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# Fluorine-18-Labeled Monoclonal Antibody Fragments: A Potential Approach for Combining Radioimmunoscinigraphy and Positron Emission Tomography

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Monoclonal antibody fragments labeled with  $^{18}\text{F}$  could be useful for PET if selective tumor uptake could be achieved within a few half-lives of this nuclide. To evaluate this possibility, the  $\text{F}(\text{ab}')_2$  fragment of Mel-14, an antibody reactive with gliomas and other tumors, was labeled by reaction with *N*-succinimidyl-4- $^{18}\text{F}$ fluorobenzoate. The in-vitro binding properties of  $^{18}\text{F}$ -labeled Mel-14  $\text{F}(\text{ab}')_2$  were nearly identical to those observed when this  $\text{F}(\text{ab}')_2$  was labeled by reaction with *N*-succinimidyl-4- $^{125}\text{I}$ iodobenzoate ( $^{18}\text{F}$ , affinity constant =  $(6.7 \pm 1.1) \times 10^8 \text{ M}^{-1}$ ;  $^{125}\text{I}$ , affinity constant =  $(8.8 \pm 0.6) \times 10^8 \text{ M}^{-1}$ ). The tissue distribution of the two labeled fragments was compared in paired-label studies performed in athymic mice with subcutaneous D-54 MG human glioma xenografts. Uptake of both nuclides in tumor was rapid with levels as high as  $18.7\% \pm 1.1\%$  injected dose/g for  $^{18}\text{F}$  and  $19.4\% \pm 1.0\%$  injected dose/g for  $^{125}\text{I}$  observed by 4 hr after injection. Tumor-to-normal tissue ratios for  $^{18}\text{F}$ -labeled Mel-14  $\text{F}(\text{ab}')_2$  at 4 hr ranged between 0.8:1 for kidneys to 40:1 for brain. These results suggest that it may be feasible to use  $^{18}\text{F}$ -labeled antibody fragments for imaging tumors with PET.

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The use of radiolabeled monoclonal antibodies (Mabs) for diagnostic and therapeutic applications remains an active area of research. Although encouraging results using labeled Mabs have often been reported (1,2), the sensitivity and specificity of tumor detection using conventional gamma camera imaging generally has not been sufficient to warrant routine clinical use. Utilization of better imaging methods is one approach for improving radioimmunoscinigraphy. For example, use of single-photon emission computed tomography (SPECT) with  $^{123}\text{I}$ -labeled anti-CEA Mab fragments has been reported to increase

lesion detectability (3,4). In some cases, lesions not observed by CT or ultrasound were identified as early as 6 hr after injection of Mab fragment.

Positron emission tomography (PET) is an imaging modality that might offer further improvements in Mab imaging. While both PET and SPECT share the advantage of being tomographic in nature, PET offers the possibility of more rigorous attenuation correction, leading to more accurate quantitative capabilities. Since one of the most important applications of Mab imaging is to determine tumor and normal tissue dosimetry prior to therapy, the ability of PET to quantitate Mab uptake could be of great value.

The selection of an appropriate positron-emitting nuclide for labeling Mabs and fragments must take into account a number of factors including the relatively slow clearance of these proteins and the need to minimize patient radiation-absorbed doses. Proteins have been labeled with a number of positron emitters, including  $^{11}\text{C}$  (5,6),  $^{68}\text{Ga}$  (7,8),  $^{89}\text{Zr}$  (9) and  $^{124}\text{I}$  (10,11). Several research groups, including our own, have considered the possibility of labeling proteins with  $^{18}\text{F}$  (12-17). Fluorine-18 has the longest half-life of the routinely available positron emitters and offers radiation dose advantages in comparison to  $^{68}\text{Ga}$  (13). Unlike  $^{89}\text{Zr}$  and  $^{124}\text{I}$ , positron emission is associated with nearly 100% of  $^{18}\text{F}$  decays, and relatively abundant higher energy gamma emissions are not present. The disadvantage of  $^{18}\text{F}$  is that its 2-hr half-life may not be appropriate for use with Mabs and fragments because of their relatively slow clearance from normal tissues.

The purpose of the present study was to investigate the pharmacokinetics of an  $^{18}\text{F}$ -labeled Mab  $\text{F}(\text{ab}')_2$  fragment in an athymic mouse human tumor xenograft model. Our goal was to determine whether tumor uptake and acceptable tumor:normal tissue ratios could be achieved in a time compatible with the half-life of  $^{18}\text{F}$ . For these studies, labeling of the Mab fragment was accomplished using *N*-succinimidyl-4- $^{18}\text{F}$ fluorobenzoate ( $^{18}\text{F}$ SFB), a reagent which we have recently described (18).

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## MATERIALS AND METHODS

### Mab Fragment

Mab Mel-14 is a murine IgG<sub>2a</sub> that is reactive with the tumor-associated proteoglycan chondroitin sulfate present in melanomas and gliomas (19). This Mab was purified from athymic mouse ascites by protein A-Sepharose affinity chromatography. Methods for the production of F(ab')<sub>2</sub> fragments of Mel-14 using pepsin have been described (20).

