Uptake of ^{99m}Tc-MIBI and ^{99m}Tc-Tetrofosmin into Malignant Versus Nonmalignant Breast Cell Lines

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The kinetics and cellular uptake of ^{99m}Tc-2-hexakis 2-methoxyisobutyl-isonitrile (MIBI) and ^{99m}Tc-1,2-bis[bis(2-ethoxyethyl)phosphino]ethane (tetrofosmin) into malignant versus nonmalignant human breast cell lines were investigated and compared. **Methods:** At specific intervals after incubation at 37°C and 22°C with ^{99m}Tc-MIBI or ^{99m}Tc-tetrofosmin, the uptake characteristics of radiotracers into human adenocarcinoma breast cell lines MCF-7 and SK-BR-3 and human breast, nontumor cell line HBL-100 were assessed. **Results:** The uptake of ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin was lower at an incubation temperature of 22°C than that at 37°C in the 3 cell lines. In MCF-7 and in SK-BR-3 cells the uptake of ^{99m}Tc-MIBI was significantly higher than the uptake of ^{99m}Tc-tetrofosmin. The uptake of ^{99m}Tc-MIBI was significantly higher into MCF-7 and SK-BR-3 cells than that into HBL-100 cells. In comparison with HBL-100 cells, uptake of ^{99m}Tc-tetrofosmin into SK-BR-3 cells was significantly higher, whereas uptake into MCF-7 cells was similar. **Conclusion:** In vitro data suggest that ^{99m}Tc-MIBI may be a better tracer than ^{99m}Tc-tetrofosmin for discrimination between malignant and nonmalignant breast disease.

Key Words: ^{99m}Tc-MIBI; ^{99m}Tc-tetrofosmin; breast tumors

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Mammography remains the diagnostic procedure of choice for breast cancer screening. However, mammography cannot be used to accurately differentiate between malignant disease and benign lesions (*1,2*), and many lesions need biopsy for final histologic diagnosis. Furthermore, because of the significant number of patients in whom mammography is inconclusive (dense or fatty breast tissue, scars, fibrocystic breasts, microcalcifications, and other architectural distortions) (*3*), there is substantial need for alternative methods to evaluate breast disease.

Many radioisotopic approaches with different radiopharmaceuticals to detect breast cancer have been described (*4–7*). To reduce other unnecessary invasive diagnostic procedures, a high specificity of scintimammography is demanded. Evaluation of the cationic lipophilic complexes ^{99m}Tc-2-hexakis 2-methoxyisobutyl-isonitrile (MIBI; Du-Pont Pharma GmbH, Bad Homburg, Germany) and ^{99m}Tc-1,2-bis[bis(2-ethoxyethyl)phosphino]ethane (tetrofosmin [Myoview]; Amersham International, Buckinghamshire, UK) for scintimammography is 1 of the most intensively studied areas of diagnostic nuclear medicine in the recent years. Studies have confirmed a role of ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin in diagnosing primary breast cancer, local recurrence, and axillary lymph node metastases (*8–10*). Besides clinical studies, research in breast imaging should also involve evaluation of the molecular aspects of breast cancer. Experimental, in vitro studies to investigate the metabolic aspects of breast cells and the uptake characteristics of these 2 cationic complexes by breast cells are necessary to better understand and define the role of scintimammography for evaluating breast disease. The aim of this study was to assess and compare the in vitro uptake characteristics of ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin into malignant versus nonmalignant human breast cell lines and to assess whether these complexes are promising for scintigraphic discrimination between malignant and nonmalignant breast disease.

MATERIALS AND METHODS

Cell Culture

The human adenocarcinoma breast cell lines MCF-7 (differentiated epithelial-like) and SK-BR-3 (poorly differentiated epithelial-like) and the human breast, nontumor, epithelial-like cell line HBL-100 were obtained from the American Type Culture Collection (Rockville, MD). MCF-7 cells were grown in RPMI-1640 medium (GIBCO BRL, Paisley, Scotland), and SK-BR-3 and HBL-100 cells were grown in McCoy's 5a medium (GIBCO BRL) supplemented with 10% Mycoplex fetal calf serum (FCS) (PAA Laboratories GmbH, Linz, Austria) and antibiotics (10 IU/mL penicillin and 50 μg/mL streptomycin) in 75-cm^2 tissue culture flasks (Falcon; Becton Dickinson Labware Europe, Meylan, France) at 37°C in a 5% CO_2/95% O_2, humidified incubator. Before the experiments, cell cultures were trypsinized and, to remove trypsin, washed once in the respective culture medium containing FCS. The concentration of cell suspensions was calculated by a Coulter Counter (Coulter Electronics Ltd., Harpenden Herts, England). The cells were resuspended at a concentration of 1×10^6 cells/mL in culture medium, transferred to polystyrene round-bottom tubes (Falcon; Becton Dickinson Labware Europe), and kept at 37°C for at least 1 h for equilibration.

