Comparison of uptake of ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin and ^{99m}Tc-Q12 into human breast cancer cell lines

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Abstract. Technetium-99m hexakis-2-methoxyisobutylisonitrile (MIBI), ^{99m}Tc-tetrofosmin and ^{99m}Tc-Q12 were all introduced for myocardial imaging but found additional applications as they are taken up by different tumours, enabling imaging of these lesions in patients. The aim of this study was to compare the uptake characteristics of these compounds in vitro in the human adenocarcinoma breast cell lines MCF-7 and ZR-75. It was shown that ^{99m}Tc-MIBI had the highest cellular uptake (15.9%±0.5% dose/mg protein after 60 min in MCF-7, and 14.2%±0.4% dose/mg protein in ZR-75), followed by ^{99m}Tc-tetrofosmin (6.8%±0.6% dose/mg protein in MCF-7, and 8.2%±0.2% dose/mg protein in ZR-75) and ^{99m}Tc-Q12 (3.2%±0.1% dose/mg protein in MCF-7, and 3.5%±0.3% dose/mg protein in ZR-75 cells). For all three compounds tenfold differences in specific activity did not influence total cell-associated radioactivity. Uptake of ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin was obviously lower at 4° C than at 37° C, whereas ^{99m}Tc-Q12 uptake showed only slight temperature dependence. When uptake was compared in cells grown to different cell densities (1 mg/ml cellular protein versus 0.3 mg/ml), no differences in uptake were detected when uptake was corrected for the amount of cellular protein present in the dishes. Furthermore, for all compounds it was shown that cellular radioactivity decreased rapidly after washing. Apart from the differences in cellular uptake of the three compounds after 60 min, no differences in residual cellular radioactivity after washing were found between the different compounds when expressed as a percentage of their 60-min uptake, suggesting that the efflux process of the radiolabelled compounds was similar. The differences in cell-associated activity after 60 min were thus presumably caused by differences in uptake. It was concluded that of the Tc-labelled compounds tested, ^{99m}Tc-MIBI had the highest cellular retention in both human breast tumour cell lines. However, for imaging in vivo not only radioactivity in the target organ is important, but also the ratio of radioactivity in the target versus that in the background. Therefore, further studies in vivo need to be performed to investigate which compound is the optimal imaging agent.

Key words: Technetium-99m methoxyisobutylisonitrile – Technetium-99m tetrofosmin – Technetium-99m Q12 – MCF-7 – ZR-75

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Introduction

Technetium-99m hexakis-2-methoxyisobutylisonitrile (MIBI) is a ^{99m}Tc-labelled lipophilic cationic complex that has been used since 1984 for myocardial perfusion imaging [1, 2]. It quickly found other applications in the area of tumour imaging in cancer patients: Müller et al. described its uptake by pulmonary metastases of thyroid cancer [3], and since then, many groups have studied its uptake by, for example, bronchial carcinoma [4], osteosarcoma [5], parathyroid adenoma [6] and breast tumours [7, 8]. Furthermore, Piwnica-Worms et al. reported in 1992 the important observation that ^{99m}Tc-MIBI is a ligand for P-glycoprotein (Pgp), the product of the human multidrug resistance gene (MDR1), which confers resistance to drugs by transporting cytotoxic agents out of cells [9]. Thus, this widely available radiopharmaceutical may be useful for imaging the Pgp status of tumours. However, the extensive hepatobiliary excretion of ^{99m}Tc-MIBI and its uptake in heart, liver, kidneys and total gastrointestinal tract [10] make imaging in the abdomen extremely difficult, and it is therefore not an ideal imaging agent.