### Radiohalogenation of Mel-14 F(ab')<sub>2</sub>

**Fluorine-18 Labeling.** The F(ab')<sub>2</sub> fragment of Mel-14 was labeled with <sup>18</sup>F by reaction with [<sup>18</sup>F]SFB, as described in detail in a previous publication (18). Briefly, aqueous [<sup>18</sup>F]fluoride was added to a solution of Krytox and K<sub>2</sub>CO<sub>3</sub>, dried, and then resolubilized in 100–200 μl of DMSO. To 1–2 mg of 4-formyl-N,N,N-trimethylanilinium triflate were added 30–90 mCi of <sup>18</sup>F and then heated at 120–140°C for 25 min. The 4-[<sup>18</sup>F]fluorobenzaldehyde was then purified as described (18), oxidized by addition of 250 μl of KMnO<sub>4</sub> solution (5% in 1 N NaOH) and then heated at 120°C. After about 3 min, 100–200 mg of sodium bisulfite and 100–200 μl of conc. HCl was added, the activity from the slurry was extracted with ethyl acetate and then dried over a column of molecular sieve. The 4-[<sup>18</sup>F]fluorobenzoic acid solution was evaporated to dryness in a 1/2-dram glass vial and 3 μmol each of dicyclohexylcarbodiimide and N-hydroxysuccinimide in 30 μl of dry THF were added. After vortexing and incubation at 25°C for 30 min, [<sup>18</sup>F]SFB was purified by HPLC using a silica gel column eluted with hexane:ethyl acetate:acetic acid (70:30:0.12). The overall radiochemical yield was about 25% (decay corrected) with a total synthesis time of 100 min.

Labeling of Mel-14 F(ab')<sub>2</sub> was accomplished by addition of [<sup>18</sup>F]SFB to 200 μg of fragment (3 mg/ml) in pH 8.5 borate buffer. After a 15–20 min reaction, between 40% and 60% of the <sup>18</sup>F activity could be coupled to the protein. The <sup>18</sup>F-labeled Mel-14 F(ab')<sub>2</sub> was purified using a 1 × 10-cm Sephadex G-25 column. Specific activities ranged between 3.3 and 4.8 mCi/mg.

**Iodine-125 Labeling.** *N*-succinimidyl-4-(trimethylstannyl)benzoate was prepared from 4-bromobenzoic acid as described (18,21). From this precursor, *N*-succinimidyl 4-[<sup>125</sup>I]iodobenzoate ([<sup>125</sup>I]SIB) was prepared via electrophilic iododestannylation using *tert*-butylhydroperoxide as the oxidant (22). To 1–2 μl of sodium [<sup>125</sup>I]iodide in 0.1 N NaOH in a conical, glass vial were added twice the volume of 3% acetic acid in chloroform, 15 μl of *tert*-butylhydroperoxide and 500 nmole of the stannyl precursor in 5 μl of chloroform. After stirring for 30 min at 25°C, the [<sup>125</sup>I]SIB was isolated by HPLC. Procedures for labeling Mel-14 F(ab')<sub>2</sub> by reaction with [<sup>125</sup>I]SIB were identical to those described above for use with [<sup>18</sup>F]SFB. Specific activities for <sup>125</sup>I-labeled Mel-14 F(ab')<sub>2</sub> ranged between 1.8 and 2.5 mCi/mg.

### Evaluation of Immunoreactivity

A single-point specific binding assay was used to evaluate the <sup>18</sup>F- and <sup>125</sup>I-labeled Mel-14 F(ab')<sub>2</sub> fragments prior to initiation of the tissue distribution experiments. In this assay, approximately 100 ng of each labeled F(ab')<sub>2</sub> were incubated in triplicate for 1.5 hr at room temperature with homogenates of both D-54 MG human glioma and antigen-negative normal rat liver. The homogenates were washed three times with phosphate-buffered saline containing 1% human serum albumin. The combined supernatants and pellets were counted for <sup>18</sup>F and <sup>125</sup>I activity using an LKB 1282 automated gamma counter. A correction was

applied for spillover of <sup>18</sup>F into the <sup>125</sup>I counting window. Specific binding was calculated by subtracting the percent activity bound to liver from the percent activity binding to tumor.

Binding affinity of <sup>18</sup>F- and <sup>125</sup>I-labeled Mel-14 F(ab')<sub>2</sub> was determined using the high antigen-expressing human glioma D-247 MG line, and the antigen-negative U-373 MG line was used to measure nonspecific binding. Cells (1 × 10<sup>5</sup>) from each line were fixed with 0.25% glutaraldehyde in 48-well plates. Serial dilution (1–1000 ng) of each labeled F(ab')<sub>2</sub> fragment were added in quadruplicate and incubated at 4°C for 2 hr. The plates were washed, and bound activity was determined using a gamma counter. Scatchard analyses of the binding data were performed using a computer program.

