Selective 2-[^{18}F]Fluorodopa Uptake for Melanogenesis in Murine Metastatic Melanomas

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The relationship between 3,4-dihydroxy-2-[^{18}F]fluoro-L-phenylalanine (2-[^{18}F]FDOPA) uptake and melanogenesis was studied using mice bearing two B16 melanomas: B16-F1 has a higher melanin synthesis ability and a slower growing rate than the higher metastatic B16-F10. A significantly higher 2-[^{18}F]FDOPA uptake by B16-F1 than by B16-F10 and a reverse relationship for the uptake of [^{14}C] 2-deoxy-2-fluoro-D-glucose and [^{3}H]thymidine were observed 1 hr postinjection. F1-to-F10 ratios of both the 2-[^{18}F]FDOPA uptake and the acid-insoluble radioactivity increased to about 5 at 6 hr, which paralleled the melanin content. FM3A mammary carcinoma showed a 2-[^{18}F]FDOPA uptake similar to the B16-F10 but without the acid-insoluble radioactivity. With D,L-DOPA loading, a 55% decreased uptake by FM3A 1 hr postinjection was significantly greater than the 20% reduction in both melanomas. O-Methylated 2-[^{18}F]FDOPA was a predominant acid-soluble metabolite in all tumors. Whole-body autoradiography discriminated the two melanomas clearly. 2-[^{18}F]FDOPA may be a promising tracer for the selective imaging of melanogenesis.

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P ositron emission tomography (PET) in the field of oncology (*1–3*) could possibly be used for measuring the viability of tumor tissue before and after treatment and provide new criteria to assess the validity of treatment protocols. Malignant melanoma is one of the most lethal of cancers. As malignant transformation occurs, the cells usually retain the ability of melanin synthesis (*4*). However, in seeking positron-emitting radiopharmaceuticals for melanoma-specific imaging, Turner et al. used carbon-11- (^{11}C) labeled neuroreceptor ligands unsuccessfully (*5*). Uptake of ^{11}C-labeled methionine and tyrosine by melanomas is not due to

specific characteristics of the melanomas, but rather general properties of the rapidly growing tumor tissues (*5,6*). Because L-3,4-dihydroxyphenylalanine (L-DOPA) is a substrate for melanin synthesis, the potential of ^{11}C-labeled L-DOPA for melanoma localization has been investigated in 1 hr (*6*). Incorporation of DOPA into melanin is assessed as the ratio of acid-insoluble-to-total radioactivity. Compared with L-DOPA, D-DOPA, which was incorporated into the melanogenesis to a lesser extent, produced a preferable melanoma image with PET (*6*). In a preliminary report on ^{18}F-labeled DOPAs (*7*), uptake by B16 melanoma and the incorporation of radioactivity into the melanin have been found; however, non-melanoma tumors also showed similar uptake to the melanoma at 1 hr after administration of the tracer. Accelerated amino acid transport and/or enhanced amino acid demand in both melanoma and non-melanoma tumors possibly explains these results.

In this paper, the selectivity of L-3,4-dihydroxy-2-[^{18}F]fluoro-L-phenylalanine (2-[^{18}F]FDOPA) for melanomas was investigated 6 hr after injection. The 2-[^{18}F] FDOPA has been shown to be a better tracer than the 6-fluoro analog (*7*). The animal models used were mice bearing B16-F1 and B16-F10 melanomas (*8*). The more highly metastatic B16-F10 melanoma has a faster growth rate and a lower melanin synthesis ability compared with the B16-F1. The melanoma uptake of [^{14}C] 2-deoxy-2-fluoro-D-glucose ([^{14}C]FDG) and [^{3}H]thymidine ([^{3}H]Thd) co-injected with 2-[^{18}F]FDOPA into the same animals can be used to discriminate between characteristics due to the rapidly growing tissues and to the melanogenesis of the models.

MATERIALS AND METHODS

Radiochemicals

2-[^{18}F]FDOPA was prepared as discussed in a previous paper (*7*). [^{14}C(U)]FDG with a specific activity of 11 GBq/mM and [methyl-^{3}H]Thd with a specific activity of 1.5 TBq/mM were purchased from American Radiolabeled Chemicals Inc., St. Louis, Mo.