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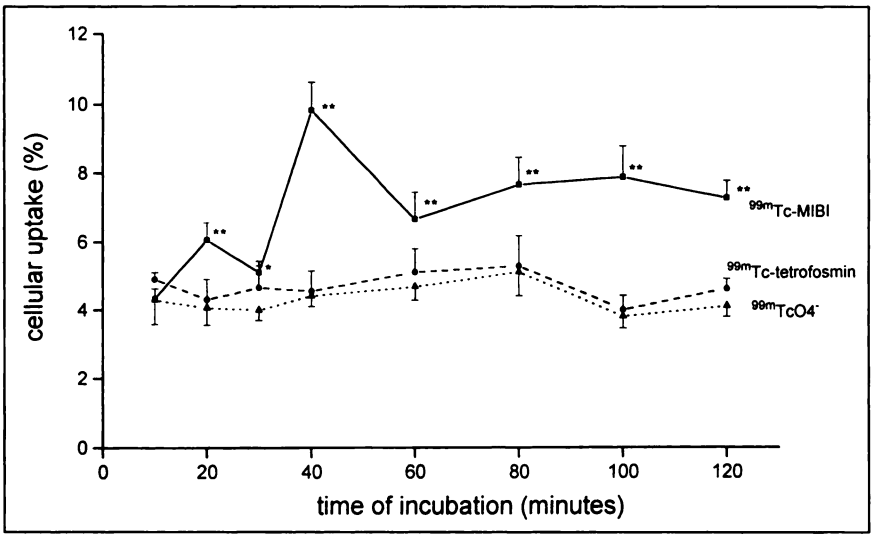

FIGURE 1. Uptake of ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin, and TcO_4^- into MCF-7 cells at 37°C of incubation (n = 6 each; 1 × 10^6 cells/mL; mean ± SD). *$P < 0.05$; **$P < 0.001$.

Radiolabeling

MIBI and tetrofosmin were labeled with ^{99m}Tc according to the kit instructions. Labeling efficiency was >96% on every occasion. Radiochemical purity was >95% as determined by thin-layer chromatography.

Cell Viability

Cell viability was checked by the trypan blue, dye-exclusion technique after preincubation and 2–3 h after incubation with the respective radiopharmaceuticals.

Experimental Design

In each experiment, the radiopharmaceutical (7.4 × 10^5 Bq) was added into a tube containing 1 × 10^6 cells/mL culture medium at a temperature of 37°C. After various incubation times (10, 20, 30, 40, 60, 80, 100, and 120 min after addition of the radiopharmaceutical) at an incubation temperature of 37°C or 22°C, cells were separated from culture medium by simple centrifugation (1200 rpm, 21°C, 10 min). The cells were then washed quickly once with the respective culture medium, and the supernatant was discarded. Six probes of each combination of the above experimental conditions (i.e., incubation time and incubation temperature) were studied. The activities in the tubes containing sedimented cells and in the tubes containing culture medium were counted by a γ well counter. The cellular uptake was calculated as the percentage of the activity counted in the cells relative to the total activity counted.

Data Analysis

Results are presented as mean ± SD. Calculation for significance was performed by a Student t test. $P < 0.05$ was considered significant.

RESULTS

Figures 1–3 show the time-dependent variation in cellular uptake of ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin, and $^{99m}TcO_4^-$ at 37°C in MCF-7, SK-BR-3, and HBL-100 cells, respectively. In MCF-7 cells (Fig. 1) and SK-BR-3 cells (Fig. 2) the uptake of ^{99m}Tc-MIBI was significantly higher ($P < 0.001$) than that of ^{99m}Tc-tetrofosmin. In HBL-100 cells (Fig. 3) the uptake of ^{99m}Tc-MIBI was slightly higher ($P < 0.001$) than that of ^{99m}Tc-tetrofosmin during the initial 30 min and after

