In Vivo Comparison of PET and SPECT Radiopharmaceuticals in Detecting Breast Cancer

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Various radiopharmaceuticals for breast cancer detection have been used for scintimammography and PET. However, few comparative studies have described the uptake of radiopharmaceuticals as a method of detecting breast cancer. The aim of this study was to assess the radiopharmaceuticals for breast cancer imaging in experimental mice implanted with breast cancer cells. **Methods:** Six radiopharmaceuticals were studied: three for PET [^{18}F-fluorodeoxyglucose (FDG), L-^{18}F-alpha-methyltyrosine (FMT) and ^{11}C-methionine (C-Met)] and three for scintimammography [^{99m}Tc-tetrofosmin (TF), ^{99m}Tc-sestamibi (MIBI) and ^{201}Tl-chloride (Tl)]. Biodistributions of six different tracers in mice implanted with MCF-7 breast cancer cells were studied 1 and 3 hr after injection. **Results:** Tumor uptake 1 hr after injection was FMT = C-Met > FDG = TF > MIBI = Tl. Thallium-201-chloride showed the highest tumor-to-blood ratio (T/B) among all radiopharmaceuticals because of its fast clearance from circulation. The T/B of the six radionuclides used in this study ranged from 1.26 for C-Met to 12.83 for Tl. Tumor-to-muscle ratio (T/M) revealed FMT = C-Met > FDG > MIBI > TF = Tl. The T/M ranged from 0.20 for TF to 2.29 for FMT. Tumor-to-lung ratio (T/L) varied from 0.45 for TF to 2.41 for FMT. FMT revealed the highest T/L of all six radiopharmaceuticals. **Conclusion:** Among radiopharmaceuticals for PET, FMT seemed to be suitable in detecting MCF-7 tumor; whereas for scintimammography, MIBI, TF and Tl appeared to have almost the same detectability of MCF-7 tumor. The results of this study strongly suggest that FMT may have a potential in breast cancer imaging.

Key Words: radiopharmaceuticals; breast cancer; L-^{18}F-alpha methyltyrosine; PET; SPECT

J Nucl Med 1998; 39:1424–1427

Mammography, ultrasonography and MRI are useful imaging techniques in detecting breast cancer. Each modality has different characteristics and disadvantages. For example, mammography is reported to have poor diagnostic sensitivity in patients with dense breast tissue (*1*). On ultrasonography, it is difficult to distinguish the difference between malignant and benign breast tumors (*2*). The sensitivity of MRI is almost 90%, however, the specificity of 70% is poor (*3,4*). Nuclear medicine images such as scintimammography and PET are expected to provide additional diagnostic information. Until now, various radiopharmaceuticals were used in detecting breast cancer with PET and SPECT. Fluorine-18-fluorodeoxyglucose (FDG) (*5,6*) and ^{11}C-methionine (C-Met) (*7,8*) have been widely used as tumor-seeking agents with PET, including breast cancer. For scintimammography, ^{99m}Tc-hexakis-isobutyl isonitrile (MIBI) is the most popular agent (*9–11*), but ^{201}Tl and ^{99m}Tc-ethylene-bis[bis(2-ethoxyethyl)]phosphin [^{99m}Tc-tetrofosmin (TF)] (*12,13*) are also used. Until now, few comparative studies have been performed that demonstrate the uptake of radiopharmaceuticals as candidates for detection of breast cancer (*14*). The aim of this study was to assess the tumor uptake of radiopharmaceuticals using experimental mice implanted with breast cancer cells.

Received Aug. 26, 1997; revision accepted Oct. 21, 1997.
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MATERIALS AND METHODS

Mice and Tumors

Five BALB/c(nu/nu) female nude mice, bred and maintained in a pathogen-free mouse colony in the Institute of Experimental Animal Research, Gunma University, Gunma, Japan, were used. They were 6 wk old at the time of the inoculation. The tumors used in this study were human breast carcinoma cells designated as MCF-7. Tumors were generated subcutaneously in the posterior region of the mice by the inoculation of 2×10^6 viable tumor cells. A 60-day release pellet containing 0.72 mg 17 β-estradiol (Innovate Research of America, Toledo, OH) was implanted subcutaneously in each mouse. When tumors grew to 10 mm in diameter, biodistribution studies were conducted.

