99mTc-sestamibi is a substrate for P-glycoprotein and the multidrug resistance-associated protein

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Summary 99mTc-sestamibi (99mTc-MIBI) is a substrate for the P-glycoprotein (P-gp) pump but it is not known whether it is a substrate for the multidrug resistance-associated protein (MRP) pump. Therefore, 99mTc-MIBI was evaluated in the GLC4 cell line and its doxorubicin-resistant MRP-, but not P-gp-, overexpressing GLC/ADR sublines as well as in the S1 cell line and its MRP2-transfected subline S1-MRP. 99mTc-MIBI concentration decreased in the GLC/ADR sublines with increasing MRP overexpression and was lower in S1-MRP than in S1. 99mTc-MIBI plus vincristine increased 99mTc-MIBI concentration in GLC4 lines compared with 99mTc-MIBI alone. 99mTc-MIBI efflux was increased with increasing MRP expression in the GLC4 lines. Glutathione depletion elevated 99mTc-MIBI concentration in GLC/ADR1500. Cross resistance for 99mTc-MIBI, used to test cytotoxicity of the Tc compound, was observed in GLC/ADR1500 vs GLC4. 99mTc-MIBI induced a synergistic effect on vincristine cytotoxicity in GLC/ADR1500. These results show that 99mTc-MIBI is involved in MRP-mediated efflux. The fact that 99mTc-MIBI efflux is influenced by MDR1 and MRP expression must be taken into account when this γ-rays-emitting complex is tested for tumour efflux measurements.

Keywords: multidrug resistance; P-glycoprotein; multidrug resistance-associated protein; 99mTc-sestamibi; drug transport

Resistance of tumours to chemotherapeutic compounds is an important problem in the clinic. Drugs such as anthracyclines, vinca alkaloids and epipodophyllotoxins are involved in the so-called multidrug resistance (MDR) (Pastan and Gottesman, 1987; Bradley et al., 1988; De Vries et al., 1989; De Jong et al., 1990; Meijer et al., 1990; Cole et al., 1992; Versantvoort et al., 1992; Schepet al., 1993).

One of the mechanisms involved in MDR is the overexpression, in tumour cells, of the ATP-dependent 170-kDa P-glycoprotein (P-gp) encoded by the MDR1 gene (Endicott and Ling, 1989). P-gp acts as a transmembrane efflux pump that transports chemotherapeutic compounds out of the cell, resulting in drug resistance. P-gp is also expressed in many normal human tissues, such as the liver (bile canaliculi), pancreas, colon, jejunum and kidney (Thiebaut et al., 1987; Sugawara et al., 1988). In normal tissues, P-gp is considered to act as a transporter of toxins.

Drugs that are substrates for P-gp are hydrophobic and mostly positively charged at neutral pH. Piwnica-Worms et al. (1993) have shown that 99mTc-sestamibi (99mTc-MIBI), a lipophilic cationic radiopharmaceutical, is also a substrate for P-gp-mediated transport (Piwnica-Worms et al., 1993; Vallabhaneni et al., 1994; Ballinger et al., 1995). Consequently, 99mTc-MIBI allowed visualization of P-gp-mediated efflux in tumours in the animal model (Piwnica-Worms et al., 1993).

Apart from P-gp, another pump, the multidrug resistance-associated protein (MRP), is involved in MDR. The MRP pump was identified and characterized as a member of the ATP-binding cassette superfamily (Cole et al., 1992; Ishikawa, 1992; Jedlickchy et al., 1994). In MRP-transfected cell lines, it was shown that this pump can be involved in drug efflux (Zaman et al., 1994). However, differences in doxorubicin accumulation between doxorubicin-sensitive and -resistant MRP-overexpressing cell lines were not observed in all cell lines (Scheper et al., 1993). It was shown with inside-out vesicles that MRP functions as a glutathione S-conjugate carrier. Multivalent anionic conjugates, such as glutathione S-conjugates are substrates for this pump (Müller et al., 1994). In contrast to P-gp, MRP is present in almost all cells of the human body.