### Tissue Distribution Studies

Athymic mice (nu/nu, BALB/c) weighing 20–25 g were obtained from a closed colony maintained at the Duke University Cancer Center Isolation Facility. Animals were injected subcutaneously in the right flank with 50 μl of D-54 MG human glioma tumor homogenate. Biodistribution studies were begun 7 to 9 days later. Two experiments were performed using the same protocol but with animals having different tumor sizes. In the first experiment, mice were injected in the tail vein with 3.5 μg (11 μCi) of <sup>18</sup>F-labeled Mel-14 F(ab')<sub>2</sub> and 2.8 μg (5 μCi) of <sup>125</sup>I-labeled F(ab')<sub>2</sub>. In the second experiment, animals received 2.5 μg (12 μCi) of <sup>18</sup>F-labeled Mel-14 F(ab')<sub>2</sub> and 2.5 μg (5 μCi) of <sup>125</sup>I-labeled Mel-14 F(ab')<sub>2</sub>. Groups of five animals were euthanized by ether overdose at 1, 2, 4 and 6 hr after injection and then dissected. Tissues of interest were removed, washed with saline, weighed and then counted for <sup>18</sup>F and <sup>125</sup>I activity. The percent injected dose (%ID) localized in each tissue was calculated by comparison to injection standards of appropriate count rate.

### Radiation Dosimetry

Calculations were performed for a hypothetical 10-mCi dose of <sup>18</sup>F-labeled Mel-14 F(ab')<sub>2</sub> and were carried out by using the standard MIRD method (23). Cumulative activities were calculated from the biodistribution data by determining the effective half-life of <sup>18</sup>F activity in each tissue; these values were multiplied by the corresponding S factors to yield the radiation-absorbed dose for each source-target organ pair. Tissues localizing more than 5% of the injected dose were considered as source organs with the remainder attributed to whole-body activity.

## RESULTS

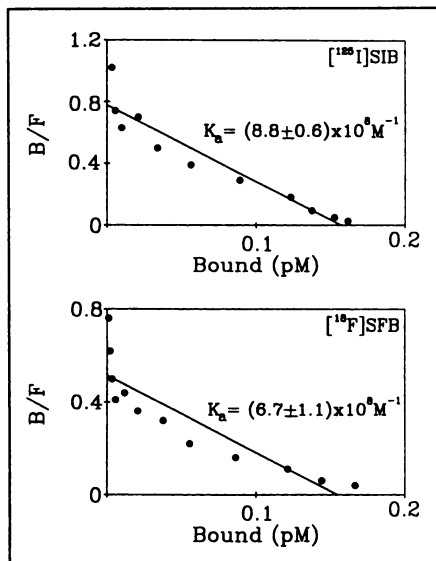
The F(ab')<sub>2</sub> fragment of Mel-14 was labeled by reaction with [<sup>18</sup>F]SFB and its in-vitro and in-vivo properties compared in paired-label format with those of Mel-14 F(ab')<sub>2</sub> labeled with [<sup>125</sup>I]SIB. A single-point homogenate assay was performed to evaluate the specific binding of the labeled F(ab')<sub>2</sub> fragments to human glioma homogenates. The specific binding values for the preparations used in the first biodistribution experiment were 61% ± 7% and 65% ± 7% for <sup>18</sup>F- and <sup>125</sup>I-labeled Mel-14 F(ab')<sub>2</sub>, respectively. For the second tissue distribution study, the specific binding percentages were 49% ± 3% for <sup>18</sup>F-labeled versus 65% ± 2% for <sup>125</sup>I-labeled Mel-14 F(ab')<sub>2</sub>. The mean specific binding for the last five preparations of the <sup>18</sup>F-labeled fragment was 58% ± 3%, a value slightly lower than that observed for Mel-14 F(ab')<sub>2</sub> labeled using [<sup>125</sup>I]

SIB ( $62\% \pm 2\%$ ). As shown in Figure 1, Scatchard analysis of the binding data for the preparation used in the second biodistribution study revealed that the affinity constant for Mel-14 F(ab')<sub>2</sub> labeled with <sup>18</sup>F, ( $6.7 \pm 1.1$ )  $\times 10^8$  M<sup>-1</sup>, was slightly lower than that of Mel-14 F(ab')<sub>2</sub> labeled with <sup>125</sup>I, ( $8.8 \pm 0.6$ )  $\times 10^8$  M<sup>-1</sup>.