Preparations of Radiopharmaceuticals

Radioactive fluorine [^{18}F] F_2 was produced by the ^{20}Ne (d, α) ^{18}F nuclear reaction with a neon/0.1% F_2 gas mixture with the BC 1720 cyclotron (Japan Steel Works, Tokyo, Japan). [^{18}F] F_2 gas was converted to ^{18}F-acetylhypofluorite ($CH_3COO^{18}F$) by being passed through the column CH_2COOK/CH_3COOH. An automated system was used to label 2-Deoxy-D-glucose with ^{18}F to produce FDG. To produce L-^{18}F-alpha-methyltyrosine (FMT), $CH_3COO^{18}F$ was bubbled into the solution of 10 mg L-alpha-methyltyrosine dissolved in 0.5 ml trifluoroacetic acid cooled by ice water. After the fluorination reaction, the solvent was vaporized and 1.0 ml water was added to dissolve the reactants. The reactants were introduced into a u-Bonda C-18 Guard pack column and then into a Lichrosorb RP-18 column (Gel Science Inc., Tokyo, Japan) to separate the labeled FMT from the free ^{18}F and unreacted FMT. We used ultraviolet signals (SIC Chromatocorder 12; SIC, Tokyo, Japan) to change the high-performance liquid chromatography flow containing L-^{18}F-alpha-methyltyrosine to the flask connected to a vacuumed rotary evaporator. After the FMT was separated into the flask connected to the rotary evaporator, the solvent was evaporated under reduced pressure and dried in a 100°C water bath. The dried reactants were dissolved in 5 ml saline and adjusted to pH 6 with $NaHCO_3$ buffer. The solution was filtered by millipore (0.22 μm) into a 5-ml sterile vial (*15*). The $^{11}CO_2$ was produced by a ^{14}N(p, α) ^{11}C reaction and then trapped with liquid nitrogen. Through a series of chemical reactions, $^{11}CO_2$ was converted to methyliodine, $^{11}CH_3I$ and then reacted with homocystein to produce C-Met (*16*). The TF was prepared from a freeze-dried kit (Nihon Medi-Physics, Tokyo, Japan) by reconstitution with 1 ml sterile sodium pertechnetate solution containing 3.7 MBq ^{99m}Tc. The vial was shaken and then stood at room temperature for 20 min. The MIBI also was prepared from a freeze-dried kit (provided by Daiichi Radioisotope Laboratories, Tokyo, Japan) and labeled according to the kit instructions.

TABLE 1
Biodistribution of PET Radiopharmaceuticals in Mice Implanted with MCF-7 Breast Cancer Cells

Organ	FDG		FMT		C-Met
	1 hr	3 hr	1 hr	3 hr	1 hr
Blood	0.13 ± 0.02	0.10 ± 0.02	0.98 ± 0.09	0.49 ± 0.04	2.73 ± 1.11
Heart	2.36 ± 0.60	3.36 ± 0.68	1.65 ± 0.14	1.21 ± 0.11	3.80 ± 1.19
Liver	0.21 ± 0.01	0.24 ± 0.04	1.48 ± 0.27	0.63 ± 0.07	8.02 ± 2.66
Kidney	0.27 ± 0.02	0.28 ± 0.06	16.31 ± 1.63	5.00 ± 0.67	7.60 ± 2.23
Spleen	0.36 ± 0.03	0.51 ± 0.08	2.23 ± 0.42	1.37 ± 0.18	7.36 ± 2.40
Stomach	0.42 ± 0.02	0.60 ± 0.13	2.74 ± 0.41	1.55 ± 0.22	6.75 ± 2.23
Intestine	0.45 ± 0.09	0.62 ± 0.16	1.11 ± 0.12	0.82 ± 0.08	5.04 ± 1.39
Lung	0.20 ± 0.02	0.65 ± 0.13	1.51 ± 0.20	1.29 ± 0.15	3.32 ± 0.91
Bone	0.54 ± 0.07	1.03 ± 0.25	1.29 ± 0.15	2.08 ± 0.42	1.88 ± 0.56
Muscle	0.85 ± 0.10	0.35 ± 0.35	1.67 ± 0.13	1.25 ± 0.08	1.56 ± 0.45
Tumor	0.51 ± 0.03	0.62 ± 0.11	3.43 ± 0.19	1.50 ± 0.15	2.60 ± 0.41

Data are expressed as percentage of injected dose per gram of tissue in five nude mice. Errors are s.e.m. FDG = ^{18}F-fluorodeoxyglucose; FMT = L-^{18}F-alpha-methyltyrosine; C-Met = ^{11}C-methionine.