Detection of protein and RNA expression for the MDR1 and MRP pump can be performed in human tumour samples. However, detection of P-gp or MRP does not necessarily provide any information about the function of these pumps in the respective tissues. Studies with modulators of MDR1, such as quinidine and cyclosporin A, as additional treatment in chemotherapy regimens, have been disappointing in patients with solid tumours (Wishart et al., 1994). Therefore, studies have been initiated in the clinic with the radiopharmaceutical 99mTc-MIBI to image the effect of modulators on MDR1-mediated efflux. This may help to select patients who might benefit most from treatment with modulators. A complicating factor is the fact that, in drug-resistant cells, P-gp and MRP can be overexpressed at the same time (Brock et al., 1995).

Currently, it is not known whether 99mTc-MIBI is also a specific substrate for MDR1. Therefore, in the present study, 99mTc-MIBI kinetics in cell lines with P-gp and different MRP expression as well as the effect of 99mTc-MIBI on cytotoxicity were analysed.

MATERIALS AND METHODS

Chemicals

Doxorubicin was obtained from Pharmacia Carlo Erba (Milan, Italy), vincristine from Eli Lilly (Indianapolis, IN, USA) and verapamil from Knoll (Almere, The Netherlands). RPMI 1640
medium, fetal calf serum (FCS) and genetin were purchased from Gibco (Paisley, UK), d,l-buthionine S,R-sulphoximine (BSO) and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) from Sigma (St Louis, MD, USA), Dulbecco’s modified Eagle medium (DMEM) and HAM F12 medium from Flow Laboratories (Irvine, UK).

Solid NH$_4^{60m}$TcO$_4$ was kindly provided by Dr Hector Knight (Mallinkrodt Medical, Petten, The Netherlands) and Cardiolite vials by Dr Stephen Haber (Du Pont Merck, Billerica, MA, USA). $^{99m}$Tc-MIBI was synthesized with a one-step Cardiolite kit formulation containing 0.075 mg of solid stannous chloride as a reducing agent for technetium (Tc) and excess hexakis(2- methoxyisobutyl)isonitrile (MIBI) as the Cu(MIBI)$_2$BF$_4$ salt. A total of 10–12 GBq $^{99m}$Tc$_2$O$_4$* obtained from a commercial molybdenum/Tc generator (Ultra-TechnexKowFM) was added to the kit reaction vial, heated at 100°C for 10 min and allowed to cool to room temperature. Radiochemical purity was >98% by thin-layer chromatography (Gelman Sciences, Ann Arbor, MI, USA) with 0.9% sodium chloride as mobile phase. In addition, macroscopic quantities of $^{99m}$Tc-MIBI for cytotoxicity experiments were prepared by the reaction of NH$_4^{60m}$TcO$_4$ with Cardiolite vials. Solid NH$_4^{60m}$TcO$_4$ (100 mg, 0.55 mmol) was dissolved in 2 ml of 0.9% sodium chloride, filtered and thereafter diluted with 0.9% sodium chloride until a final volume of 5 ml. As $^{99m}$Tc-MIBI is a β-emitter ($t_{1/2} = 2.1 \times 10^8$ a, β $= 0.3$ MeV), this stock solution was calibrated using a β-counter (Packard Instruments, Downers Grove, IL, USA). A Cardiolite kit was dissolved in 0.65 ml of stannous chloride solution (8.4 μmol ml$^{-1}$ Sn$^{2+}$). Thereafter, 37 MBq Na$^{99m}$TcO$_4$ eluate and 15 μl of the NH$_4^{60m}$TcO$_4$ stock solution (1.1 μmol, 0.2 mg) was added. The kit reaction vial was heated at 100°C for 15 min and allowed to cool to room temperature, producing an almost quantitative yield of the $^{99m}$Tc(MIBI)$_6^*$ complex. The reaction mixture was loaded onto a C-18 Sep Pak cartridge (Waters Associates, Milford, MA, USA). The Sep Pak cartridge was pre-wet with 5 ml of ethanol, followed by a 10-ml water rinse. The cartridge was washed with 10 ml of 0.9% sodium chloride and the pure complex was eluted with ethanol/0.9% sodium chloride (95:5.5 ml). The radiochemical purity was >97% by thin-layer chromatography. The chemical structure was confirmed by ion-spray mass spectrometry (Nermag, Argenteuil, France). This yielded a single peak at m/z = 777 with characteristic major fragments at 664 ($^{99m}$Tc(MIBI))$^+$ and 551 ($^{99m}$Tc(MIBI))$^2$. The solution was evaporated (37°C, under a nitrogen atmosphere). $^{99m}$Tc-MIBI$_{Cl}$ was dissolved in ethanol/0.9% sodium chloride 1:9 (v/v). The solution was calibrated using the abovementioned β-counter. The final concentrations of the different solutions were between 3 and 7 nm $^{99m}$Tc-MIBI. In addition, to avoid ethanol-induced effects in $^{99m}$Tc-MIBI cytotoxicity experiments, the stock solution of $^{99m}$Tc-MIBI in ethanol was diluted to 300 μM $^{99m}$Tc-MIBI as the highest concentration (≤1% ethanol in the MTA controls).