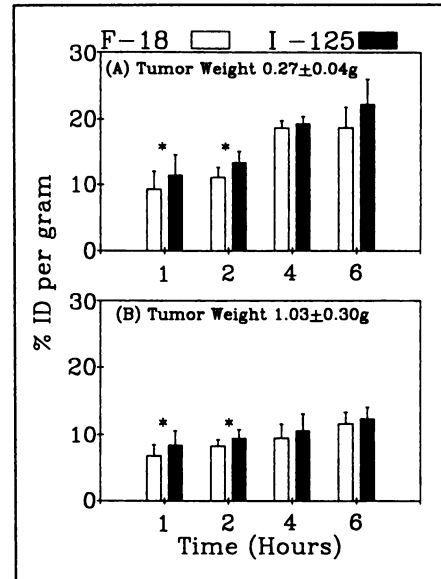
Paired-label tissue distribution studies were performed in athymic mice bearing subcutaneous D-54 MG xenografts to compare the tumor selectivity of Mel-14 F(ab')<sub>2</sub> labeled with [<sup>18</sup>F]SFB with the selectivity of the fragment labeled with [<sup>125</sup>I]SIB. The mean tumor weight in the first experiment was  $0.27 \pm 0.04$  g and was  $1.03 \pm 0.30$  g in the second experiment. In both studies, tumor uptake of <sup>18</sup>F and <sup>125</sup>I increased with time; however, tumor accumulation after one half-life of <sup>18</sup>F was between 59% and 77% of the levels observed at 6 hr (Fig. 2). For example, in the second experiment, tumor uptake of <sup>18</sup>F increased from  $8.2\% \pm 1.0$  %ID/g at 2 hr to  $11.6 \pm 1.7$  %ID/g at 6 hr.

With the smaller tumors, accumulation of <sup>18</sup>F reached  $18.8 \pm 3.0$  %ID/g at 6 hr compared with  $22.3 \pm 3.7$  %ID/g for <sup>125</sup>I-labeled Mel-14 F(ab')<sub>2</sub>. Tumor uptake of <sup>18</sup>F was 3%–19% lower than that of <sup>125</sup>I; these differences were considered to be significant by paired t-test at 1 and 2 hr ( $p < 0.05$ ). Similar behavior was observed in the experiment performed with the larger tumors except that the %ID/g tumor localization was lower at all time points. For example, at 6 hr, uptake of <sup>18</sup>F in tumor was  $11.6 \pm 1.7$  %ID/g compared with  $12.4 \pm 1.7$  %ID/g for radioiodinated Mel-14 F(ab')<sub>2</sub> (difference not significant). Tumor localization of <sup>18</sup>F was 6–19% lower than that of <sup>125</sup>I with these differences considered to be significant at 1 and 2 hr ( $p < 0.05$ ).

The distribution of <sup>18</sup>F activity in normal tissues following the injection of <sup>18</sup>F-labeled Mel-14 F(ab')<sub>2</sub> was similar in both studies, and the results observed in the second experiment are presented in Table 1. Highest concentra-



**FIGURE 1.** Determination of the affinity constant ( $K_a$ ) of Mel-14 F(ab')<sub>2</sub> after labeling with <sup>18</sup>F and <sup>125</sup>I.



**FIGURE 2.** Paired-label localization of <sup>18</sup>F- and <sup>125</sup>I-labeled Mel-14 F(ab')<sub>2</sub> in D-54 MG subcutaneous human glioma xenografts implanted in athymic mice. Differences considered to be statistically significant ( $p < 0.05$ ) are indicated (\*).

tions of activity were seen in blood and lungs, with <sup>18</sup>F levels declining in these tissues to about half of initial levels by 4 hr. The tissue distribution data were also calculated as %ID/g per organ and used to estimate normal tissue dosimetry for a hypothetical 10-mCi dose of <sup>18</sup>F-labeled Mel-14 F(ab')<sub>2</sub>. As shown in Table 2, the radiation-absorbed dose to the kidneys was predicted to be highest at about 6 rad with spleen next at 0.82 rad. Doses of less than 0.5 rad were calculated for liver, lung and whole body.

The selectivity of tumor uptake of radiolabeled Mel-14 F(ab')<sub>2</sub> fragments was evaluated by calculating tumor:normal tissue uptake ratios. The results from the first experiment are summarized in Figure 3. In general, tumor-to-tissue ratios for both <sup>18</sup>F and <sup>125</sup>I increased with time and, with the exception of the blood and kidneys, values greater than 2:1 were seen by 4 hr after injection. At 4 hr tumor-to-muscle ratios were 14:1 and tumor-to-brain ratios were 40:1 for both labeled Mel-14 F(ab')<sub>2</sub> fragments (data not shown). In most cases, tumor:normal tissue ratios for the two nuclides were quite similar. Exceptions were tumor-to-liver and tumor-to-intestine ratios at 2–6 hr, which were significantly higher ( $p < 0.05$ , paired t-test), and tumor-to-kidney ratios at 1–4 hr, which were significantly lower ( $p < 0.05$ ), for the <sup>18</sup>F-labeled fragment.