Animal Models

Melanoma B16-F1 and B16-F10 [a more highly metastatic cell line (8)] cells, which were supplied by Dr. O. Yoshie (9), were used within two passages after recovery from frozen stocks. Male 8-wk-old C57BL/6 mice were subcutaneously injected with 8.0×10^6 B16-F1 cells on their inside left thigh and with 6.0×10^6 B16-F10 cells on their opposite thigh. Within 2 wk of inoculation, the mice bearing two B16 melanomas with scarce necrosis were used for experimentation. The doubling times of melanomas in cell culture in vitro were measured. Also, in vivo tumor growth rates were measured using five mice as described previously (10). C3H/He mice with FM3A mammary carcinoma were also prepared by subcutaneous injection of tumor cells on their back.

Tissue Distribution

B16 melanoma-bearing mice were injected intravenously with 3.0 MBq (0.30 μmole) of 2-[^{18}F]FDOPA, 23 kBq of [^{14}C] FDG, and 120 kBq of [^{3}H]Thd. The mice were killed by cervical dislocation at various time intervals. FM3A-bearing mice given 2-[^{18}F]FDOPA were killed 1 and 6 hr postinjection. Another group of B16-bearing mice and FM3A-bearing mice were injected with 2-[^{18}F]FDOPA together with 17 μmole of D,L-DOPA and killed 1 hr postinjection. Blood was removed by heart puncture using a syringe. Tissues were dissected, counted for ^{18}F, and weighed. After decay of ^{18}F radioactivity, the tissues were burned in an automated sample-combustion system (Aloka ASC-113) and the radioactivity of ^{14}C and ^{3}H in the forms of $^{14}CO_2$ and ^3H_2O, respectively, was measured with a liquid scintillation counter. The tissue uptake of radioactivity was expressed as the differential absorption ratio (DAR), i.e., (counts of tissue/g tissue) × (g body weight/total injected counts), to correct the data for the injected doses of the tracers and for body weight of the mice. Tumor-to-tissue uptake ratios in each animal group were calculated.

Metabolic Study

Mice given 8.3–11 MBq (0.35 to 0.60 μmole) of 2-[^{18}F] FDOPA were killed at indicated times, and blood and tumor samples were obtained. Plasma and tumor samples (0.1–0.4 g) were homogenized in ice-cold 1 ml of 10% trichloroacetic acid (TCA). The homogenate was centrifuged at 6000 g for 3 min and divided into the supernatant (fraction 1) and the pellet. The pellet was washed in 1 ml of 5% TCA three times. From the percentages of pellet total radioactivity, tumor uptake data, and specific activity of the tracer, concentrations of the tracer (μg equivalent to 2-[^{18}F]FDOPA per gram of tissue) in the final pellet were calculated as the melanin incorporation fraction.

Labeled matabolites in fraction 1 were analyzed by high-performance liquid chromatography (HPLC). A Nova-Pak C_{18} column equipped in an RCM 8 × 10 module (Waters) was eluted with the reverse-phase ion pair solution with a gradient modifier at room temperature at a constant flow rate of 2 ml/min. The initial solvent (buffer A) was 0.8% acetic acid, 1 mM sodium octylsulfate, and 1 mM EDTA and the stronger elution solvent (buffer B) was a mixture of the buffer A and methanol (30:70, v/v). A mixture of buffer A and buffer B (33/67, v/v) was eluted for the first 10 min, after which a solvent with a linear gradient of composition (33%–100%) of

buffer B was eluted for 25 min. During the last 10 min, buffer B was delivered. The elution profile was detected with a radioactivity monitor (Ramona-D equipped with an IM-2020X flow cells for $^3H/^{14}C$ detection, Raytest), and the radioactivity in each 1.0 ml effluent was measured with a NaI(Tl) gamma scintillation counter. Recovery of radioactivity by HPLC was essentially quantitative.

3-O-Methyl-2-[^{18}F]fluorodopa (pb^{18}F]MeFDOPA) and 2-[^{18}F]fluorodopamine ([^{18}F]FDA) were prepared enzymatically using catechol-O-methyltransferase (COMT) in rat celebellum homogenate (11) and aromatic amino acid decarboxylase in rat striatum homogenate (12), respectively. Retention times of labeled compounds in the HPLC analysis were 11.2 min for 2-[^{18}F]FDOPA, 27.6 min for [^{18}F]MeFDOPA, and 33.2 min for [^{18}F]FDA.

