 6.2) was added to stop the reaction. Using the same procedures with ^{1}H-NMR and MS for identification of 4-PCA, the ^{1}H-NMR data (CD_3OD, 400 MHz) were δ 2.72–2.75 (t, J = 5.0 Hz, 2H, -CH_2-NH_2), 2.86–2.89 (t, J = 6.0 Hz, 2H, -S-CH_2-), 6.74–6.77 (d, J = 8.6 Hz, 2H, aromatic) and 7.16–7.19 (d, J = 9.0 Hz, 1H, aromatic) and MS data revealed an MH(+) of 296 and a calculated MH(+) of 296 for $C_8H_{10}INOS$.

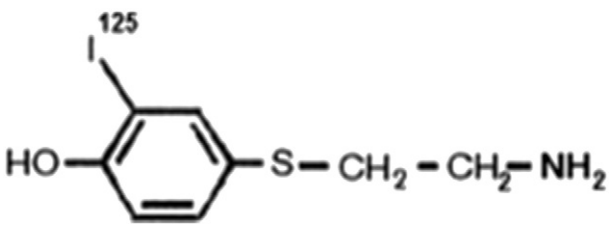

Fig. 1. Structure of ^{125}I-PCA.

2.3. Melanoma cells

At 37 °C and under 5% CO_2, B16 melanoma cells (Riken Cell Bank, Ibaraki, Japan) were incubated in Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich, St. Louis, MO) including 10% fetal calf serum (Dainippon Sumitomo, Osaka, Japan).

2.4. Biodistribution in B16 melanoma-bearing mice

Animal studies were approved by the Animal Care Committee at Kanazawa University and were conducted in accordance with the international standards for animal welfare and institutional guidelines. We prepared melanoma cells at 5×10^6 cells per 100 μl and injected them subcutaneously into the right thigh of 6-week-old C57BL6 male mice (SLC Inc., Hamamatsu, Japan). Mice were housed for about ten days under a 12-h light/12-h dark cycle with free access to food and water. Mice were fasted with no food overnight with water supplied ad libitum before experiments. At about ten days later, the size of the melanoma became palpable, and 37 kBq ^{125}I-PCA, ^{125}I-3-Iodo-α-methyl-L-tyrosine (^{125}I-AMT) [13,14] or ^{123}I-*m*-iodobenzylguanidine (^{123}I-MIBG) (Fujifilm RI Pharma Co., Ltd., Tokyo, Japan) [15], ^{67}Ga-citrate (Fujifilm RI Pharma Co., Ltd., Tokyo, Japan) [14] was injected via the tail vein in five tumor-bearing mice. Mice were sacrificed at 5, 15, 30, 60 and 120 min postinjection. After blood was sampled via cardiocentesis, brain, pancreas, spleen, stomach, intestine, kidney, liver, heart, lung, skin muscle and melanoma were excised. Radioactivity in weighed tissue samples was measured using a gamma counter (ARC-380; Aloka, Tokyo, Japan). Data are expressed in terms of injected dose (%ID/organ) for stomach and intestine and injected dose per g wet tissue (%ID/g tissue).

2.5. In vitro assay with B16 melanoma cells

Transport assays were performed using the methods of Nishii et al. and Kobayashi et al., with some modifications [12,16]. Briefly, B16 melanoma cells were seeded onto 24-well cell culture multiwell plates at a density of 5×10^5 cells/ml/well. Assays were conducted at 24 h after seeding. After culture medium was removed, each well was incubated with 1 ml of HEPES buffer (pH 7.4) for 10 min at 37 °C. Cells were then incubated with 0.5 ml of incubation medium containing 18.5 kBq ^{125}I-PCA for 30 min at 37 °C (control conditions). Inhibition studies of tyrosinase, DNA polymerase and membrane active transport were carried out using 0.5 mM phenylthiourea (Sigma, St. Louis, MO), 0.5 mM thymidine (Nacalai Tesque), 0.5 mM ouabine (Sigma) and 1 mM L-tyrosine (Sigma), respectively. B16 melanoma cells were incubated for 30 min using ^{125}I-PCA and these inhibitors. At the end of

Table 1
Biodistribution of ^{125}I-PCA in B16 melanoma-bearing C57BL6 mice.