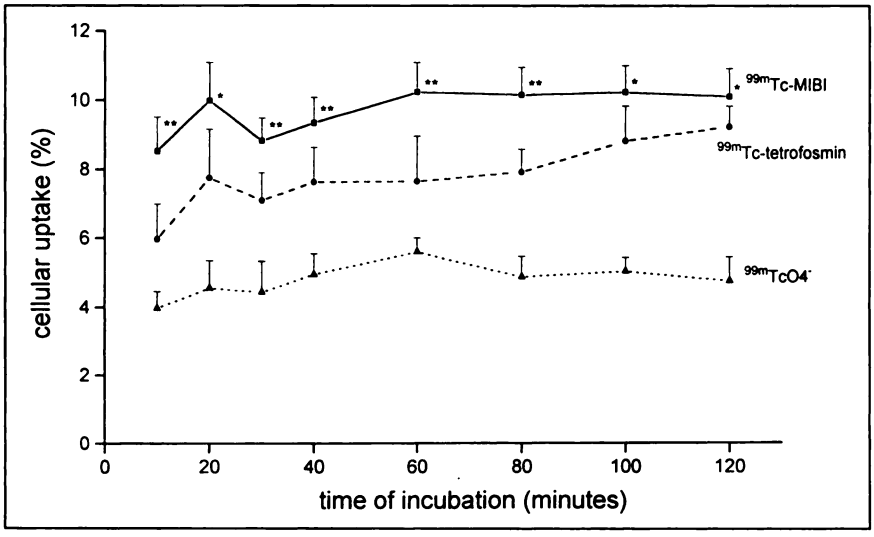

FIGURE 2. Uptake of ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin, and TcO_4^- into SK-BR-3 cells at 37°C of incubation (n = 6 each; 1 × 10^6 cells/mL; mean ± SD). *$P < 0.05$; **$P < 0.001$.

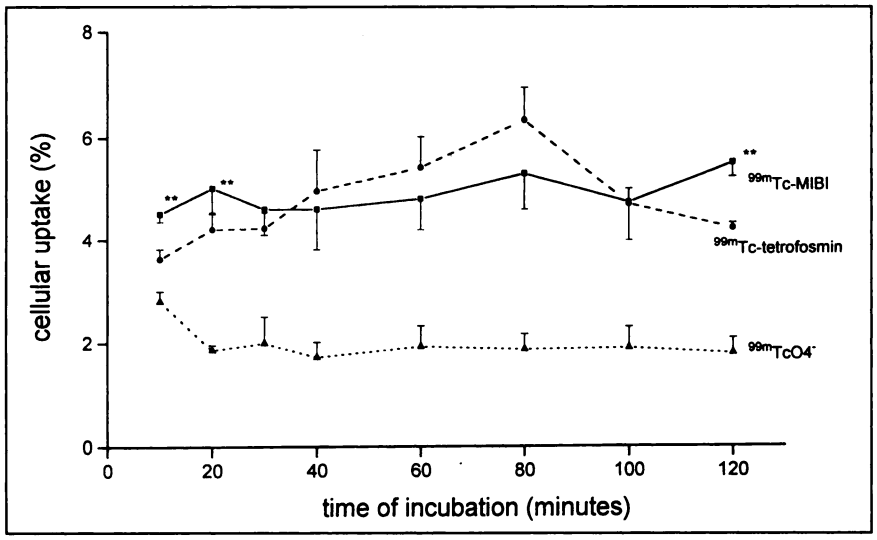

FIGURE 3. Uptake of ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin, and TcO_4^- into HBL-100 cells at 37°C of incubation (n = 6 each; 1 × 10^6 cells/mL; mean ± SD). $^{**}P < 0.001$.

100 min, but it was lower ($P < 0.05$) between 30 and 100 min. In the 3 cell lines uptake of ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin was lower at 22°C (MCF-7 cells, 4.5% ± 0.3% to 5.2% ± 0.6% and 3.8% ± 0.3% to 5.1% ± 0.6%; SK-BR-3 cells, 6.5% ± 0.7% to 9.2% ± 0.9% and 3.9% ± 0.4% to 5.6% ± 0.4%; HBL-100 cells, 4.5% ± 0.8% to 4.8% ± 0.6% and 3.4% ± 0.3% to 4.1% ± 0.3%, respectively) than that at 37°C (Figs. 1–3). $^{99m}TcO_4^-$ was included in this study as a negative control. Cellular uptake was lower than that of ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin in all 3 cell lines (Figs. 1–3) and did not show temperature dependence.