Measurement of Melanin Contents

Melanin content was measured by a method of Oikawa and Nakayasu (13). About 200 mg of melanoma tissue (180–224 mg) were obtained from five mice and homogenized together in water. The homogenate was treated with two volumes of concentrated HCl. The black pigment was collected by centrifugation, refluxed in 6 N HCl for 3 days, and then washed with water and with acetone. The purified melanin preparations were weighed.

Autoradiography and Histologic Examination

B16 melanoma-bearing mice were injected with 47 MBq (4.8 μmole) of 2-[^{18}F]FDOPA. Whole-body autoradiography (WBARG) was performed 60 min after injection as described previously (14). Small pieces of tumor samples from another mouse were frozen, cut into 5-μm thick slices, and stained with hematoxylin-eosin. The melanin content in B16-F1 and B16-F10 tissues was compared microscopically.
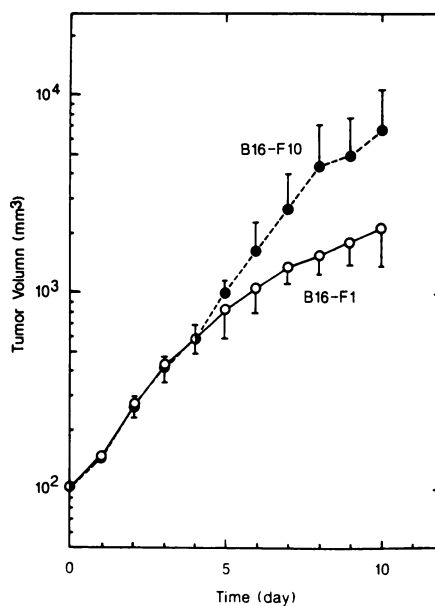

FIGURE 1
Growth curves of B16-F1 (O) and B16-F10 (●). The day on which tumor volume exceeded 100 mm³ was designated as Day 0. Mean ± s.d. (n = 5).

RESULTS

Doubling times of the B16-F1 and B16-F10 cells in culture were 16.9 hr and 13.5 hr, respectively. In Figure 1, growth curves of the two melanomas implanted in C57BL/6 mouse are shown. The doubling times of B16-F1 and B16-F10 were 2.8 days and 1.5 days, respectively, in the time point of experiments (5–7 days in Fig. 1).

Tissue distribution of the three tracers are summa-

rized in Table 1. The [^{3}H]Thd uptake levels in both B16-F1 and B16-F10 were the highest among the three tracers. B16-F10 showed a significantly higher uptake of [^{3}H]Thd (~30% increment) than B16-F1. On the contrary, a higher uptake of 2-[^{18}F]FDOPA by B16-F1 and a slower decrease of radioactivity were found. As previously reported (7), ratios of the acid-insoluble fraction to the total ^{18}F uptake in both melanomas increased with time, and the ratios for B16-F1 were larger than those for B16-F10. When the acid-insoluble

TABLE 1

Tissue Distributions of Radioactivity in B16-F1- and B16-F10-Bearing Mice after i.v. Injection of 2-[^{18}F]FDOPA, [^{14}C]FDG, and [^{3}H]Thd