## DISCUSSION

One approach for improving radioimmunodiagnosis is to utilize more sophisticated imaging techniques. Because of the enhanced sensitivity and quantitative capabilities associated with PET, there has been a considerable interest in using this modality for radioimmunoscintigraphy and as a means for obtaining dosimetry data prior to radioimmunotherapy. One of the problems that must be overcome is the lack of an ideal positron-emitting nuclide for labeling Mabs. Longer lived nuclides such as <sup>89</sup>Zr and <sup>124</sup>I

**TABLE 1**  
Tissue Distribution of  $^{18}\text{F}$  in Athymic Mice Bearing D-54 MG Tumors Following Injection of Mel-14  $\text{F}(\text{ab}')_2$  Labeled with  $^{18}\text{F}$ SFB

Tissue	%ID/g*			
	1 hr	2 hr	4 hr	6 hr
Liver	6.82 ± 0.70	5.94 ± 0.85	5.02 ± 0.91	4.18 ± 0.20
Spleen	4.07 ± 0.61	3.37 ± 0.29	2.57 ± 0.50	2.29 ± 0.36
Lungs	20.80 ± 3.48	15.93 ± 3.87	9.48 ± 2.08	8.72 ± 2.47
Heart	6.64 ± 1.08	6.13 ± 0.86	4.17 ± 0.92	3.98 ± 0.93
Kidneys	8.93 ± 1.48	8.20 ± 1.19	6.02 ± 1.14	5.60 ± 0.68
Stomach	1.48 ± 0.24	1.73 ± 1.15	2.30 ± 0.42	1.14 ± 0.18
Small intestine	1.84 ± 0.32	2.06 ± 0.35	2.00 ± 0.30	1.53 ± 0.17
Large intestine	0.81 ± 0.15	1.67 ± 0.80	2.44 ± 0.28	1.43 ± 0.33
Thyroid	3.06 ± 0.46	2.40 ± 0.70	1.65 ± 0.80	2.06 ± 0.55
Muscle	1.19 ± 0.18	1.24 ± 0.65	1.46 ± 0.48	1.21 ± 0.18
Bone	3.15 ± 0.70	2.14 ± 0.29	1.58 ± 0.29	1.62 ± 0.37
Blood	31.89 ± 0.11	28.15 ± 2.56	18.55 ± 2.91	16.46 ± 1.74
Brain	0.70 ± 0.11	0.63 ± 0.10	0.47 ± 0.09	0.35 ± 0.06
Tumor	6.75 ± 1.69	8.22 ± 1.01	9.48 ± 2.08	11.62 ± 1.74

\* Mean ± s.d. for five animals per group.

(9-11) are well-matched with the relatively slow pharmacokinetics of Mabs; however, emission of high-energy gamma rays, low positron abundance and limited availability detract from the utility of  $^{89}\text{Zr}$  and  $^{124}\text{I}$  for PET.

In the current study, we have investigated an alternative strategy—labeling a Mab fragment with  $^{18}\text{F}$ . This nuclide is routinely available and has excellent decay properties for PET imaging. The question that must be resolved is whether sufficient tumor uptake and target-to-normal tissue ratios can be achieved within a time frame compatible with the half-life of  $^{18}\text{F}$ . Although several groups, including our own, have reported methods for labeling Mabs with  $^{18}\text{F}$  (12-17), only limited data are available concerning the in-vitro binding specificity and the in-vivo tumor localizing capability of  $^{18}\text{F}$ -labeled Mabs or fragments. Herein, we have investigated these properties using Mel-14  $\text{F}(\text{ab}')_2$  labeled with  $^{18}\text{F}$ SFB in athymic mice bearing subcutaneous human glioma xenografts.

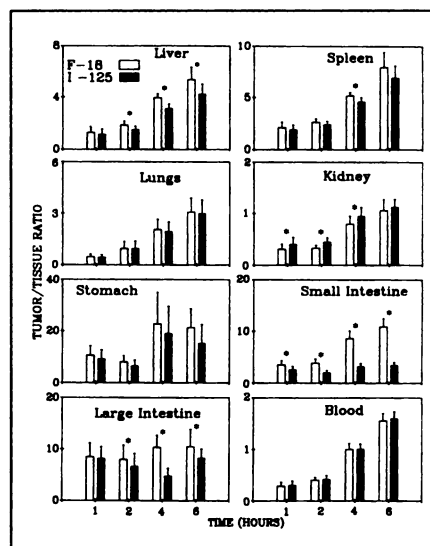
Selection of the  $^{18}\text{F}$ SFB method was predicated on several factors. Unlike several other methods (13), reasonable yields can be achieved in about 2 hr total synthesis time and without exposure of the Mab for extended periods of time to an elevated temperature (47°C). The latter may have an adverse effect on Mab immunoreactivity; how-

ever, the magnitude of this potential problem cannot be ascertained since the in-vitro binding properties of Mabs labeled with methyl 3- $^{18}\text{F}$ fluoro-5-nitrobenzimidate and 4- $^{18}\text{F}$ fluorophenacyl bromide have not been reported.