Figures 4 and 5 show the uptake of ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin at 37°C into MCF-7 and SK-BR-3 cells, respectively, compared with the uptake into HBL-100 cells. The uptake of both complexes was significantly higher ($P < 0.001$) into SK-BR-3 cells than that into MCF-7 cells. The uptake of ^{99m}Tc-MIBI (Fig. 4) into MCF-7 and SK-BR-3 cells was significantly higher ($P < 0.001$) than that into HBL-100 cells. ^{99m}Tc-MIBI uptake into MCF-7 cells at 37°C was increased sharply from 30 to 40 min, with the maximum uptake (9.82% ± 0.81%) at 40 min and a tendency toward reaching a plateau after 80 min. The uptake of ^{99m}Tc-MIBI increased into SK-BR-3 cells within 60 min (10.22% ± 0.85%) of incubation and reached a plateau thereafter. The uptake of ^{99m}Tc-tetrofosmin (Fig. 5) into SK-BR-3 cells was significantly higher ($P < 0.001$) than that into MCF-7 and HBL-100 cells, whereas the uptake was almost identical between MCF-7 and HBL-100 cells. ^{99m}Tc-tetrofosmin exhibited an increasing uptake into SK-BR-3 cells at 37°C, reaching the maximum at 120 min (9.21% ± 0.60%); only moderately increased uptake into MCF-7 and HBL-100 cells was evident within 80 min (5.28% ± 0.90% and 6.33% ± 0.61%, respectively), with uptake decreasing thereafter.

Cell viability, as checked by the trypan blue, dye-exclusion technique, did not differ significantly between preincubation and after incubation with the radiopharmaceuticals.

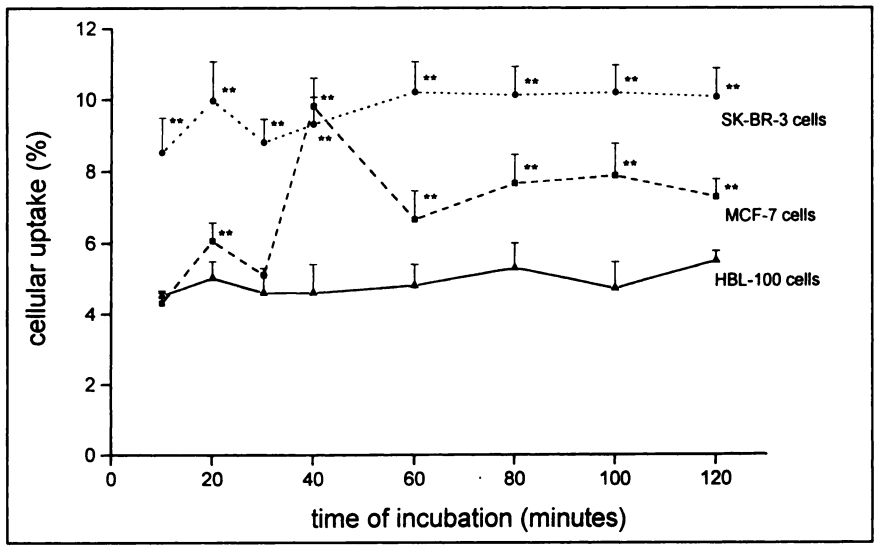

FIGURE 4. Uptake of ^{99m}Tc-MIBI at 37°C into MCF-7, SK-BR-3, and HBL-100 cells (n = 6 each; 1 × 10^6 cells/mL; mean ± SD). **Significantly higher uptake ($P < 0.001$) compared with uptake into HBL-100 cells.

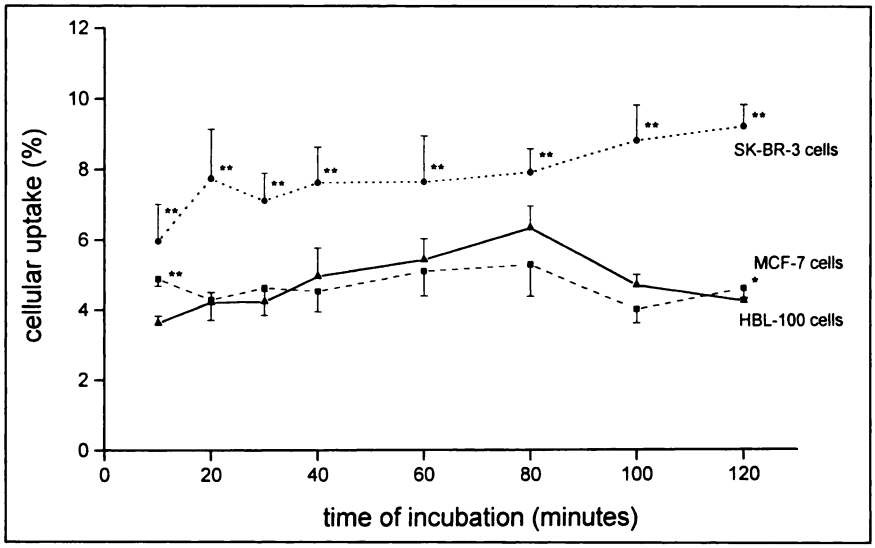

FIGURE 5. Uptake of ^{99m}Tc-tetrofosmin at 37°C into MCF-7, SK-BR-3, and HBL-100 cells (n = 6 each; 1 × 10^6 cells/mL; mean ± SD). **Significantly higher uptake (P < 0.001) compared with uptake into HBL-100 cells.