Organ or tumor	Time (min)				
	5	15	30	60	120
Blood	5.52 ± 1.06	4.07 ± 0.19	2.53 ± 0.43	1.56 ± 0.05	0.46 ± 0.07
Brain	1.89 ± 0.15	1.58 ± 0.17	1.16 ± 0.10	0.45 ± 0.08	0.21 ± 0.08
Pancreas	11.38 ± 3.26	4.53 ± 0.75	2.37 ± 0.54	0.74 ± 0.16	0.25 ± 0.15
Spleen	7.99 ± 2.68	3.50 ± 0.38	1.99 ± 0.78	1.08 ± 0.45	0.28 ± 0.07
Stomach*	0.49 ± 0.15	0.55 ± 0.16	0.75 ± 0.31	0.77 ± 0.25	0.53 ± 0.19
Intestine*	6.82 ± 1.76	8.15 ± 2.22	9.33 ± 1.65	12.77 ± 2.73	16.48 ± 2.92
Kidney	33.30 ± 1.91	17.78 ± 2.71	9.80 ± 1.31	3.83 ± 1.38	1.28 ± 0.47
Liver	24.04 ± 3.77	12.47 ± 2.38	6.21 ± 0.84	2.24 ± 0.86	1.36 ± 0.42
Heart	6.25 ± 0.17	2.93 ± 0.49	1.60 ± 0.23	0.76 ± 0.34	0.32 ± 0.11
Lung	23.63 ± 3.35	18.01 ± 2.44	13.61 ± 1.40	7.02 ± 0.59	2.20 ± 0.83
Skin	3.11 ± 0.47	1.84 ± 0.33	1.34 ± 0.24	0.52 ± 0.13	0.45 ± 0.05
Muscle	3.22 ± 0.31	1.57 ± 0.42	0.85 ± 0.12	0.31 ± 0.09	0.17 ± 0.07
Melanoma	3.12 ± 0.72	3.26 ± 1.03	3.66 ± 0.86	3.44 ± 0.43	3.25 ± 0.43

Percent injected dose per g tissue (%ID/g), mean ± standard deviation of data from five mice.
* %ID/organ calculated from %ID/g and measured organ weights.

incubation, each well was rapidly washed twice with 1 ml of ice-cold incubation medium. Cells were then solubilized in 0.5 ml of 0.1 N NaOH, and radioactivity was measured with a liquid scintillation counter (LSC-5100; Aloka, Tokyo, Japan). Subsequently, cells were detached from one another with trypsin to facilitate further counting. All experimental conditions were examined in quadruplicate assays.

2.6. Partition coefficients

Partition coefficients of ^{125}I-PCA were measured using 2.0 ml of n-octanol as the organic phase and 2.0 ml of 0.1 M phosphate buffer (pH 7.0 for tumor tissue and pH 7.4 for plasma) as the aqueous phase. The n-octanol and buffer were pre-mixed twice using a mechanical

