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Biphasic accumulation kinetics of [^{99m}Tc]-hexakis-2-methoxyisobutyl isonitrile in tumour cells and its modulation by lipophilic P-glycoprotein ligands

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Abstract

Aim: To study the accumulation and washout kinetics of [^{99m}Tc]-hexakis-2-methoxyisobutyl isonitrile (^{99m}Tc-MIBI) in MDR positive and MDR negative tumour cells and how this is modified by lipophilic P-glycoprotein ligands. *Methods:* The tumour cells were incubated in the presence and absence of the ligands and the uptakes of ^{99m}Tc-MIBI, rhodamine 123 and 2-[^{18}F]fluoro-2-deoxy-D-glucose (^{18}FDG) were measured. *Results:* The accumulation of ^{99m}Tc-MIBI in the tumour cells followed biphasic kinetics. Verapamil and cyclosporin A increased the membrane fluidity and significantly enhanced the ^{99m}Tc-MIBI uptake of the MDR negative cells, while the rhodamine 123 uptake was not affected. Verapamil significantly increased the uptake of rhodamine 123 and ^{18}FDG but did not modify that of ^{99m}Tc-MIBI in the MDR positive cells. Cyclosporin A significantly increased the ^{18}FDG uptake of the MDR positive and negative tumour cells; these effects were ouabain-sensitive. Depolarization of the cytoplasmic membrane, acidification of the extracellular medium and the administration of CCCP decreased the accumulation of ^{99m}Tc-MIBI and rhodamine 123 uptake in the tumour cells. *Conclusions:* Lipophilic P-glycoprotein ligands modified the biphasic accumulation kinetics of the ^{99m}Tc-MIBI uptakes of MDR negative and positive tumour cells in different and complex ways and could therefore mask the P-glycoprotein pump-dependent changes in tracer accumulation.

Keywords: Biphasic ^{99m}Tc-MIBI uptake; ^{18}FDG; Rhodamine 123; Lipophilic ligand; Multidrug resistance

1. Introduction

Earlier biophysical studies established that ^{99m}Tc-hexakis-2-methoxyisobutyl isonitrile (^{99m}Tc-MIBI) is a Nernstian probe of the membrane potential (Piwnica-Worms et al., 1990; Pauwels et al., 1998). The net accumulation and unidirectional uptake rates of ^{99m}Tc-MIBI are thermodynamically driven by negative mitochondrial inner matrix and plasma membrane potentials (Piwnica-Worms et al., 1995;

Abbreviations: Pgp; P-glycoprotein; MDR; multidrug resistance; MRP1; multidrug resistance-associated protein; ^{18}FDG; 2-[^{18}F]fluoro-2-deoxy-D-glucose; ^{99m}Tc-MIBI; [^{99m}Tc]-hexakis-2-methoxyisobutyl isonitrile; R123; rhodamine 123; VER; verapamil; CSA; cyclosporin A; CCCP; carbonyl cyanide *m*-chlorophenylhydrazone; PET; positron emission tomography; SPET; single photon emission computed tomography

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Pauwels et al., 1998) thereby concentrating the agent within cells in a similar manner as for other lipophilic cationic probes of the membrane potential.

However, little is known about the possible distribution of ^{99m}Tc-MIBI between its intracellular free state and states bound to intracellular structures and organic cell components, a phenomenon affecting the intracellular accumulation of the tracer.

It is well documented that malignant tumours display an increased accumulation of this radiopharmaceutical (Pauwels et al., 1998; Burak et al., 2003). ^{99m}Tc-MIBI has been shown to be a substrate for the MDR1 gene coded P-glycoprotein (Pgp) and multidrug resistance-associated protein (MRP1) pumps (Piwnica-Worms et al., 1995; Kostakoglu et al., 1998; Utsunomiya et al., 2000). Consequently, the accumulation of ^{99m}Tc-MIBI is reduced in cancer cells expressing Pgp or MRP1. These findings lend support to the hypothesis that the ^{99m}Tc-MIBI accumulation assay may be an appropriate in vivo tool for exploration of the Pgp status of tumours. However, certain of the experimental results are somewhat controversial, and thus do not provide firm evidence of a general and straightforward relationship between the extent of Pgp expression and the ^{99m}Tc-MIBI accumulation levels in the individual tumours (Kostakoglu et al., 1998; Kinuya et al., 2003; Del Vecchio et al., 2003).