An additional advantage of the  $^{18}\text{F}$ SFB method is that the Mab fragment could be labeled with retention of immunoreactivity at specific activities that should be adequate for clinical studies. In contrast, with other  $^{18}\text{F}$  protein acylation agents labeled by isotopic exchange, such as 2, 3, 5, 6-tetra-fluorophenyl-pentafluorobenzoate (17), it is unlikely that Mabs could be labeled with adequate specific activity and immunoreactivity because of the presence of a large excess of carrier. Based on clinical PET studies with  $^{18}\text{F}$ FDG (24) and the dosimetry estimates performed in this work, an injected dose of  $^{18}\text{F}$ -labeled  $\text{F}(\text{ab}')_2$  of about 10 mCi would be anticipated. In a pre-

**TABLE 2**  
Radiation Absorbed Dose Estimated for a Hypothetical Dose of 10 mCi  $^{18}\text{F}$ -Labeled Mel-14  $\text{F}(\text{ab}')_2$

Tissue	Absorbed Dose (rad)
Liver	0.33
Kidney	5.99
Lung	0.19
Spleen	0.78
Blood (whole body)	0.24



**FIGURE 3.** Tumor-to-normal tissue uptake ratios for  $^{18}\text{F}$ - and  $^{125}\text{I}$ -labeled Mel-14  $\text{F}(\text{ab}')_2$  in an athymic mouse xenograft model. Differences considered to be statistically significant ( $p < 0.05$ ) are indicated (\*).

vious report, gliomas were imaged in patients receiving radioiodinated Mel-14 F(ab')<sub>2</sub> at doses of 5–20 mg (25). This suggests that a specific activity of 0.5–2 mCi/mg of <sup>18</sup>F-labeled Mel-14 F(ab')<sub>2</sub> would be required, a level well below that already achieved in this study (3.3–4.8 mCi/mg) using [<sup>18</sup>F]SFB.

An additional reason for utilizing [<sup>18</sup>F]SFB for labeling the Mab fragment is that this method results in the formation of 4-[<sup>18</sup>F]fluorobenzoyl protein conjugates. Previous studies with Mabs labeled with other halogens using the same strategy have suggested that this general approach offers certain advantages. For example, by using an 81C6 Mab 3-[<sup>131</sup>I]iodobenzoyl conjugate, we have demonstrated increased in-vivo retention of label on the Mab and higher tumor uptake in comparison to the same Mab labeled with a conventional method (26). In addition, by using Mel-14 F(ab')<sub>2</sub> labeled with the alpha-emitting halogen <sup>211</sup>At in an analogous fashion, immunoreactivity retention and selective tumor uptake was demonstrated (27). In the present study, the 4-[<sup>18</sup>F]fluorobenzoate *N*-succinimidyl ester was used instead of the *meta*-substituted analog of the <sup>131</sup>I-labeled and <sup>211</sup>At-labeled compounds noted above because of the greater ease of the fluorodenitration reaction of *para*-substituted compounds (28). Although our selection of the *meta* isomers was based on a slightly lower degree of dehalogenation (29), Wilbur has reported that 4-[<sup>131</sup>I]iodobenzoyl Mab conjugates are also relatively inert to dehalogenation (30) and that tumor localization could also be achieved using 4-[<sup>211</sup>At]astatobenzoyl Mab fragment conjugates (31).

Although the synthetic scheme for the <sup>18</sup>F-labeled *N*-succinimidyl ester is different than that of the iodo and astato analogs, a common feature of these approaches is that they avoid exposure of the Mab to oxidants and reductants, possibly helping maintain immunoreactivity. Because of the short half-life of <sup>18</sup>F, a simple, single-point binding assay was performed as an initial measure of immunoreactivity after labeling. In all but one experiment, the specific binding to human glioma homogenates of <sup>18</sup>F-labeled Mel-14 F(ab')<sub>2</sub> was identical to that of fragment labeled by reaction with [<sup>125</sup>I]SIB. The results for both nuclides were in excellent agreement with those reported previously for Mel-14 F(ab')<sub>2</sub> [<sup>131</sup>I]iodobenzoyl and [<sup>211</sup>At]astatobenzoyl conjugates (28) and higher than those achieved with direct, electrophilic iodination (20). In addition, the affinity constant measured for <sup>18</sup>F-labeled Mel-14 F(ab')<sub>2</sub> was comparable to that determined in parallel for the F(ab')<sub>2</sub> labeled using [<sup>125</sup>I]SIB, suggesting that this Mab fragment could be labeled by reaction with [<sup>18</sup>F]SFB with good retention of immunoreactivity.