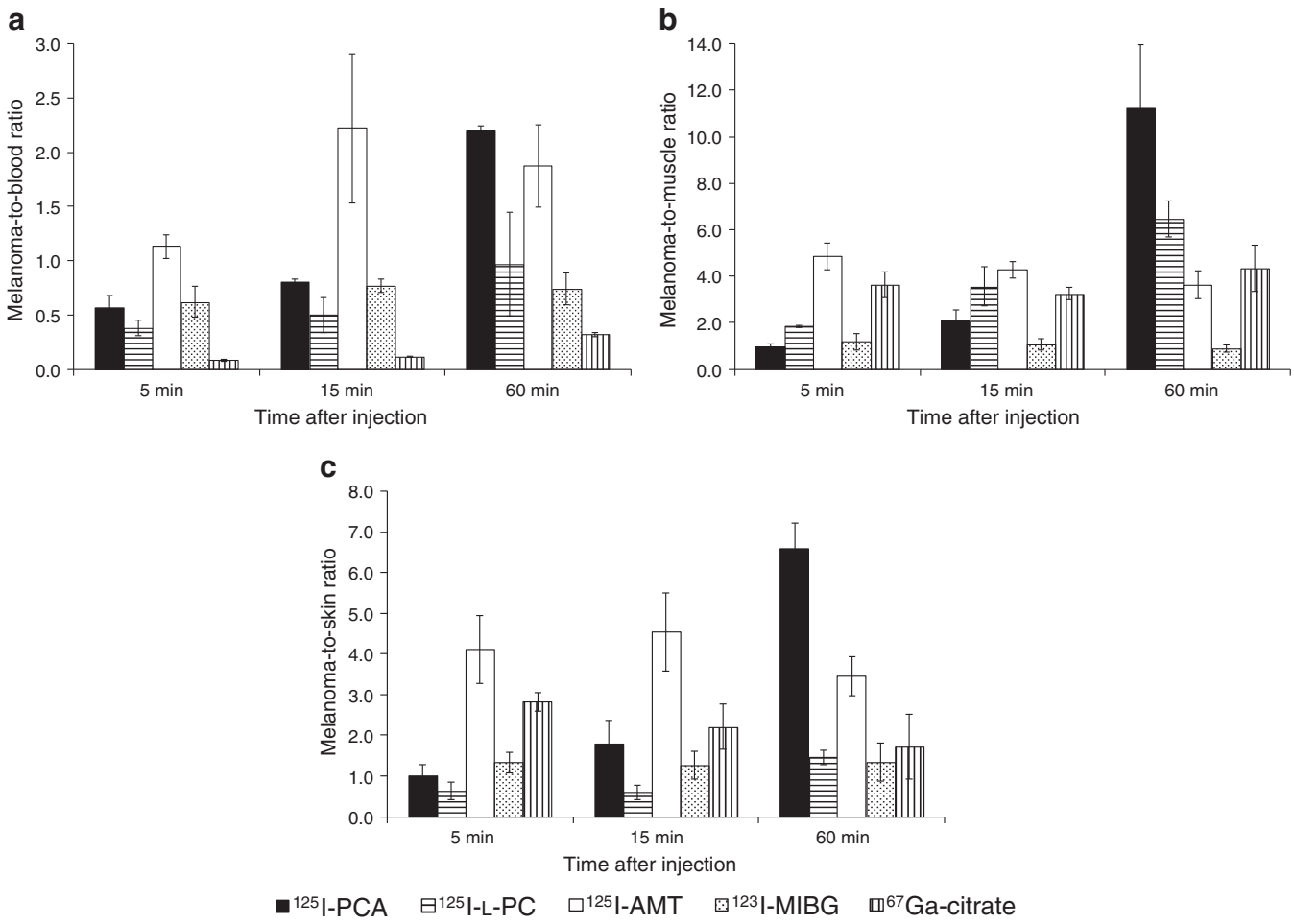

Fig. 2. Melanoma-to-blood ratios (a), melanoma-to-muscle ratios (b) and melanoma-to-skin ratios (c) in B16 tumor-bearing C57BL6 mice at 5, 15 and 60 min after injection. ^{125}I-PCA increased gradually after injection and showed higher values than other radiotracers at 60 min after injection for all ratios.

Table 2
In vitro assay in B16 melanoma cells using inhibitors.

