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-$^{99m}$m hexakis-2-methoxyisobutyylonitrile (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-$^{99m}$m tetrofosmin – Technetium-$^{99m}$m Q12 – MCF-7 – ZR-75


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-
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. Foecken, 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 mM NaHCO$_3$, 2 mM 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 nM 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 confluence.

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