Animal Experiments

A 100-μl solution of radionuclides containing 0.37 MBq positron emitter and 0.37 MBq conventional radionuclides for SPECT was injected intravenously into the tail vein of the mice. The combinations of positron emitters and radionuclides were as follows: TF and FMT, MIBI and FDG and C-Met and Tl. Blood samples were taken 1 and 3 hr after injection of radiotracer. After blood sampling, the mice were killed; tissues were weighed and measured for radioactivity in a gamma well counter. Radioactive measurement of the tissue samples was conducted 1 hr after the mice were killed for positron emitters and repeated for SPECT radiopharmaceuticals at 21 hr. Radionuclide accumulation in the tissue was calculated as percentage of injected dose per gram (%ID/g) of tissue: percentage of injected dose per gram = radioactivity in 1 g of tissue/total injected dose × 100. Based on the results of %ID/g in tissue and tumor, tumor-to-blood ratio (T/B), tumor-to-muscle ratio (T/M) and tumor-to-lung ratio (T/L) were also calculated.

Statistical Analysis

Differences of mean %ID/g tracer uptake between 1 and 3 hr, and among organs observed in this study, were estimated statistically by Mann-Whitney test of nonparametric analysis and unpaired Student's t-test. A significant difference was defined as $p < 0.05$.

RESULTS

In FDG biodistribution studies, heart uptake was the highest among all organs. Tumor FDG uptake was 0.51 %ID/g 1 hr after injection and 0.62 %ID/g 3 hr after injection (Table 1). Blood radioactivity was the lowest among all tissues examined.

In FMT biodistribution studies, renal uptake was the highest with 16.3 and 5.00 %ID/g 1 and 3 hr after injection, respectively. Tumor FMT uptake 1 hr after injection was 3.43 %ID/g and decreased to 1.50 3 hr after injection. Stomach and spleen revealed uptake relatively as high as tumor and also showed reduction of FMT uptake 3 hr after injection. Blood and muscle uptakes were 0.98 and 1.67 %ID/g, respectively, which were significantly higher than those of FDG.

In C-Met biodistribution studies, liver uptake was the highest among all organs. Tumor, muscle and blood C-Met uptakes 1 hr after injection were 2.60, 1.56 and 2.73 %ID/g, respectively. Tumor C-Met uptake was almost the same as tumor FMT uptake and was significantly higher than tumor FDG uptake.

In TF biodistribution studies, uptake in the intestines was the highest (Table 2). Tumor TF uptake was 0.63 %ID/g 1 hr after injection and 0.44 %ID/g 3 hr after injection. No significant reduction was observed. Blood and muscle uptakes were 0.25 and 3.29 %ID/g, respectively.

Results of MIBI studies showed that the highest MIBI uptake

TABLE 2
Biodistribution of SPECT Radiopharmaceuticals in Mice Implanted with MCF-7 Breast Cancer Cells

Organ	TF		MIBI		Tl	
	1 hr	3 hr	1 hr	3 hr	1 hr	3 hr
Blood	0.25 ± 0.12	0.26 ± 0.10	0.13 ± 0.07	0.09 ± 0.05	0.03 ± 0.01	0.04 ± 0.00
Heart	14.22 ± 3.10	6.79 ± 1.41	2.55 ± 0.44	4.37 ± 0.78	0.21 ± 0.36	1.58 ± 0.15
Liver	2.81 ± 0.79	1.06 ± 0.31	3.57 ± 0.83	3.83 ± 0.99	0.59 ± 0.18	0.86 ± 0.19
Kidney	10.03 ± 1.69	4.85 ± 0.69	5.53 ± 0.91	4.29 ± 0.78	2.69 ± 0.64	6.34 ± 0.60
Spleen	0.57 ± 0.11	0.44 ± 0.05	0.38 ± 0.10	0.03 ± 0.06	0.56 ± 0.13	1.05 ± 0.17
Stomach	5.07 ± 0.92	6.40 ± 1.23	1.80 ± 0.31	2.50 ± 0.51	0.45 ± 0.19	1.08 ± 0.18
Intestine	33.64 ± 4.60	15.32 ± 3.47	3.18 ± 0.95	9.26 ± 2.34	0.85 ± 0.24	1.32 ± 0.16
Lung	1.47 ± 0.30	0.78 ± 0.64	0.39 ± 0.08	0.45 ± 0.09	0.56 ± 0.14	0.77 ± 0.07
Bone	2.38 ± 0.62	0.72 ± 0.63	0.44 ± 0.09	0.96 ± 0.28	0.44 ± 0.15	0.77 ± 0.08
Muscle	3.29 ± 0.59	2.30 ± 0.41	0.59 ± 0.12	1.02 ± 0.30	0.53 ± 0.16	0.92 ± 0.09
Tumor	0.63 ± 0.10	0.44 ± 0.05	0.26 ± 0.05	0.36 ± 0.10	0.30 ± 0.18	0.63 ± 0.05

Data are expressed as percentage of injected dose per gram of tissue in five nude mice. Errors are s.e.m. TF = ^{99m}Tc-tetrofosmin; MIBI = ^{99m}Tc-sestamibi; Tl = ^{201}Tl-chrolide.