	% of control			
Inhibitors	Phenylthiourea	Thymidine	Ouabine	L-tyrosine
^{125}I-PCA	38.4 ± 11.8**	11.5 ± 3.6**	102.2 ± 19.5	90.3 ± 10.3
^{125}I-L-PC	55.2 ± 12.3*	68.4 ± 12.1*	113.7 ± 15.8	77.6 ± 14.9
^{123}I-AMT	84.8 ± 5.4	88.9 ± 18.2	72.6 ± 0.9	58.3 ± 2.5*

* $P < 0.05$.
** $P < 0.01$ vs. control.

mixer for 1 min at room temperature. Then, 20 μl of ^{125}I-PCA in saline was added and mixed twice for 1 min at room temperature. The radioactivity of 200 μl of each phase was measured using a gamma counter after centrifugation. As reference, the partition coefficients of ^{125}I-L-PC, ^{123}I-AMT and *N*-isopropyl-*p*-[^{123}I]iodoamphetamine (^{123}I-IMP) were used [12,13].

2.7. Statistical analysis

Data are presented as means and standard deviation (SD). *P* values were calculated using a two-tailed paired Student's *t*-test for comparison between two groups. *P* values of less than 0.01 or 0.05 were considered to be significant.

3. Results

3.1. Synthesis of 4-PCA

On TLC analysis, Rf values of 4-PCA, cystamine and phenol were respectively 0.50–0.60, 0.10–0.20 and 0.65–0.75 in methanol and acetate (100:1). The yield of 4-PCA was 8.7%.

3.2. Radiosynthesis of ^{125}I-PCA

On TLC analysis, Rf values of ^{125}I-PCA and ^{125}I$^-$ were respectively 0.40–0.50 and 0.75–0.85. The labeling efficiency of ^{125}I-PCA was more than 73%. On HPLC analysis, retention times were about 5 min for ^{125}I$^-$, 30 min for unlabelled 4-PCA, and 40 min for ^{125}I-PCA. After purification, no-carrier added ^{125}I-PCA was greater than 95% pure and separated from unlabeled 4-PCA.

3.3. Biodistribution in B16 melanoma-bearing mice

^{125}I-PCA peaked in B16 melanoma cells at 30 min after injection and higher values in B16 melanoma cells were seen when compared with blood, and these levels were retained in B16 melanoma cells beyond 30 min after injection (Table 1). In normal tissues, higher uptake of ^{125}I-PCA was observed in the kidney, liver and lung at 5 min after injection, but ^{125}I-PCA was quickly washed out. Radioactivity in blood also disappeared quickly, and in the stomach, it did not subsequently increase. Fig. 2 shows the melanoma-to-blood ratios, melanoma-to-muscle ratios and melanoma-to-skin ratios using ^{125}I-PCA, ^{125}I-L-PC, ^{125}I-AMT, ^{123}I-MIBG and ^{67}Ga-citrate at 5, 15 and 60 min after injection. ^{125}I-PCA showed the highest ratios of all radiotracers after 60 min of injection.

Table 3
Partition coefficients at pH 7.0 and 7.4.

pH	7.0	7.4
^{125}I-PCA	−0.34 ± 0.05	−0.43 ± 0.03
^{125}I-L-PC	−0.28 ± 0.04	−0.37 ± 0.04
^{123}I-AMT	−0.88 ± 0.00	−0.91 ± 0.00
^{123}I-IMP	0.72 ± 0.03	0.94 ± 0.01

Log (n-octanol/0.1 M phosphate buffer).

3.4. In vitro assay in B16 melanoma cells

As shown in Table 2, accumulation of ^{125}I-PCA and ^{125}I-L-PC was significantly inhibited by phenylthiourea and thymidine, but was not inhibited by ouabine and L-tyrosine. The influence of ^{125}I-PCA on phenylthiourea and thymidine was greater than that of ^{125}I-L-PC.

3.5. Partition coefficients

Partition coefficients of ^{125}I-PCA were not significantly different from ^{125}I-L-PC, but were greater than those of ^{123}I-AMT and lower than those of ^{123}I-IMP (Table 3).