| | | Uptake*, DAR | | |
		1 hr (n = 5)	2 hr (n = 5)	6 hr (n = 6)
B16-F1	2-[^{18}F]FDOPA	2.27 ± 0.43†	2.02 ± 0.65§	0.81 ± 0.31§
		(6.4)†	(8.4)†	(23.3 ± 4.6)†
	[^{14}C]FDG	2.06 ± 1.07	1.78 ± 0.44	1.81 ± 0.70
	[^{3}H]Thd	2.46 ± 0.42***	2.40 ± 0.34***	1.95 ± 0.46**
B16-F10	2-[^{18}F]FDOPA	1.50 ± 0.31	0.98 ± 0.09	0.15 ± 0.05
		(3.5)†	(6.5)†	(14.2 ± 6.7)‡
	[^{14}C]FDG	2.90 ± 0.96	1.94 ± 0.35	1.50 ± 0.55
	[^{3}H]Thd	3.29 ± 0.59	2.89 ± 0.23	2.80 ± 0.56
Blood	2-[^{18}F]FDOPA	0.37 ± 0.04	0.18 ± 0.02	0.028 ± 0.008
	[^{14}C]FDG	0.088 ± 0.029	0.052 ± 0.007	0.047 ± 0.008
	[^{3}H]Thd	0.41 ± 0.06	0.42 ± 0.04	0.39 ± 0.05
Brain	2-[^{18}F]FDOPA	0.33 ± 0.09	0.23 ± 0.05	0.048 ± 0.018
	[^{14}C]FDG	1.35 ± 0.46	0.94 ± 0.15	0.41 ± 0.11
	[^{3}H]Thd	0.43 ± 0.08	0.40 ± 0.02	0.35 ± 0.01
Heart	2-[^{18}F]FDOPA	0.30 ± 0.07	0.16 ± 0.03	0.023 ± 0.004
	[^{14}C]FDG	4.87 ± 2.14	3.04 ± 1.41	2.39 ± 1.84
	[^{3}H]Thd	0.49 ± 0.09	0.47 ± 0.08	0.36 ± 0.05
Lung	2-[^{18}F]FDOPA	0.38 ± 0.08	0.18 ± 0.02	0.043 ± 0.019
	[^{14}C]FDG	0.52 ± 0.07	0.39 ± 0.04	0.41 ± 0.09
	[^{3}H]Thd	0.44 ± 0.05	0.42 ± 0.05	0.43 ± 0.06
Liver	2-[^{18}F]FDOPA	0.76 ± 0.20	0.28 ± 0.07	0.056 ± 0.008
	[^{14}C]FDG	0.17 ± 0.02	0.15 ± 0.02	0.12 ± 0.02
	[^{3}H]Thd	0.79 ± 0.12	0.57 ± 0.06	0.48 ± 0.07
Pan-creas	2-[^{18}F]FDOPA	2.36 ± 0.54	1.51 ± 0.24	0.084 ± 0.064
	[^{14}C]FDG	0.39 ± 0.06	0.32 ± 0.05	0.29 ± 0.06
	[^{3}H]Thd	0.39 ± 0.04	0.37 ± 0.04	0.36 ± 0.05
Spleen	2-[^{18}F]FDOPA	0.39 ± 0.06	0.22 ± 0.05	0.037 ± 0.010
	[^{14}C]FDG	0.35 ± 0.10	0.34 ± 0.07	0.35 ± 0.09
	[^{3}H]Thd	2.06 ± 0.49	1.26 ± 0.26	1.47 ± 0.35
Small in-tes-tine	2-[^{18}F]FDOPA	0.59 ± 0.10	0.38 ± 0.08	0.070 ± 0.016
	[^{14}C]FDG	0.28 ± 0.08	0.22 ± 0.04	0.16 ± 0.02
	[^{3}H]Thd	2.14 ± 0.15	2.14 ± 0.11	1.97 ± 0.18
Kidney	2-[^{18}F]FDOPA	1.35 ± 0.31	1.04 ± 0.14	0.64 ± 0.17
	[^{14}C]FDG	0.23 ± 0.18	0.24 ± 0.11	0.16 ± 0.02
	[^{3}H]Thd	0.71 ± 0.15	0.46 ± 0.08	0.41 ± 0.04
Muscle	2-[^{18}F]FDOPA	0.37 ± 0.04	0.18 ± 0.01	0.046 ± 0.016
	[^{14}C]FDG	0.63 ± 0.04	0.51 ± 0.16	0.65 ± 0.12
	[^{3}H]Thd	0.35 ± 0.07	0.36 ± 0.05	0.40 ± 0.09

* Mean ± s.d. Figures in parentheses show % incorporation of acid-insoluble fraction.
† Mean (n = 3) and ‡ mean ± s.d. (n = 6).
Student's t-test for difference between B16-F1 uptake and B16-F10 uptake: § p < 0.001; † p < 0.01; ** p < 0.02; *** p < 0.05.

TABLE 2

B16-F1-to-B16-F10 Uptake Ratios of 2-[^{18}F]FDOPA, [^{14}C]FDG, and [^{3}H]Thd

		F1-to-F10 ratio*		
		1 hr	2 hr	6 hr
2-[^{18}F]FDOPA	Total uptake	1.52	2.06	5.50
	Acid-insoluble uptake	4.83	5.23	5.30
[^{14}C]FDG	Total uptake	0.71	0.92	1.20
[^{3}H]Thd	Total uptake	0.75	0.83	0.70

* Mean (n = 5–6).

fraction was expressed as the microgram equivalent to FDOPA per gram of tissue, the concentrations incorporated into the melanine fraction were 0.60 (n = 3) at 1 hr and 0.99 (n = 3) at 2 hr; 1.1. ± 0.2 (n = 6) at 6 hr for B16-F1; and 0.12 (n = 3) at 1 hr, 0.19 at 2 hr, and 0.22 ± 0.11 (n = 6) at 6 hr for B16-F10. In the case of [^{14}C]FDG, a higher radioactivity level in B16-F10 at 1 hr postinjection decreased gradually, although the level in B16-F1 was nearly constant. The [^{14}C]FDG uptake by brain decreased rapidly with time.