^{99m}Tc-MIBI belongs to a class of compounds that have a core atom of radioactive technetium in their structures. Other compounds also introduced for myocardial perfusion imaging are: ^{99m}Tc-tetrofosmin, a lipo-

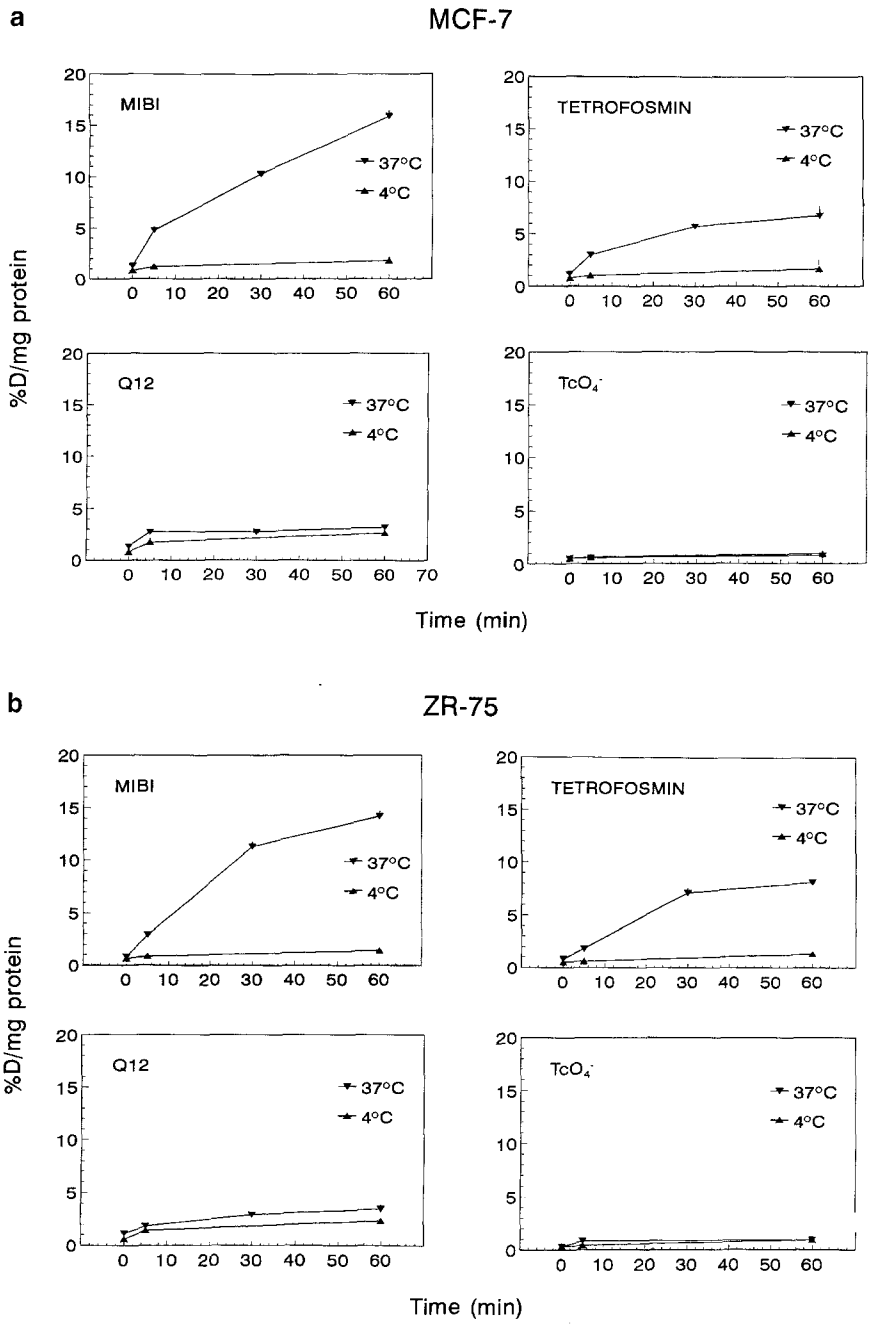

Fig. 1. Time course of cellular radioactivity, expressed as % dose/mg cellular protein, of ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin, ^{99m}Tc-Q12 and $^{99m}TcO4^-$ in **a** MCF-7 cells and **b** ZR-75 cells, at both 37° C and 4° C

philic diphosphine, and ^{99m}Tc-Q12, a mixed ligand complex of the "Q"-series of non-reducible Tc(III)cations. ^{99m}Tc-tetrofosmin has also been reported to be suitable for clinical evaluation of breast tumours [11] and for functional imaging of multidrug resistance [12], and its pharmacokinetic advantages over ^{99m}Tc-MIBI for cardiac imaging [13] may also apply to tumour imaging. ^{99m}Tc-Q12 is also a transport ligand recognized by the human MDR P-glycoprotein [14].