The biodistribution of <sup>18</sup>F-labeled Mel-14 F(ab')<sub>2</sub> was compared to coinjected F(ab')<sub>2</sub> labeled with [<sup>125</sup>I]SIB over 1–6 hr to determine whether selective uptake of <sup>18</sup>F could be achieved within a reasonably short time period. In addition, the potential for using <sup>18</sup>F-labeled F(ab')<sub>2</sub> for predicting the dosimetry of a therapeutic nuclide, <sup>131</sup>I,

could be ascertained directly. However, before attempting this two-step strategy in patients, potential complications related to the formation of a human antimouse response (HAMA) must be considered. HAMA can be minimized by a number of approaches, including the use of Mab fragments, human/chimeric Mabs and giving the second injection of Mab only a few days following the first (32).

In two separate experiments, rapid uptake of <sup>18</sup>F in subcutaneous human glioma xenografts was seen as early as 2 hr, with levels increasing gradually until 6 hr after injection. These results are in agreement with previous studies with Mel-14 F(ab')<sub>2</sub> indicating nearly constant, tumor levels over 4–24 hr, with a gradual decrease thereafter (20,26). In both experiments, tumor uptake of <sup>18</sup>F was only 3%–19% lower than that observed for co-injected <sup>125</sup>I-labeled Mel-14 F(ab')<sub>2</sub>. This might reflect the slightly lower immunoreactivity measured for the <sup>18</sup>F-labeled F(ab')<sub>2</sub> fragment.

There was a significant difference in the percent injected dose per gram of tumor uptake of both nuclides between the two experiments. With <sup>18</sup>F, it might be possible to attribute this observation to differences in immunoreactivity; this is not the likely explanation, since the specific binding of the <sup>125</sup>I-labeled fragment was nearly identical in both studies. The most likely cause for this up to two-fold difference in tumor accumulation is the nearly fourfold difference in mean tumor weight between the two studies. Shah et al. (33) have shown that accumulation of radioiodinated Mab B72.3 decreased significantly with increasing tumor weight, presumably due to permeability differences related to necrosis. When the %ID/g tumor for each animal was plotted *versus* tumor weight for both nuclides, a good correlation was seen ( $r = 0.93$  for both experiments, data not shown), supporting the explanation that differences in tumor uptake between the two experiments were due to factors associated with tumor size.

If PET imaging with <sup>18</sup>F-labeled Mab fragments is to be feasible, favorable tumor-to-normal-tissue ratios must be achievable within a relatively short time after injection. As expected, contrast between tumor and normal tissues increased with time. High levels of activity were persistent in the kidney, a characteristic that has been observed previously with F(ab')<sub>2</sub> fragments (18,27,30). With the exception of the blood and kidney, tumor-to-normal-tissue ratios greater than 2:1 were seen in all tissues by 4 hr and ratios greater than 10:1 were seen in the intestines, stomach, muscle and brain at this time. It is interesting to note that the tumor-to-intestine ratios for <sup>18</sup>F-labeled Mel-14 F(ab')<sub>2</sub> were higher and tumor-to-kidney ratios were slightly lower than those of co-administered radioiodinated F(ab')<sub>2</sub>. A possible explanation would be the difference in lipophilicity of [<sup>18</sup>F]fluorobenzoyl and [<sup>125</sup>I]iodobenzoyl catabolites, and studies are in progress to address this question. Because of the imaging advantages inherent in PET, including its tomographic nature, target-to-nontarget ratios need not be as high as those required using conven-

tional gamma camera imaging. Although it is recognized that extrapolation of animal data to humans is often misleading, these results suggest that detection of tumors might be feasible at many anatomic locations using  $^{18}\text{F}$ -labeled Mab fragments.

One advantage of using  $^{18}\text{F}$  for labeling Mabs and fragments is that, despite its longer half-life, radiation doses should be lower than those encountered if 68-min  $^{68}\text{Ga}$  is used as the label (13). Dosimetry calculations were performed using these biodistribution data in anticipation of potential clinical studies with  $^{18}\text{F}$ -labeled Mel-14 F(ab')<sub>2</sub>. Bladder doses were not calculated because of difficulties in obtaining realistic cumulative activity values in the athymic mouse model. With  $^{18}\text{F}$ -labeled Mel-14 F(ab')<sub>2</sub>, kidneys received the highest dose, about 0.6 rad/mCi, a value about sevenfold lower than that reported for [ $^{18}\text{F}$ ]FDG (24). As a further comparison, the kidney dose calculated from clinical studies with 6-[ $^{18}\text{F}$ ]fluorodopamine was 0.9 rad/mCi (34), a value 50% higher than that calculated for  $^{18}\text{F}$ -labeled Mel-14 F(ab')<sub>2</sub>. For other tissues, radiation-absorbed doses were similar or less than those reported for other  $^{18}\text{F}$ -labeled tracers that have been administered to patients (24,34); however, since the results for  $^{18}\text{F}$ -labeled Mel-14 F(ab')<sub>2</sub> were obtained in mice, they should be considered as only rough estimates of those expected in patients. More comprehensive dosimetric evaluation of  $^{18}\text{F}$ -labeled Mab fragments will be performed in dogs. These animals will be catheterized so that estimation of bladder dose will be possible.