Uptake ratios of B16-F1 to B16-F10 are summarized in Table 2. The ratio of total 2-[^{18}F]FDOPA uptake increased with time. The ratio of acid-insoluble radioactivity was nearly constant and paralleled the F1-to-F10 ratio of the melanin content: 2.39 mg/g tissue of B16-F1 and 0.51 mg/g tissue of B16-F10. The F1-to-F10 ratio of [^{14}C]FDG also increased because the gradual clearance of ^{18}F from B16-F10 and [^{3}H]Thd was constant.

The effect of D,L-DOPA on 2-[^{18}F]FDOPA uptake was studied in two melanomas and in a FM3A mammary carcinoma. FM3A also served as the non-mela-

noma control (Table 3). FM3A showed an uptake of 2-[^{18}F]FDOPA similar to the B16-F10 melanoma. About 90% of radioactivity in the FM3A and B16-F10 tissues 1 hr postinjection was cleared by 6 hr, although 36% of radioactivity in B16-F1 remained. The corresponding figure for pancreas was only 3.6% (Table 1). A significant decreased uptake by the DOPA loading was found 1 hr after injection; about 20% reduction for both B16-F1 and B16-F10 and 55% for FM3A.

Labeled metabolites in the acid-soluble fraction of plasma and tumor samples were analyzed by HPLC (Table 4). Ratios of the acid-insoluble radioactivity in the plasma and FM3A were less than 1.0% of total radioactivity. Most of the radioactivity of the acid-soluble metabolites in melanomas, FM3A, and plasma was detected as [^{18}F]MeFDOPA. Fluorine-18-Me-FDOPA and [^{18}F]FDA were eluted at the same retention times as the authentic samples prepared enzymatically. 2-[^{18}F]fluorodihydroxyphenylacetic acid ([^{18}F] FDOPAC) and 2-[^{18}F]fluorohomovanillic acid ([^{18}F] FHVA) were tentatively identified by comparing their retention times to those of the corresponding derivatives of DOPA.

Melanoma imaging was demonstrated 1 hr postinjection by WBARG (Fig. 2A). The grain density obtained by radioactivity in B16-F1 was measured to be 1.7 times higher than that in the B16-F10. In a photograph of the section (Fig. 2B), the black B16-F1 shows a higher melanin synthesis ability than the dark brown B16-F10. In photomicrographs of tumor specimens (Fig. 3), a higher melanin deposit was found in the B16-F1 than in the B16-F10.

TABLE 3

Effect of D,L-DOPA Loading on Tumor Uptake of Radioactivity After Intravenous Injection of 2-[^{18}F]FDOPA into Tumor-Bearing Mice

		Uptake, DAR*	
		1 hr	6 hr
B16-F1		2.27 ± 0.43	0.81 ± 0.31
B16-F1	+ DOPA	1.80 ± 0.11¶	
B16-F10		1.50 ± 0.31‡	0.15 ± 0.05†
B16-F10	+ DOPA	1.18 ± 0.03¶	
FM3A		1.58 ± 0.27‡	0.18 ± 0.06†
FM3A	+ DOPA	0.71 ± 0.30§	

* Mean ± s.d. (n = 4 to 6).

Student's t-test for difference between B16-F1 and each of other tumors: † p < 0.001; ‡ p < 0.01, and for difference between the control and the DOPA-loading group; § p < 0.001; ¶ p < 0.05.