TABLE 3
Comparison of Tumor-to-Blood, Tumor-to-Muscle and Tumor-to-Lung Ratios 1 Hr After Injection Among Six Radiopharmaceuticals

Ratio	FDG	FMT	C-Met	TF	MIBI	Tl
T/B	4.12 ± 0.62	3.60 ± 0.34	1.26 ± 0.26	3.34 ± 0.07	4.49 ± 1.31	12.83 ± 0.54
T/M	0.60 ± 0.07	2.29 ± 0.04	1.69 ± 0.13	0.20 ± 0.03	0.44 ± 0.26	0.54 ± 0.09
T/L	1.04 ± 0.10	2.41 ± 0.33	0.79 ± 0.07	0.45 ± 0.05	0.70 ± 0.08	0.52 ± 0.04

Errors are s.e.m. T/B = tumor-to-blood ratio; T/M = tumor-to-muscle ratio; T/L = tumor-to-lung ratio; FDG = ^{18}F-fluorodeoxyglucose; FMT = L-^{18}F-alpha-methyltyrosine; C-Met = ^{11}C-methionine; TF = ^{99m}Tc-tetrofosmin; MIBI = ^{99m}Tc-sestamibi; Tl = ^{201}Tl-chloride.

was observed in the kidneys (approximately 5 %ID/g). Tumor uptake was 0.26 and 0.36 %ID/g 1 and 3 hr after injection, respectively.

Liver and cardiac uptakes were relatively high (3.57 and 2.68 %ID/g 1 hr after injection, respectively). Muscular uptake of MIBI 1 hr after injection was < 1.0 %ID/g.

In Tl biodistribution studies, the kidneys had the highest accumulation of Tl 1 hr after injection, 2.69 %ID/g. Cardiac uptake was 1.21 %ID/g 1 hr after injection. Tumor Tl uptake was relatively low, 0.30 %ID/g 1 hr after injection, and increased to 0.63 %ID/g 3 hr after injection. Blood Tl radioactivity accumulation was 0.03 %ID/g 1 hr after injection, the lowest of all radionuclides used in this study.

Tumor uptakes of FMT and C-Met were significantly higher than tumor uptakes of other radionuclides. TF had almost the same tumor uptake as FDG, which was the highest of the three SPECT radiopharmaceuticals. Thus, tumor uptake can be represented as: FMT = C-Met > FDG = TF > MIBI = Tl. The T/B of the six radionuclides used in this study ranged from 0.87 for C-Met to 12.8 for Tl (Table 3). Tl showed the highest T/B of the six radiopharmaceuticals examined, and C-Met was the lowest. The T/M ranged from 0.20 for TF to 2.29 for FMT (Table 3). The T/M revealed FMT = C-Met > FDG > MIBI > TF = Tl. The T/L was also evaluated and varied from 0.45 for TF to 2.41 for FMT. FMT had the highest T/L of the radiopharmaceuticals.

DISCUSSION

The detectability of radiopharmaceuticals in finding breast cancer was assessed using MCF-7 human breast carcinoma cells implanted in mice. Wahl et al. (*17*) evaluated the tumor localization of FDG, C-Met, Tl and MIBI using Lewis rats bearing RMT rat breast carcinoma cells, and uptake of FDG was greater than that of other tracers. In this study, MCF-7 human breast carcinoma cells were used, and FMT and TF were added to the four radiopharmaceuticals previously studied. Among PET imaging radiopharmaceuticals, the tumor uptake of FMT, an aminoacid analog, was significantly greater than that of FDG. In studies of human patients with breast cancer, FDG is commonly used and demonstrates excellent sensitivity, more than 95% (*5,6*). C-Met PET has potential for evaluation of breast cancer (*7*). In our study of mice, tumor C-Met uptake was significantly higher than that of FDG. FMT is an aminoacid tracer, a new radiotracer developed in our institute, that has the potential to become a primary tumor-detecting agent because of its high tumor uptake and T/B and T/M.