4. Discussion

In this study, ^{125}I-PCA accumulated in and was retained by malignant melanoma cells (Table 1). Tyrosine analogues, which are tyrosinase substrates, are good candidates for developing drugs for melanoma-targeting chemotherapies [17]. 4-PCA is a better substrate for tyrosinase than 2-PCA because the structure of 4-PCA is more similar to tyrosine than 2-PCA [3,5,18]. Miura et al. showed that the affinity of 4-PCA, a precursor of ^{125}I-PCA, was higher than that of L-PC [5]. As shown in Table 2, ^{125}I-PCA has a greater affinity for tyrosinase in melanocytes than ^{125}I-L-PC, as radioactivity of ^{125}I-PCA was significantly inhibited by phenylthiourea, which is an inhibitor of tyrosinase, and thymidine, which is a DNA polymerase inhibitor, as compared with ^{125}I-L-PC. Yamada et al. reported that 4-PCA for chemotherapy is oxidized by tyrosinase to the *o*-quinone form via the catechol derivative, and that some of the quinones then conjugate with sulfhydryl enzymes, including DNA polymerase [7]. Meristem cells incorporate thymidine and then complete DNA replication before cell division. However, there was no cell accumulation of ^{125}I-PCA through the amino acid transporter. Accumulation of ^{125}I-AMT was inhibited by ouabine and L-tyrosine, but was not inhibited by phenylthiourea and thymidine. In addition, the lipophilicity of ^{125}I-PCA was markedly lower than that of ^{123}I-IMP, has high cell transportation by high lipophilicity (Table 3). Therefore, ^{125}I-PCA accumulated in melanoma cells due to its high affinity for both tyrosinase and DNA polymerase.

In B16 melanoma-bearing mice (Table 1), ^{125}I-PCA accumulated in melanoma and was retained after 30 min of injection. ^{125}I-PCA showed faster washout from blood and was quickly excreted by the kidney. ^{125}I-PCA also accumulated in normal tissues such as the pancreas and lung when compared to ^{125}I-L-PC [12], but was excreted quickly from normal tissues. With regard to stability of ^{125}I-PCA, radioactivity in the stomach typically increases if radioiodine-labeled tracers undergo deiodination. As shown in Table 1, radioactivity did not increase. Therefore, in vivo stability of ^{125}I-PCA is estimated to be higher. It is important for SPECT imaging to improve melanoma-to-blood ratios, melanoma-to-muscle ratios and melanoma-to-skin ratios. For all ratios, ^{125}I-PCA increased gradually after injection and showed peak values at 60 min after injection, as compared with other SPECT tracers that are able to detect melanoma (Fig. 2). Thus, ^{125}I-PCA is an effective imaging radiotracer for malignant melanoma.

5. Conclusion

^{125}I-PCA is a better substrate for tyrosinase and DNA polymerase and has higher uptake and longer retention in malignant melanoma cells when compared with ^{125}I-L-PC. Therefore, $^{123/131}$I-PCA has good potential for diagnosis for malignant melanoma.

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References

[1] Pawelek JM. Factors regulating growth and pigmentation of melanoma cells. J Invest Dermatol 1976;66:201–9.

[2] Ito S, Inoue S, Yamamoto Y, Fujita K. Synthesis and antitumor activity of cysteinyl-3, 4-dihydroxyphenylalanines and related compounds. J Med Chem 1981;24:673–7.

[3] Ito S, Kato T, Ishikawa K, Kasuga T, Jimbow K. Mechanism of selective toxicity of 4-S-cysteinylphenol and 4-S-cysteaminylphenol to melanocytes. Biochem Pharmacol 1987;36:2007–11.

[4] Jimbow K, Miura T, Ito S, Ishikawa K. Phenolic melanin precursors provide a rational approach to the design of antitumor agents for melanoma. Pigment Cell Res 1989;2:34–9.