TABLE 4

Percentages of Radioactive Metabolites in the Acid-Soluble Fractions in Plasma and Tumor Tissues After Intravenous Injection of 2-[^{18}F]FDOPA into Tumor-Bearing Mice

		Percentages of metabolites*				
		FDOPAC (%)	FDOPA (%)	FHVA (%)	MeFDOPA (%)	FDA (%)
Plasma	1 hr	1.8	1.1	9.4	68.5	1.4
B16-F1	1 hr	0.8	0.9	1.8	89.1	1.0
	6 hr	0.4	1.2	1.8	89.2	0.9
B16-F10	1 hr	1.3	2.3	3.8	85.5	1.1
	6 hr	2.6	0.7	2.9	71.1	1.5
FM3A	1 hr	0.7	0.3	1.5	94.9	0.9
	6 hr	2.5	1.0	3.7	71.7	5.0

* Mean (n = 3). Percentage of each metabolite to the acid-soluble radioactivity applied on the column were calculated. Percentages of the acid-insoluble radioactivity to the total radioactivity of melanomas were presented in Table 1. The ratios in plasma and FM3A were negligible.

FIGURE 2
Autoradiographic distribution of radioactivity in a B16-F1/F10 bearing mouse 1 hr after i.v. injection of 2-[^{18}F]FDOPA (A) and a photograph of the section (B). Autoradiogram (A) was scanned with Chromoscan 3 (Joyce-Loeble); the optical density was digitized.

FIGURE 3
Photomicrographs of B16-F1 (A) and B16-F10 (B) (x 100). Bar = 100 μm.

DISCUSSION

In order to clarify the relationship between the selective 2-[^{18}F]FDOPA uptake by melanoma and the melanogenesis in it, two sublines of murine B16 melanoma were used in this study. B16-F10 exhibits a higher metastatic potential (8) and a lower melanin synthesis ability compared with the B16-F1. The different melanogenesis between two sublines has been proven by the visual demonstration in a photograph of a whole-body section (Fig. 2B) and microphotographs of tumor pieces (Fig. 3) and by chemical analysis. When considering proliferation rates of two melanoma lines, the B16-F10 shows more rapidly growing rates in cell culture in vitro (1.3 times) and in animal models in vivo (1.9 times). The characteristics of the rapidly growing B16-F10 tissue could be assessed by the higher uptake of both [^{3}H] Thd (1.3 times) and [^{14}C]FDG (1.4 times).

An outstanding contrast has been found for 2-[^{18}F] FDOPA uptake. A significant higher uptake and a higher incorporation into melanogenesis have been demonstrated in B16-F1 than in B16-F10 and FM3A, as the non-melanoma control, which is primarily due to a higher melanin synthesis ability of the B16-F1. Because of rapid clearance of the labeled metabolites from normal tissues and tumors with no or low ability of melanogenesis compared to the B16-F1, the selectivity is enhanced later after injection. The 2[^{18}F]FDOPA uptake by tumors may be partially due to the enhanced amino acid requirement in tissues (15), and the amino acid transport system may mediate the tumor uptake of 2-[^{18}F]FDOPA (7). With DOPA loading, a 55%
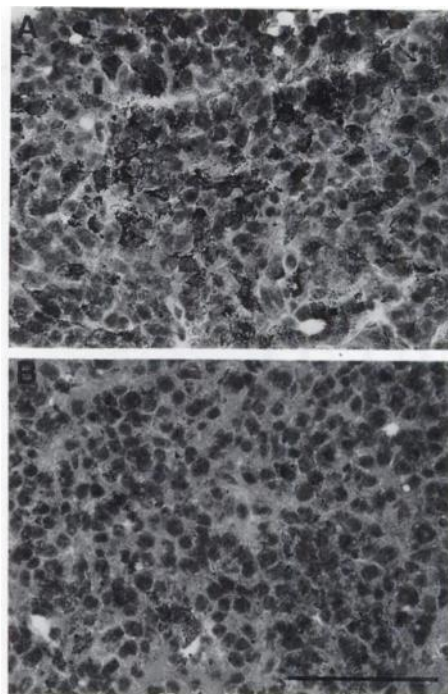
reduction of 2-[^{18}F]FDOPA uptake by FM3A 1 hr postinjection is a striking contrast to an ~20% reduction by both melanoma lines. Melanomas may have a certain affinity for the 2-[^{18}F]FDOPA and its metabolites as described below in connection with melanogenesis when compared with FM3A.