The purpose of this study was to compare the uptake characteristics of ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin and ^{99m}Tc-Q12 during 60 min in well-characterized in vitro tumour models: the human adenocarcinoma breast cell lines MCF-7 and ZR-75.

Materials and methods

Cell culture. The MCF-7 and ZR-75 cell lines were obtained from Dr. J.A. Foekens, Dr. Daniel den Hoed Cancer Centre, Rotterdam, The Netherlands, and grown in RPMI-1640 (phenolred-free, Gibco, Grand Island, N.Y.) medium (pH 7.5), supplemented with 24 m*M* $NaHCO_3$, 2 m*M* glutamine, 10% heat-inactivated fetal calf serum (FCS), and 10 µg/ml insulin in 75 cm^2 flasks in a 5% CO_2, 37° C, humidified incubator. For ZR-75 cells, 1 n*M* 17β-oestradiol was also added to the medium. Before the experiment, subconfluent cell cultures were trypsinized and transferred to six-well plates. Unless otherwise stated, cells in the six-well plates were used for the experiments after reaching confluency.

Radiolabelling. MIBI (Dupont, Billerica, Mass.), tetrofosmin (Amersham International, Aylesbury, Buckinghamshire, UK) and

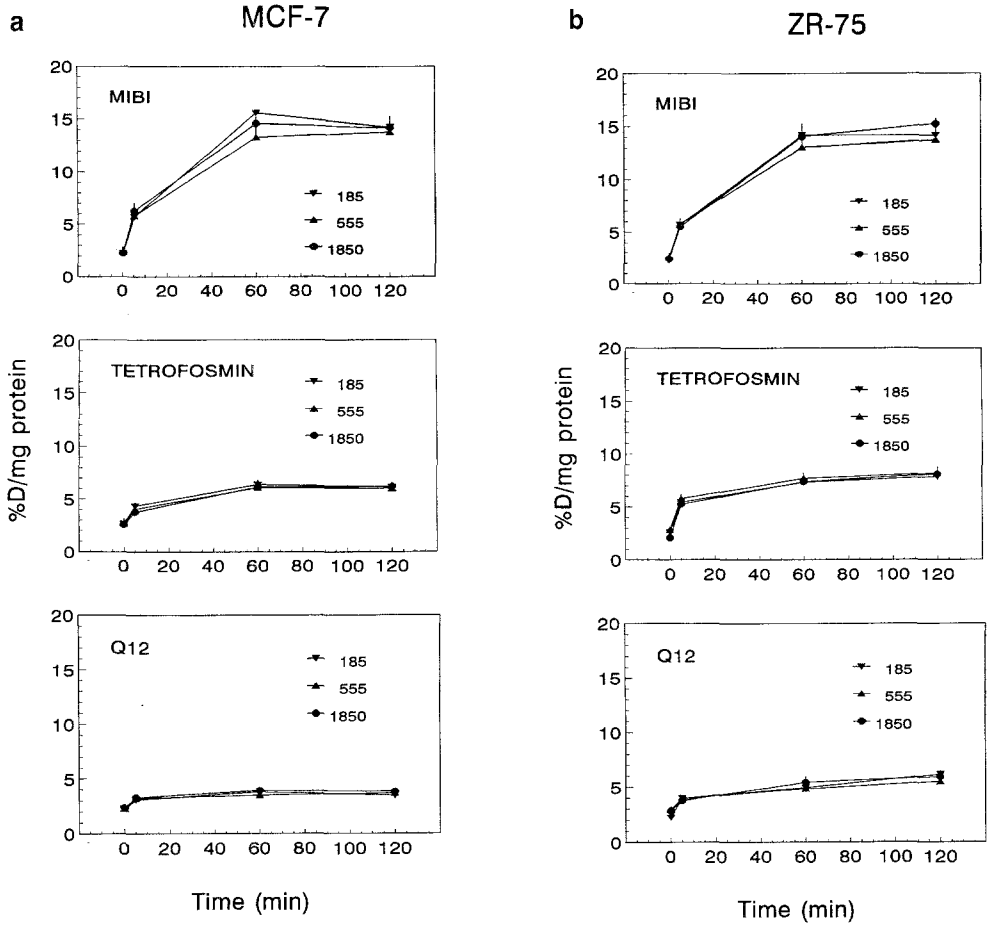

Fig. 2. Influence of specific activity (185, 555 or 1850 MBq/kit) on cellular radioactivity of ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin and ^{99m}Tc-Q12 in **a** MCF-7 cells and **b** ZR-75 cells

Q12 (Mallinckrodt Medical, Petten, The Netherlands) were labelled according to the kit instructions; labelling efficiency was always greater than 95%. 1850 MBq ^{99m}Tc was added to each kit. In experiments dealing with the effects of specific activity, kits were radiolabelled by addition of 185, 555 or 1850 MBq.

Experimental design. Before the experiment, cells were washed and incubation was started by addition of 1 ml incubation medium/well (incubation medium is culture medium without FCS, but with 0.2% bovine serum albumin) with 97.5 kBq of either substance (unless otherwise stated).