**Cell lines**

The human ovarian cancer cell line, A2780 and its 92-fold doxorubicin-resistant MDR1-overexpressing but MRP-negative subline A2780AD were cultured in RPMI 1640 medium/10% FCS in a humidified atmosphere with 5% carbon dioxide at 37°C (Zijlstra et al, 1987). Stable resistance in the A2780AD cell line was assured by culturing this line with 2 μM doxorubicin twice a week. Before use, A2780AD was cultured without doxorubicin for 14 days.

The human small-cell lung cancer cell line GLC$_4$ and its doxorubicin-resistant MRP-overexpressing, P-gp-negative sublines GLC$_4$/ADR$_{10}$, GLC$_4$/ADR$_{10a}$ and GLC$_4$/ADR$_{150a}$ with two-fold, 10-fold and 150-fold resistance, respectively, to doxorubicin were cultured in RPMI 1640 medium/10% FCS in a humidified atmosphere with 5% carbon dioxide at 37°C (Zijlstra et al, 1987; De Jong et al, 1990; Meijer et al, 1991; Versantvoort et al, 1995a). GLC$_4$ expresses a low level of MRP. MRP mRNA was overexpressed in all GLC$_4$-resistant sublines with a higher expression with increasing doxorubicin resistance (Müller et al, 1994). Resistance to doxorubicin in these resistant cell lines was assured by culturing GLC$_4$/ADR$_{10}$ and GLC$_4$/ADR$_{150a}$, respectively, with 0.018 μM and 0.59 μM doxorubicin once every 3 weeks and GLC$_4$/ADR$_{150a}$ with 1.2 μM doxorubicin twice a week. Before use, the resistant cell lines were cultured without doxorubicin for 21 days. In addition, on a regular basis, mdrl-RNA expression and P-gp expression were characterized in GLC$_4$ and GLC$_4$ sublines. Both were always negative.

The human non-small-cell lung cancer cell line SW-1573/S1 and its stable MRP-overexpressing subline S1-MRP were kindly provided by Professor Dr P Borst and Dr G Zaman, Dutch Cancer Institute, Amsterdam, The Netherlands. They were cultured in DMEM medium/10% FCS in a humidified atmosphere with 5% carbon dioxide at 37°C. The MRP-overexpressing cell line was obtained after transfection of SW-1573/S1 cells with an expression vector containing MRP cDNA and a neo gene (pRc/RSV-MRP), followed by selection with genetin (Zaman et al, 1994). In addition, the MRP expression was negative in S1 and positive in S1-MRP.

**Cellular $^{99m}$Tc-MIBI accumulation and efflux**

Cells (2 x 10$^6$) from A2780, GLC$_4$, S1 and their resistant sublines were incubated in polystyrene tubes for 1 h at 37°C with 64 fm $^{99m}$Tc-MIBI in 5 ml of RPMI 1640/10% FCS. The different cells lines were initially incubated with $^{99m}$Tc-MIBI for 15 min and 1 h. Because the steady state of $^{99m}$Tc-MIBI was reached within 1 h of incubation, further experiments were performed with 1-h drug incubation. To study modulating effects, A2780 and its resistant subline were incubated simultaneously with $^{99m}$Tc-MIBI (64 fm) and verapamil (50 μM), and GLC$_4$ and its resistant sublines were incubated without doxorubicin for 14 days.
incubated with $^{99m}$Tc-MIBI (64 fm) and vincristine (20 μM) for 1 h. After the incubation, the cells were washed with 5 ml of ice-cold phosphate-buffered saline (PBS) followed by centrifugation (5 min, 180 g, 4°C). The wash step as described above was repeated three times. The cellular $^{99m}$Tc-MIBI was measured in water with a γ-counter (LKB Wallac, Turku, Finland). Correction of the extracellular adhesion of $^{99m}$Tc-MIBI to the cells was performed by subtracting the results obtained after $^{99m}$Tc-MIBI incubation for 5 min at 4°C. Extracellular adhesion of $^{99m}$Tc-MIBI was always less than 5% compared with the cellular accumulation of $^{99m}$Tc-MIBI.