Because of a high affinity for COMT (16), 2-[^{18}F] FDOPA is not used for the study of brain dopamine metabolism (17). Most radioactivity was detected as a O-methylated form in the plasma as previously observed in rats (12) and also in both plasma and tumors even 1 hr postinjection in this study. When considering that the melanin incorporation fraction for the first 2 hr was ~90% of that measured at 6 hr postinjection and that [^{18}F]MeFDOPA is a predominant metabolite in plasma and melanoma tissues at a later time (Table 4), 2-[^{18}F]FDOPA could be a primary chemical form incorporated into melanogenesis, as L-DOPA is the best substrate for tyrosinase, a key enzyme of melanin synthesis (18). The contribution of labeled metabolites into melanogenesis may be minor. The tyrosinase may have less affinity for [^{18}F]FDA than [^{18}F]FDOPA (18,19). Because of the gradual increase of the melanin incorporation fraction after 2 hr, the direct incorporation into melanogenesis of the [^{18}F]MeFDOPA, which cannot be converted to a quinone form, may not be ruled out.

The potential of melanoma imaging has been demonstrated by WBARG. Because of the rapid radioactivity clearance from the normal tissues, a clear B16-F1

image was obtained even at 1 hr postinjection. Imaging at late times will minimize background interference and increase melanoma selectivity. Imaging seems to be promising for visualization of widespread metastases with a high ability of melanin synthesis in the body. Imaging at 6 hr, about three times longer than the half-life of ^{18}F is applicable for PET studies, although the time scale is not applicable to ARG studies. In the field of single-photon emission computed tomography, radiolabeled iodothiouracil, a false melanin precursor, is proposed as a promising agent for the melanoma imaging (20). Preferably, imaging is performed 24 hr after injection of the tracer, and the melanoma uptake level is comparable to the case of 2-[18F]FDOPA at 6 hr postinjection. Recently, successful imaging of malignant melanomas in humans has been performed using a conventional gamma camera and radiolabeled monoclonal antibodies (21). This immunoscintigraphy is due to the specific recognition between monoclonal antibodies and melanoma-specific antigens in the plasma membrane of the cells. Gallium-67 is also used in tumor detection in the field of conventional nuclear medicine (22), but it does not have such selective affinity for melanomas.

It should be pointed out that the results in this paper also represent the potential usefulness of ^{18}F-labeled FDG and ^{11}C-labeled Thd not only for detecting but also for assessing the metabolism of melanomas with different growing rates with PET. For this purpose, the 60-min time scale is appropriate for PET studies, because the highest melanoma uptake of both tracers and large B16-F10/B16-F1 uptake ratios during the 6 hr were achieved 1 hr postinjection. A longer time for [18F] FDG studies may be rather undesirable. A relatively rapid decrease of radioactivity in B16-F10 during the 6 hr after injection of [14C]FDG is explained as follows; a higher glucose-6-phosphatase activity present in the B16-F10 hydrolyzed [14C]FDG-6-phosphate, a metabolically trapped form, to [14C]FDG, which was washed out gradually from the tissue. Moreover, the decrease of [14C]FDG uptake is outstanding in the brain.

In tissue culture studies, the melanin synthesis ability of melanoma cells did not necessarily parallel their growth rates (23); however, the quantitative assessment of melanogenesis in vivo could be of great value for the diagnosis of melanoma.

In conclusion, the selective uptake of 2-[18F]FDOPA by melanoma in connection with melanogenesis has been demonstrated. With PET, functional imaging using this tracer and metabolic imaging using other tracers like [18F]FDG and [11C]Thd could offer promising diagnostic methods for melanoma.

ACKNOWLEDGMENTS

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SELF-STUDY TEST
Radiobiology and Radiation Protection
ANSWERS

ITEM 1: ALARA Philosophy in Nuclear Medicine Practice
ANSWER: **D**

The Food and Drug Administration (FDA) is responsible for establishing the safety and efficacy of drugs, including radiopharmaceuticals, prior to allowing their commercial distribution. As part of the safety evaluation, the toxicity of the pharmaceutical portion of the radiopharmaceutical is tested at the usual doses administered to a patient and at doses many times greater than would ever be administered to a patient. The package insert (label) furnished by the manufacturer (and approved by the FDA) provides a range of suggested dosages; this range is a guideline, not a legal stipulation. Physicians may choose to vary from the conditions of the package label, using either lower or higher dosages than suggested, based on their clinical assessment of the needs of individual patients. A physician's decision to vary substantially from the recommendations in the package insert should be made only after careful consideration, since justification of these doses becomes the physician's responsibility.