Cells were incubated at 37° C for indicated periods of time. Cellular uptake was stopped by removing the incubation medium from the cells, washing with 2 ml ice-cold phosphate-buffered saline (PBS), and lysis of the cells in 1 ml 0.1 *N* NaOH. Removal of cellular radioactivity from the dish was more than 99.5% complete after correction for the amount of radioactivity retained in wells without cells. Total cellular uptake was determined by measuring cell lysate radioactivity in a LKB-1282-Compugamma-system and was expressed as percent of the applied dose per mg cellular protein. The latter was determined using a commercially available kit (Biorad, The Netherlands). All activities were corrected for decay.

Nuclear binding experiments were performed essentially as cellular uptake experiments, as described above, with the following modifications: after incubation, medium was discarded and cells were washed twice with ice-cold PBS. Cells were then scraped from the wells with a rubber policeman, and resuspended in 2 ml ice-cold PBS. Cells were pelleted by centrifugation (300 *g*, 4° C, 7 min) and subsequently solubilized in 1 ml PBS, 0.5% Triton X-100. After 2 min vortexing, nuclei were spun down (900 *g*, 4° C, 5 min) and washed twice in PBS, 0.5% Triton X-100. After the final wash, radioactivity of the nuclear pellet was measured to determine nuclear binding.

Data are expressed as mean±SD for incubations assayed in triplicate, with each experiment performed 3–4 times.

Results

Figures 1a and 1b show the time-dependent increase in cellular uptake of ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin, ^{99m}Tc-Q12 and $^{99m}TcO4^-$ both at 37° C and 4° C in MCF-7 and ZR-75 cells. Cell-associated radioactivity, expressed as % dose per mg cellular protein, increased with time. It is shown that ^{99m}Tc-MIBI had the highest cellular uptake, followed by ^{99m}Tc-tetrofosmin and ^{99m}Tc-Q12. At 37° C ^{99m}Tc-MIBI had an uptake of 15.9%±0.5% dose/mg protein after 60 min in MCF-7, and 14.2%±0.4% dose/mg protein in ZR-75 cells. ^{99m}Tc-tetrofosmin uptake was 6.8%±0.6% dose/mg protein in MCF-7, and 8.2%±0.2% dose/mg protein in ZR-75 cells and ^{99m}Tc-Q12 uptake was 3.2%±0.1% dose/mg protein in MCF-7, and 3.5%±0.3% dose/mg protein in ZR-75 cells. Nuclear binding was very low: for ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin and ^{99m}Tc-Q12 it was less than 0.3% of total cellular binding after 60 min, indicating that nuclear binding did not contribute to total cellular uptake of the tested compounds.