The cellular accumulation was expressed as attomol $^{99m}$Tc-MIBI per 10⁶ cells. For efflux studies, 2 × 10⁶ cells from these cell lines were incubated in 5 ml of RPMI 1640/10% FCS for 1 h at 37°C with 64 fm $^{99m}$Tc-MIBI as described above. Thereafter, the cells were washed with RPMI 1640/10% FCS at 37°C. After 0, 10 or 30 min, the efflux of $^{99m}$Tc-MIBI was terminated by adding ice-cold PBS followed by centrifugation (5 min, 180 g, 4°C) and measurement of the cellular $^{99m}$Tc-MIBI. After correction for extracellular adhesion of $^{99m}$Tc-MIBI, efflux was expressed as % $^{99m}$Tc-MIBI in the cells related to the amount of $^{99m}$Tc-MIBI after 1 h of $^{99m}$Tc-MIBI incubation. Three to six independent experiments were performed, each in duplicate.

To check the effect of difference in cellular $^{99m}$Tc-MIBI accumulation on cellular efflux of $^{99m}$Tc-MIBI, 2 × 10⁶ cells from GLC2 and GLC/ADR₁₀₀x were incubated with 8 MBq (64 fm). Because accumulation of $^{99m}$Tc-MIBI was found to be four fold higher in GLC₂ than in GLC/ADR₁₀₀x, GLC₂ and GLC/ADR₁₀₀x were incubated at 4°C for 1 h with 8 MBq (64 fm) and 2 MBq (16 fm) respectively. After incubation with $^{99m}$Tc-MIBI at equal levels, the efflux study was started as described above at a temperature of 37°C.

The effect of glutathione depletion on $^{99m}$Tc-MIBI accumulation was analysed in GLC₂ and GLC/ADR₁₀₀x. Cells were precultured for 24 h in the presence of 25 μM of the glutathione synthesis inhibitor BSO. After 24 h, glutathione is no longer detectable in these lines, without growth delay or loss of viability (Meijer et al, 1991). After 24 h, the cell lines were incubated for 1 h with $^{99m}$Tc-MIBI as described above. Three independent experiments were performed, each in duplicate.

**Cytotoxicity assay**

The microculture tetrazolium assay (MTA) was used as described before (Steel and Peckham, 1979). Cells, 3750 and 10 000 per well for GLC₂ and GLC/ADR₁₀₀x respectively, were incubated for 1 h with $^{99m}$Tc-MIBI in a concentration range from 0.5 μM to 300 μM in 0.1 ml of culture medium. Thereafter, the cells were washed three times by removal of medium after centrifugation (10 min, 180 g) followed by addition of fresh medium and were cultured for 4 days. The percentage cell survival was calculated as the mean of test samples/mean of untreated samples. Controls consisted of media without cells (background extinction) and cells incubated with medium instead of the drug. Two independent experiments were performed, each in quadruplicate. From these survival curves, the $^{99m}$Tc-MIBI concentrations were determined that inhibited cell survival by 10% (IC₁₀) or 25% (IC₂₅).

IC₁₀ and IC₂₅ concentrations of $^{99m}$Tc-MIBI were used to test the modulating effect of $^{99m}$Tc-MIBI on vincristine cytotoxicity in GLC₂ and GLC/ADR₁₀₀x. Survival curves were performed in GLC₂ and GLC/ADR₁₀₀x cell lines for 1-h exposure to vincristine (range 0 μM to 0.60 μM) plus and minus $^{99m}$Tc-MIBI (IC₁₀ or IC₂₅). Modulating effects were analysed by isobologram analysis according to Steel and Peckman (1979). Three independent experiments were performed, each in quadruplicate.