In summary, a Mab F(ab')<sub>2</sub> fragment could be labeled with  $^{18}\text{F}$  using the [ $^{18}\text{F}$ ]SFB method with retention of in-vitro binding specificity and affinity. Selective uptake of  $^{18}\text{F}$ -labeled Mel-14 F(ab')<sub>2</sub> in a human tumor xenograft in athymic mice could be achieved in a time frame compatible with the relatively short half-life of this nuclide. Although preliminary in nature, these results suggest that PET imaging of tumors using [ $^{18}\text{F}$ ]fluorobenzoyl Mab fragment conjugates may be possible.

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## SELF-STUDY TEST

# Radiobiology and Radiation Protection

### ANSWERS

(0.05 Sv/year) limit. ICRP 26 recommends that both internal and external doses be included in dose estimates. Although this seems a straightforward requirement, internal dosimetry is still more an art than a science, and there is substantial controversy within the health physics community about how the internal dose should be handled in the case of long-lived radionuclides that also have long biological half-lives.

The de minimis concept was included in the proposed revisions to Part 20 of Title 10, Code of Federal Regulations, but there was no discussion of a numerical definition of de minimis occupational exposure in ICRP 26.

#### ITEMS 18-20: Thyroid Blocking of Populations

ANSWERS: 18, F; 19, T; 20, F

After the accident at Chernobyl in April 1986, the Russian authorities administered KI to many persons (thought to be 100,000). In the U.S., there is no national stockpile of KI and few states have emergency stores of KI. Guidelines for administration of KI to members of the general public have been developed by the FDA, the NCRP, and the American Thyroid Association. Each has come up with different projected "action" thresholds, i.e., the expected thyroidal doses at which general population "blocking" should be considered; these range from 5-25 rems to up to 100 rems anticipated doses to the thyroid.

In the event of an accident, it is the relevant local health authority that has the responsibility for making recommendations to its population, although the FDA and the Federal Emergency Management Agency (FEMA) would play supporting roles in the decision and distribution process. As in any other health question, the problem is one of balancing the benefits of diminished radiation dose to the thyroid if KI is administered early (ideally, before or within a few hours after significant environmental contamination by radioiodine) versus the probability of allergic or other adverse pharmacologic responses to KI. Because the radiation induction of thyroid cancer, on the one hand, and allergic events on the other are both rare events, the decision is not easy, and the public perception of risk needs to be taken into account.

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#### ITEMS 21-25: Medical Management of Patients Contaminated with Radionuclides

ANSWERS: 21, T; 22, F; 23, F; 24, F; 25, T

Although accidents involving radiation injury are rare events, the Joint Commission on the Accreditation of Healthcare Organizations (JCAHO) has required, since February 1978, that all hospitals have a procedure for handling these emergencies. The Joint Commission's *Accreditation Manual for Hospitals* states:

*The hospital shall have written plans for the timely care of casualties arising from both external and internal disasters, and shall document the rehearsal of these plans.*

Rarely are injuries due to ionizing radiation life threatening, either to the victim or to the treating staff. The effects of these injuries become manifest over a period of time (as a function of dose, dose rate, and portion of the body irradiated) and, except for agents to block organ uptake (iodide) or to remove internally deposited radionuclides (chelators), there is no treatment that can interrupt this injury process.

When the ambulance arrives, the driver and other personnel should remain with the vehicle until they have been monitored for contamination. Information as to the nature of the accident, the type of contamination, and any prior attempts at decontamination should be obtained from those who accompany the patient. The patient's airway, breathing, and circulation should be checked immediately. If critically injured, the patient should be taken directly to the radiation emergency area of the emergency room with attention to avoidance of gross contamination of the emergency room. When the injuries are not critical or life-threatening, the patient should be surveyed in or near the ambulance. Contaminated clothing should be removed near the vehicle, tagged, and retained for a later survey.

The external contamination by beta and gamma emitters should be measured over the whole body. Next, one should determine if wounds are contaminated by direct measurements over them. In addition, swab samples of the body orifices should be taken, always before the patient is washed and showered. If internal contamination is suspected, all urine, feces, vomitus, and wound secretions should be collected for radioassay. Surgical clothing (i.e., scrub suits, gowns, mask, and 2 pairs of gloves) and waterproof shoe covers are reasonable protective measures for medical personnel. Those using decontamination liquids should wear waterproof aprons as well.

To establish a baseline for evidence of radiation injury, a complete blood count, including platelets and white cell differential count, should be performed immediately; counts should be repeated at 12- to 24-hr intervals if indicated by the rate of change in the absolute lymphocyte count. The determination of the absolute lymphocyte count is essential, because circulating lymphocytes are extremely radiosensitive and the decline in their numbers furnishes the earliest and most accurate indication of radiation injury.

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Note: For further in-depth information, please refer to the syllabus pages included at the beginning of *Nuclear Medicine Self-Study Program I: Part I*.