The Nuclear Regulatory Commission (NRC) is responsible for ensuring the safe use of by-product material. Its rules stipulate that only those individuals with appropriate education and experience are licensed to use radioactive materials in humans for diagnosis, therapy, and research. The NRC relies on the FDA to establish the safety and efficacy of radiopharmaceuticals and does not attempt to include or exclude radiopharmaceuticals based on its own independent judgment. The NRC has established groups of radiopharmaceuticals that require similar levels of experience, types of instrumentation, and radiation protection precautions. A physician may use any or all of the radiopharmaceuticals in a group for which he or she has gained approval, and may use any new radiopharmaceutical that is subsequently added to that group (as a result of approval by the FDA). The NRC does not stipulate or suggest any dosage ranges for any radiopharmaceuticals, whether diagnostic or therapeutic. The physician is expected to be familiar with the package insert and with the standard of care nationwide with respect to each radiopharmaceutical. The physician's clinical judgment is the deciding factor in determining the actual dosage given a patient.

Radiation protection regulations in the United States are based on the conservative assumption that radiation effects at low doses can be predicted from high-dose effects by extrapolating the dose-response curve from the high-dose region to zero-dose. A consequence of this assumption is that some small effect must be presumed for all doses, even very small doses where the occurrence of the effect may not be established. Many radiation protection specialists have argued for years that surely there must be some radiation dose that is so small (de minimis) that any expected effect would constitute a negligible additional risk to the exposed population. The implication of the de minimis dose is that efforts by radiation users and regulators alike would not need to be expended in order to reduce the radiation dose below this level. The de minimis concept, however, does not apply to the situation posed in this question, because the dilemma is whether or not the dosage administered to patients can be increased above that

normally used.

In the usual procedure for skeletal imaging with ^{99m}Tc-MDP, imaging is delayed until several hours have elapsed after administration of the radiopharmaceutical. This delay period allows that portion of the ^{99m}Tc-MDP not taken up in bone to be cleared via urinary excretion from the soft tissues, yielding an enhanced target-to-background ratio and improved image appearance. As with all diagnostic radiopharmaceuticals, the actual amount of ^{99m}Tc-MDP injected is small (in the range of 1 mg); an increase from 20 mCi to 50 mCi would have essentially no pharmacologic effect and probably would cause no difference in the distribution of ^{99m}Tc-MDP between bone and extraskeletal structures. There is no evidence that the excess radiopharmaceutical would be preferentially excreted into urine rather than going to bone.

Although it is true that the FDA and the NRC do not regulate the dosage range of a radiopharmaceutical that a physician may use, it is not true that these agencies would sanction the routine use of a radiopharmaceutical at dosage levels substantially different from those suggested in the package insert or reported in the literature. The ALARA (as *low as reasonably achievable*) philosophy applies equally to clinical practice as it does to radiation protection of workers and the environment. If radiation or radioactive materials must be used in order to obtain clinically important information or to effect therapy, there must be a clear benefit to the patient. Furthermore, the patient should not be subjected to unnecessary amounts of radiation, because the benefits derived from the radiation might be eroded by the additional risk of the unwarranted radiation. In an individual patient, the decision may be made to use more than 20 mCi, perhaps even as much as 50 mCi, but that decision should be made for that particular patient and not for a general class of patients. For example, if an elderly patient has difficulty remaining motionless long enough for satisfactory imaging with the lower dosage, a higher dosage may be warranted in order to obtain diagnostic-quality images. This decision to use a higher dosage would be in keeping with the ALARA philosophy because the benefit-risk ratio for this patient would be unacceptable at the lower dosage but acceptable at the higher dosage.

ITEM 2: Maximum Dosage of Radiopharmaceuticals
ANSWER: **E**

FDA regulations do not stipulate dosage levels of any pharmaceutical, whether it is radioactive or not. Radiopharmaceuticals are subjected to the same review procedure as nonradioactive pharmaceuticals, i.e., the filing of a "Notice of Claimed Investigational Exemption for a New Drug" (IND) to authorize the premarketing clinical research on the drug and the subsequent approval of a "New Drug Application" (NDA), which authorizes commercial distribution of the drug. The FDA regulations do not directly impose any limits on the dosage of a pharmaceutical any time during this process. Rather, dosage range during clinical investigation is predicated on available preclinical evidence, and the dosage range suggested in the package insert reflects the scientific evidence submitted to FDA in support of the claims of safety and effectiveness for particular