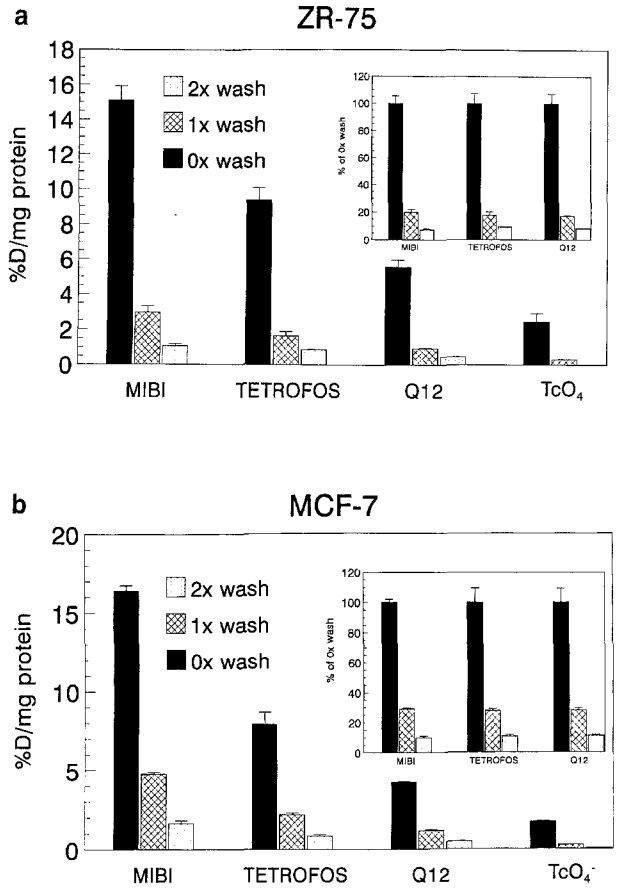

Fig. 3. Cellular radioactivity of ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin, ^{99m}Tc-Q12 and ^{99m}TcO4$^-$ in **a** MCF-7 cells and **b** ZR-75 cells after zero, one or two washing steps. Cells were incubated with label during 60 min (*black bar*). After this incubation period, cells were washed and incubated for 60 min with medium without label (*cross-hatched bar*). The whole procedure was repeated once (*dotted bar*). *Inset*: cellular radioactivity after washing, expressed as % of the 60 min uptake