**Statistics**

Statistical significance was determined with the paired and unpaired Student’s t-test. Only P-values < 0.05 were considered to be significant.

**RESULTS**

**Cellular $^{99m}$Tc-MIBI accumulation in A2780 and the P-gp-overexpressing cell line A2780AD**

Cellular accumulation after 1-h exposure to 64 fm $^{99m}$Tc-MIBI was much higher in A2780 (mean ± s.d. 35 × 10⁻⁴ ± 5 × 10⁻⁴ attomol per 10⁶ cells) than in A2780AD (0.43 × 10⁻⁴ ± 0.03 × 10⁻⁴ attomol per 10⁶ cells) (P < 0.0005). Co-incubation with verapamil increased the cellular $^{99m}$Tc-MIBI accumulation to 15 × 10⁻⁴ ± 3 × 10⁻⁴ attomol per 10⁶ cells in A2780AD (P < 0.0025). Verapamil did not affect the cellular $^{99m}$Tc-MIBI accumulation in A2780.

**Cellular $^{99m}$Tc-MIBI accumulation and efflux in cell lines with different MRP expression**

After equal $^{99m}$Tc-MIBI accumulation of GLC₂ and GLC/ADR₁₀₀x at 10 min after starting the efflux study, the cellular content of $^{99m}$Tc-MIBI in GLC/ADR₁₀₀x was 46% compared with GLC₂. This illustrates that, at initially the same cellular $^{99m}$Tc-MIBI accumulation, increased efflux exists in GLC/ADR₁₀₀x compared with GLC₂.

Figure 1 shows that increasing doxorubicin resistance in GLC/ADR₁₀₀x, GLC/ADR₁₀₀x and GLC/ADR₁₀₀x coincided with a decreasing $^{99m}$Tc-MIBI accumulation after 1 h of $^{99m}$Tc-MIBI exposure. Compared with GLC₂, the cellular concentration of $^{99m}$Tc-MIBI was lower, being 35%, 20% and 1.5% in GLC/ADR₁₀₀x, GLC/ADR₁₀₀x and GLC/ADR₁₀₀x respectively. Under the same conditions, the cellular $^{99m}$Tc-MIBI accumulation was 0.13 ± 0.04

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Results

Cellular Glu-MIBI concentration (A) and exposure. Each point represents the mean ± s.d. of values obtained in three independent experiments, each performed in duplicate. At t = 10 min, the cellular Glu-MIBI concentration was lower in GLC4/ADR than in GLC4 (P < 0.025), there was no significant difference between GLC4/ADR and GLC4/ADR10, and the cellular concentration in GLC4/ADR100 was lower than in GLC4/ADR100 (P < 0.05). Figure 3.

Figure 4. Efflux of Glu-MIBI in S1 (C) and S1-MRP (O) cells. Glu-MIBI content was determined after 1-h Glu-MIBI incubation and at 10 min and 30 min with drug-free medium and expressed as a % of the radioactivity present after 1-h drug exposure. Each point represents the mean ± s.d. of three independent experiments, each performed in duplicate. At t = 10 min, the cellular Glu-MIBI concentration was lower in S1-MRP than in S1 (P < 0.0025).

Figure 5. Cell survival after 1-h incubation with Glu-MIBI in GLC4 (C) and GLC4/ADR100 (O). Each point represents the mean ± s.d. of two independent experiments, each performed in quadruplicate. From a concentration of 200 μM Glu-MIBI, significantly more cytotoxicity was observed in GLC4 than in GLC4/ADR100 (P < 0.0005).

Effects of glutathione depletion on accumulation and efflux of Glu-MIBI

Glutathione depletion by BSO followed by 1 h of Glu-MIBI exposure increased the Glu-MIBI concentration in GLC4 to 182% compared with undepleted GLC4 cells. In glutathione-depleted GLC4/ADR100 cells, the Glu-MIBI concentration increased even more. This increase resulted in a cellular Glu-MIBI concentration that differed, no longer significantly, from the cellular Glu-MIBI concentration in GLC4 (Figure 2).