Discordant results can be due to a variety of reasons. Many of the ^{99m}Tc-MIBI accumulation studies involved the use of Pgp substrates and modulators (e.g. verapamil (VER) and cyclosporin A (CSA)) that are transported by and/or influence the function of the Pgp pump. These molecules are often of highly hydrophobic nature (Krishna and Mayer, 2000; Takara et al., 2002), which may have effects on the ^{99m}Tc-MIBI accumulation and washout kinetics of both MDR positive (MDR$^+$) and MDR negative (MDR$^-$) cancer cells. Some of the most recent publications have drawn attention to the complexity of the reverting and other effects of VER when ^{99m}Tc-MIBI is used (Cayre et al., 1999; Rodrigues et al., 2001; Arbab et al., 2003; Márián et al., 2003). In tumours, MDR does not usually appear in an all or none fashion; there is extensive heterogeneity in the different cell types and even within a single cell type (Kostakoglu et al., 1998). ^{99m}Tc-MIBI images provide information on a summed tracer accumulation in which multidirectional effects are averaged. It is therefore essential to examine the effects of different ligands in the uptake of ^{99m}Tc-MIBI in both MDR$^+$ and MDR$^-$ tumor cells.

^{18}FDG is the most commonly used PET radiotracer in tumour diagnostics, which visualizes the changes of glucose metabolic rate in tissues (Wahl, 1996; Mankoff and Bellon, 2001). The high metabolic rate is usually characteristic of rapidly dividing tumour cells and is generally manifested in an increased ^{18}FDG uptake (Wahl, 1996). It was shown earlier that the MDR$^+$ cells and the tumour xenografts of the same line exhibit higher ^{18}FDG uptake than the MDR$^-$ cells and the tumours. Activation of the Pgp pump by VER is also manifested in an increased glucose metabolism while CSA treatment did not show Pgp dependent ^{18}FDG elevation (Márián et al., 2003). The increase of the ^{18}FDG uptake of VER treated MDR$^+$ cells is thought to be the result of an elevated the ATP-ase activity (Márián et al., 2003). Broxterman et al. (1990) have also shown that the effects of CSA and VER on energy metabolism in multidrug resistant tumour cells is different.

Detailed studies of the transport and accumulation processes of ^{99m}Tc-MIBI may be instrumental in the development of a measuring protocol for the assessment of the Pgp status of malignant cells, a parameter of utmost importance, influencing the management of tumour patients. For a better understanding of ^{99m}Tc-MIBI accumulation phenomena, we set out to examine the accumulation and washout kinetics of ^{99m}Tc-MIBI and the accumulation of ^{18}FDG in tumour cells under different extracellular and intracellular conditions. These effects were compared with the changes in mitochondrial membrane potential accompanying these treatments, as measured using the validated mitochondrial membrane potential-sensitive dye rhodamine 123 (R123).

2. Materials and methods

2.1. Reagents

All of the chemicals applied were of analytical or spectroscopic grade. Propidium iodide (PI), rhodamine 123, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), trimethylammonio-diphenyl-hexatriene (TMA-DPH), were obtained from Molecular Probes (Eugene, OR). D-Glucose, bovine serum albumin (BSA), digitonin (DIG), verapamil, cyclosporin A, ouabain and inorganic chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Phosphate buffered saline (PBS) contained: 140 mM NaCl, 5 mM KCl, 8 mM Na_2HPO_4, 3 mM NaH_2PO_4 and 5 mM D-glucose at pH 7.4. Buffers of different potassium concentrations contained 5 mM KCl, 135 mM NaCl or 120 mM KCl, 20 mM NaCl and were buffered with 10 mM HEPES (pH 7.4).

2.2. Cells

Human epidermoid carcinoma cell line KB-3-1 and its vinblastine-selected Pgp-expressing counterpart KB-V1, mouse fibroblast cell line NIH 3T3, human lymphoid B-cell line JY and hamster vas deferens smooth muscle cell line DDT1 MF-2 were used.

The KB-3-1, KB-V1 and NIH 3T3 cells were grown as monolayer cultures at 37 °C in an incubator containing 5% CO_2 and maintained by regular passage in Dulbecco's minimal essential medium (supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 units ml^{-1} penicillin and 100 μg ml^{-1} streptomycin).

The JY cells were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 units ml^{-1} penicillin and 100 μg ml^{-1} streptomycin at 37 °C in a 5% CO_2 atmosphere.