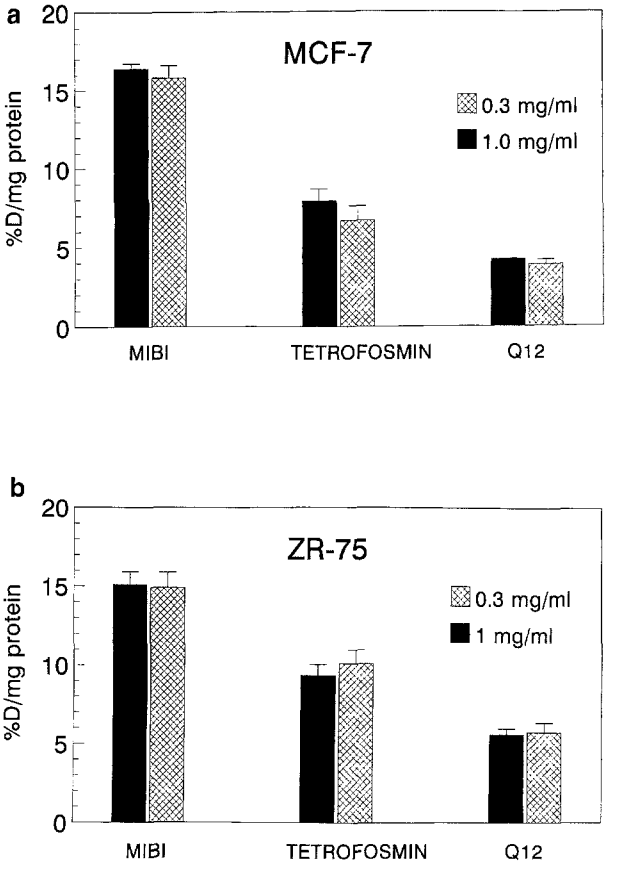

Fig. 4. Cellular radioactivity of ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin and ^{99m}Tc-Q12, after 60-min incubation, in near-confluent (1.0 mg/ml cellular protein) and non-confluent (0.3 mg cellular protein/ml) MCF-7 cells (**a**) and ZR-75 cells (**b**)

^{99m}TcO4$^-$ was included in this experiment as a negative control; cell-associated radioactivity was indeed very low and did not show temperature dependence, whereas cellular radioactivity of ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin was obviously lower at 4° C than at 37° C. ^{99m}Tc-Q12 uptake showed only slight temperature dependence.

Figures 2a and 2b show the time-dependent increase in cellular uptake of ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin and ^{99m}Tc-Q12, at different specific activities, into both breast tumour cell lines. It is shown that for all three compounds that differences in specific activity in the range tested did not influence total cell-associated radioactivity.

Figures 3a and 3b show cellular radioactivity after no, one or two washing steps for both breast tumour cell lines. Cells were incubated with label for 60 min. After this incubation period, cells were washed twice and incubated for 60 min with medium without label. The whole procedure was repeated once. It is shown that cellular radioactivity decreased rapidly after the washing procedures. Apart from the differences in cellular uptake after 60 min between the compounds, no significant differences were found in residual cellular radioactivity after washing between the different compounds when expressed as a percentage of their 60 min uptake.

Figures 4a and 4b show the differences in cellular uptake after 60 min of incubation in cells grown to confluence (1 mg/ml cellular protein) and in non-confluent grown cells (0.3 mg/ml cellular protein). No differences were detected in cellular uptake of ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin and ^{99m}Tc-Q12 at the various cell densities when the uptake was corrected for the amount of cellular protein present in the dishes.

Discussion

^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin and ^{99m}Tc-Q12 were all introduced for myocardial imaging, but found additional applications as they are taken up by different tumours, enabling imaging of these lesions in patients [3–8, 11].

The studies described here revealed great differences in cell-associated radioactivity of human breast tumour cells after incubation for 60 min with ^{99m}Tc-MIBI,

^{99m}Tc-tetrofosmin or ^{99m}Tc-Q12. This may have been caused by differences in either uptake in or efflux from the cell, as cell-associated radioactivity of a radiolabelled compound as measured at a certain time point is the net result of uptake minus efflux of the compound and its metabolites, if any, from the cell. With regard to ^{99m}Tc-MIBI, Chiu et al. [15] have demonstrated that it is sequestered within the cytoplasm and mitochondria of cultured mouse fibroblasts and that its net cellular retention is determined by the electrical potentials across the membrane of both the cell and the mitochondria. These results suggest that normal and abnormal tissues with a large number of mitochondria per cell show higher ^{99m}Tc-MIBI uptake than tissues with fewer mitochondria. Investigating the myocellular uptake mechanism of ^{99m}Tc-MIBI in cultured chick myocardial cells, Piwnica-Worms and Holman found that the transport of this compound involves passive diffusion across plasma and mitochondrial membranes, and that at equilibrium it is largely sequestered within mitochondria by the transmembrane potential [16]. Microautoradiography studies demonstrated that ^{99m}Tc-MIBI is clustered in the area around the nucleus. No radioactivity was detectable on the plasma membrane and nuclear envelope, in agreement with the results obtained in this study [17]. As for other organs or tissues, the mechanisms of ^{99m}Tc-MIBI uptake are not precisely known. However, it is likely that many factors are simultaneously involved. These include the biochemical characteristics of ^{99m}Tc-MIBI: its cationic charge and lipophilicity, the degree of local blood flow, transcapillary exchange, interstitial transport and the membrane potential of both mitochondria and cell membranes.