DISCUSSION

The need for early detection remains a challenge to additional diagnostic procedures, as do the low specificity and the high rate of false-positive results using mammography as the screening method. Although the ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin uptake mechanisms by tumor cells are not understood completely, the mechanisms have been hypothesized to be related to mitochondria (*3,11,12*) and determined by a combination of blood flow and the metabolic status of cells (*3,11–13*). Even if blood flow is a limiting factor, tumor tissue characteristics play a key role, and retention of these tracers can occur if tumor cells are viable and metabolically active (*11*). In this study, the uptake of ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin was lower in the 3 cell lines at an incubation temperature of 22°C than that at 37°C. Cellular uptake of $^{99m}TcO_4^-$ was significantly lower than the uptake of the cationic tracers in all cell lines. These results confirm that the uptake of these tracers by breast cells is related to cellular metabolic activity. Our in vitro data show a significantly higher uptake of ^{99m}Tc-MIBI than that of ^{99m}Tc-tetrofosmin in MCF-7 and SK-BR-3, the 2 malignant cell lines. This might indicate that ^{99m}Tc-MIBI is a better complex for detecting breast cancer. These data are concordant with the findings of Arbab et al. (*11*) in HBL2, an Epstein-Barr virus–negative lymphoma B-cell line, and in SW-13, a small cell carcinoma of the adrenal cortex cell line, where it was found that only a small fraction of ^{99m}Tc-tetrofosmin accumulated inside the mitochondria whereas most ^{99m}Tc-MIBI exhibited an intramitochondrial localization. Recent reports suggest different mechanisms of cellular uptake of these tracers (*12,14*). Cellular uptake and retention of ^{99m}Tc-MIBI seem to occur in response to the electrical potentials generated across the membrane bilayers of both the cell and the mitochondria, and up to 90% of the radiotracer activity is found in the mitochondria. Although still under investigation, it was shown that ^{99m}Tc-tetrofosmin crosses the cell membrane in a nonspecific manner that is dependent on its lipophilicity and sarcolemmal and mitochondrial transmembrane electrical potentials (*14*). Higher uptake of ^{99m}Tc-MIBI into mitochondria might strengthen the hypothesis that malignant cells can be traced better with ^{99m}Tc-MIBI because of the higher number or higher activity of mitochondria (or both) in these cells (*11*). However, for imaging in vivo, not only radioactivity in the target but also the ratio of radioactivity in the target to that in the background are important.

In a ^{99m}Tc-tetrofosmin study performed on 18 patients with palpable breast masses, Adalet et al. (*8*) reported that none of the normal breasts showed abnormal uptake of the tracer and suggested that ^{99m}Tc-tetrofosmin is a promising tracer for the differentiation between benign and malignant breast masses. Kao et al. (*9*), in a ^{99m}Tc-MIBI study performed on 38 patients with palpable breast masses, found the sensitivity, specificity, and diagnostic accuracy to be 84%, 100%, and 87%, respectively, in differentiating benign from malignant lesions. False-positive results of ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin have been reported in some fibroadenomas with high cellularity (*15*). However, it has been suggested that the likelihood of breast cancer is elevated on positive scintimammography (*16*) and that suspicious lesions on mammography and sonography with lack of tracer accumulation make breast cancer unlikely (*10*).

Our in vitro results show a significantly higher uptake of ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin in the poorly differentiated adenocarcinoma breast cell line SK-BR-3 compared with that in the nontumor, breast cell line HBL-100. However, although the uptake of ^{99m}Tc-MIBI was significantly higher in the differentiated adenocarcinoma breast cell line MCF-7, the uptake of ^{99m}Tc-tetrofosmin was quite similar compared with that in the HBL-100 cell line.

CONCLUSION

These in vitro data suggest that ^{99m}Tc-MIBI may be a better indicator than ^{99m}Tc-tetrofosmin for the discrimination

between malignant and nonmalignant breast disease. Our data provide encouraging preclinical perspectives and a rationale for further, more detailed in vivo evaluation of ^{99m}Tc-MIBI scintimammography in breast disease.

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