Among SPECT agents, tumor TF uptake was higher than that of MIBI and Tl. In an in vitro study using the MCF-7 tumor cell line, tumor MIBI uptake was reported to be higher than that of TF (*14*). This finding was inconsistent with our results, which may be due to a different tumor biological environment such as vascularity and tumor bed between in vitro and in vivo studies. However, no significant difference in T/B as a factor of image contrast was demonstrated among radiopharmaceuticals except Tl, which quickly cleared from circulation. The T/M is another important factor in assessing image quality; it is an index that reflects the image contrast of the tumor to that of surrounding muscle tissue. Of the PET imaging radiopharmaceuticals, the T/M of FMT was significantly higher than that of the other two PET radiopharmaceuticals, indicating that FMT would be useful for imaging breast cancer. MIBI also showed a significantly greater T/M than the other two SPECT radiopharmaceuticals. If the T/M is low, radionuclide uptake by axillary metastatic lesions might be obscured by the surrounding muscle tissue, resulting in a low detectability for axillary lymph node metastasis. The ability to detect chest wall invasion also would be poor if the T/M was low, because the delineation of the tumor would be difficult.

The T/L, which contributes to image contrast, is also evaluated. Image contrast would be poor if lung uptake is high enough compared with that of the breast lesion. FMT showed the highest T/L of the radiopharmaceuticals. This suggests the potential of FMT to detect breast cancer. In biodistribution studies, cardiac uptake affects the detection of left primary breast lesions or metastatic lesions of the retroparasternal lymph node. Lower cardiac uptake would provide better detectability of these lesions. Cardiac uptake of FMT and C-Met was significantly lower than that of FDG and the three SPECT radiopharmaceuticals. Liver uptake of radiotracers may affect lesions located in the lower portion of the right breast. Metastatic liver tumors of breast cancer are also difficult to detect by CT, because the findings of metastatic lesions vary on CT and the lesions show poor contrast enhancement (*18*). Lower liver uptake of radiopharmaceuticals is preferred so that metastatic liver lesions and right primary breast cancer can be diagnosed. Liver uptakes of PET radiopharmaceuticals were lower than those of SPECT radiopharmaceuticals. Of the six radiotracers used in this study, liver FDG uptake was significantly lower than that of the other five radiopharmaceuticals.

Delayed Tl scans obtained 3–5 hr after injection in patients with thyroid and lung cancer were clinically useful to characterize tumor malignancy (*19,20*). In this study, FDG, TF, MIBI and Tl revealed a retention mechanism in breast cancer cells. Only tumor FMT uptake showed a significant reduction in the level of radioactivity between 1 and 3 hr after injection, which may reflect the presence of a washout mechanism in breast cancer. Delayed imaging performed 3 hr after injection for differentiating breast cancer from a benign breast tumor must be evaluated to investigate this phenomenon.

CONCLUSION

Of the radiopharmaceuticals for PET imaging, FMT seems to be suitable in detecting MCF-7 tumor; whereas for scintimammography, MIBI, TF and Tl appear to have almost the same detectability for MCF-7 tumor. The results of this study strongly suggest that FMT may have the potential for imaging breast cancer.

ACKNOWLEDGMENTS

This study was presented in part at the 44th Annual Meeting of the Society of Nuclear Medicine, San Antonio, TX, June 1–5, 1997.

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(continued from page 7A)

FIRST IMPRESSIONS
Swollen Left Scrotum Following Chronic Ambulatory Peritoneal Dialysis

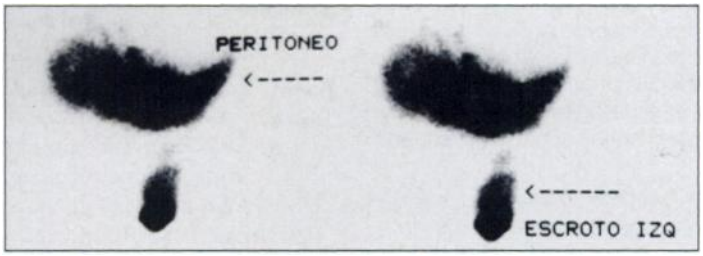

Figure 1.

PURPOSE
A 73-yr-old man with a history of diabetes mellitus and secondary chronic renal failure was started on a chronic abdominal peritoneal dialysis program; 6 mo later, swelling of the left scrotum was observed. Nuclear peritoneography was performed in the anterior, upright position, showing tracer in the left scrotum (Fig. 1) demonstrating communication with the peritoneal cavity. Subsequently, indirect and direct hernias were found at surgery containing 100 ml ascitic fluid.

TRACER
Technetium-99m-MAA (74 MBq; 2 mCi)

ROUTE OF ADMINISTRATION
Intraperitoneal by catheter

TIME AFTER INJECTION
1 hr

INSTRUMENTATION
GE Starcam 3200i gamma camera (GE Medical Systems, Milwaukee, WI) with LEAP collimation

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