The exact mechanisms of cellular uptake and retention of ^{99m}Tc-tetrofosmin and ^{99m}Tc-Q12 have not been studied, but the same factors as for ^{99m}Tc-MIBI may play a role.

As shown in Figures 3a and 3b, we did not find a significant difference in cell-associated activity of ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin, ^{99m}Tc-Q12 and ^{99m}TcO4$^-$ after two washing steps, when expressed as a percentage of their 60 min uptake, suggesting that the efflux process of the radiolabelled compounds is similar and that the quantitative differences in cell-assciated activity are caused by differences in uptake. It has recently been reported that the retention of ^{99m}Tc-MIBI also depends on the activity of the 170-kPa Pgp coded by MDR1, which functions as an ATP-dependent efflux pump for many cytotoxic substances, mostly lipophilic cations. ^{99m}Tc-MIBI is reported to be a ligand for this MDR1 Pgp [9], as accumulation of the complex in cells was inversely related to the level of Pgp in these cells, and as verapamil and cyclosporin A, multidrug-resistant reversal agents, enhanced accumulation of ^{99m}Tc-MIBI many fold. It has been reported that ^{99m}Tc-tetrofosmin and ^{99m}Tc-Q12 are also ligands for the Pgp protein [12, 14]. Functional imaging of Pgp activity may play a role in chemotherapy regimens for maximal benefit of the patient. The detection of tumours which have Pgp-mediated resistance is important to prevent the patient being exposed to toxic chemotherapy which is not likely to be effective.

As it has been reported that diffusion plays a role in uptake through the plasma membrane, we also investigated the influence of cell density in the wells using breast tumour cells that were either used when grown to confluence (1 mg/ml cellular protein) or when grown non-confluent (0.3 mg/ml cellular protein). Although it may be supposed that in the case of non-confluent grown cells more plasma membrane area is available for diffusion than in tightly confluent grown cells, under the conditions used in our study this did not influence cell-associated radioactivity after 60-min incubation (Fig. 4).

To investigate whether the differences in cellular uptake could be explained by differences in the specific activity of the labels used, we performed studies in the breast cell lines with different specific activities of each compound (Fig. 2). On a molar base, the difference between the highest (MIBI) and lowest (tetrofosmin) compound mass per kit is by about a factor of 10. As shown, however, tenfold differences in specific activity did not influence the cell-associated radioactivity of the three radiolabelled compounds.

In these experiments, data are expressed as % dose/mg cellular protein. It appeared that differences in uptake of the tested compounds did not change when uptake was expressed as % dose/μg DNA (not shown), which cancels differences introduced by variations in cell sizes from culture to culture.

From our experiments it can be concluded that of the Tc-labelled compounds tested, ^{99m}Tc-MIBI has the highest cellular uptake during 60 min in both human breast tumour cells, theoretically making it the most suitable compound for imaging. However, it has been reported that when ^{99m}Tc-tetrofosmin was compared with ^{99m}Tc-MIBI in a 1-day, rest and dipyridamole stress myocardial single-photon emission tomographic imaging setting, no significant differences were observed in the quality or the diagnostic interpretation of the images [13]. In vivo not only radioactivity in the target organ is important for imaging, but also the ratio of radioactivity in the target versus that in the background. The extensive hepatobiliary excretion of ^{99m}Tc-MIBI and its localization in heart, liver, kidneys and total gastrointestinal tract [10] may have contributed to the aforementioned finding. The biodistribution of ^{99m}Tc-MIBI also poses a problem for imaging in the abdomen, and the reported advantageous pharmacokinetics in vivo of ^{99m}Tc-tetrofosmin over ^{99m}Tc-MIBI for cardiac imaging [13] may also apply to tumour imaging. Therefore, further studies in vivo need to be performed to identify the optimal imaging agent.

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