[5] Miura S, Ueda T, Jimbow K, Ito S, Fujita K. Synthesis of cysteinylphenol, cysteaminylphenol, and related compounds, and *in vivo* evaluation of antimelanoma effect. Arch Dermatol Res 1987;279:219–25.

[6] Pankovich JM, Jimbow K. Tyrosine transport in a human melanoma cell line as a basis for selective transport of cytotoxic analogues. Biochem J 1991;280:721–5.

[7] Yamada I, Seki S, Ito S, Suzuki S, Matsubara O, Kasuga T. The killing effect of 4-S-cysteaminylphenol, a newly synthesised melanin precursor, on B16 melanoma cell lines. Br J Cancer 1991;63:187–90.

[8] Alena F, Iwashina T, Gili A, Jimbow K. Selective in vivo accumulation of N-acetyl-4-S-cysteaminylphenol in B16F10 murine melanoma and enhancement of its in vitro and in vivo antimelanoma effect by combination of buthionine sulfoximine. Cancer Res 1994;54:2661–6.

[9] Gili A, Thomas PD, Ota M, Jimbow K. Comparison of in vitro cytotoxicity of N-acetyl and N-propionyl derivatives of phenolic thioether amines in melanoma and neuroblastoma cells and the relationship to tyrosinase and tyrosine hydroxylase enzyme activity. Melanoma Res 2000;10:9–15.

[10] Tandon M, Thomas PD, Shokravi M, Singh S, Samra S, Chang D, et al. Synthesis and antitumour effect of the melanogenesis-based antimelanoma agent N-propionyl-4-S- cysteaminylphenol. Biochem Pharmacol 1998;55:2023–9.

[11] Bastiaannet E, Wobbes T, Hoekstra OS, van der Jagt EJ, Brouwers AH, Koelemij R, et al. Prospective comparison of [^{18} F]fluorodeoxyglucose positron emission tomography and computed tomography in patients with melanoma with palpable lymph node metastases: diagnostic accuracy and impact on treatment. J Clin Oncol 2009; 27:4774–80.

[12] Nishii R, Kawai K, Flores II LG, Kataoka H, Jinnouchi S, Nagamachi S, et al. A novel radiopharmaceutical for detection of malignant melanoma, based on melanin formation: 3-iodo-4-hydroxyphenyl-L-cysteine. Nucl Med Commun 2003;24:575–82.

[13] Kawai K, Fujibayashi Y, Saji H, Yonekura Y, Konishi J, Kubodera A, et al. A strategy for the study of cerebral amino acid transport using iodine-123-labled amino acid radiopharmaceutical: 3-iodo-alpha-metyl-L-tyrosine. J Nucl Med 1991;32:819–24.

[14] Kloster G, Bockslaff H. L-3-123I-alpha-methyltyrosine for melanoma detection: a comparative evaluation. Int J Nucl Med Biol 1982;9:259–69.

[15] Osei-Bonsu A, Kokoschka EM, Ulrich W, Sinzinger H. 131I-metaiodobenzylguanidine (mIBG) for bronchial oat cell cancer and melanoma detection? Eur J Nucl Med 1989; 15:629–31.

[16] Kobayashi M, Hashimoto F, Ohe K, Nadamura T, Nishi K, Shikano N, et al. Transport mechanism of ^{11}C-labeled L- and D-methionine in human-derived tumor cells. Nucl Med Biol 2012;39:1213–8.

[17] Jimbow K, Iwashina T, Alena F, Yamada K, Pankovich J, Umemura T. Exploitation of pigment biosynthesis pathway as a selective chemotherapeutic approach for malignant melanoma. J Invest Dermatol 1993;100:231S–8S.

[18] Pankovich JM, Jimbow K, Ito S. 4-S-cysteaminylphenol and its analogues as substrates for tyrosinase and monoamine oxidase. Pigment Cell Res 1990;3:146–9.