Cell survival

Cell survival curves of GLC4 and GLC4/ADR100 cell lines after exposure to Glu-MIBI are shown in Figure 5. Over the Glu-MIBI concentration range tested, more cytotoxicity was observed in GLC4 than in GLC4/ADR100. The Glu-MIBI IC10 and IC25 were 50 μM and 85 μM, respectively, for GLC4 and 100 μM and > 300 μM, respectively, for GLC4/ADR100. A modulation effect of Glu-MIBI (IC10 and IC25) was observed on vincristine cytotoxicity in GLC4/ADR100, but not in GLC4. Isobologram analysis showed a synergistic effect of Glu-MIBI on vincristine cytotoxicity in GLC4/ADR100 (data not shown).

DISCUSSION

The present study demonstrates that in vitro Glu-MIBI is not only a substrate for P-gp but also for MRP. The earlier results of Piwnica-Worms et al. (1993), which suggest that Glu-MIBI is a substrate for P-gp, were confirmed. Co-incubation of Glu-MIBI and verapamil resulted in an increased cellular concentration of Glu-MIBI in the P-gp-overexpressing cell line A2780AD, while no effect was observed in the doxorubicin-sensitive parental cell line.

Evidence that Glu-MIBI is also a substrate for MRP has been obtained along various lines. In sublines of the human small-cell lung carcinoma cell line GLC4, with varying degrees of doxorubicin resistance and MRP content, it was shown that Glu-MIBI accumulation was lower when MRP expression increased. Versantvoort et al. (1995a) observed, just as for Glu-MIBI, a lower daunorubicin accumulation when MRP expression increased in the GLC4 cell lines. Increased MRP expression in...
these cell lines was also shown to correlate with increased \(^{99m}\text{Tc-MIBI}\) efflux. In addition, incubation of GLC\(_C\) and GLC/ADR\(_{150}\) with \(^{99m}\text{Tc-MIBI}\) at 4°C to reach equal accumulation in absolute terms confirmed increased \(^{99m}\text{Tc-MIBI}\) efflux with increased MRP expression. This indicates that \(^{99m}\text{Tc-MIBI}\) efflux is not dependent on the absolute cellular levels of \(^{99m}\text{Tc-MIBI}\), but efflux is dependent on the rate of transport per minute. Co-incubation of \(^{99m}\text{Tc-MIBI}\) with vincristine resulted in higher \(^{99m}\text{Tc-MIBI}\) levels in all GLC\(_C\) cell lines. The fact that \(^{99m}\text{Tc-MIBI}\) also increased by vincristine co-incubation in the parental GLC\(_C\) cell line can be explained by a low MRP expression in this cell line. The effects of vincristine are most likely due to partial blocking of \(^{99m}\text{Tc-MIBI}\) efflux by vincristine.

Doxorubcin resistance in GLC/ADR\(_{150}\) is multifactorial and considered to be due to an increase in MRP, an increased detoxification and decreased DNA topoisomerase II level (Zijlstra et al., 1987; Timmer-Bosscha et al., 1989; Simon et al., 1994). Therefore, we also analysed the effect of \(^{99m}\text{Tc-MIBI}\) on the non-small-cell lung carcinoma cell line S1 and its MRP-transfected subline S1-MRP. Just as in the GLC\(_C\) cell lines, a lower accumulation and increased efflux of \(^{99m}\text{Tc-MIBI}\) was observed in S1-MRP compared with the parental S1 cell line.

Recently, it was shown that MRP functions as a glutathione S-conjugate carrier (Jedlitschky et al., 1994; Leier et al., 1994; Müller et al., 1994). This finding has stimulated studies with MDR drugs in MRP-overexpressing cell lines after glutathione depletion with BSO (Meier et al., 1991; Versantvoort et al., 1995b; Zaman et al., 1995). Zaman et al. (1995) observed a complete reversal of resistance to doxorubicin, daunorubicin, vincristine and etoposide after glutathione depletion in the MRP-transfected cell line S1-MRP. Glutathione depletion also resulted in an increased \(^{99m}\text{Tc-MIBI}\) accumulation from MRP-transfected cells. These BSO effects were not observed in P-gp-overexpressing cell lines (Versantvoort et al., 1995b). In the present study, it was shown that glutathione depletion in GLC/ADR\(_{150}\) almost fully restored the cellular \(^{99m}\text{Tc-MIBI}\) level to the level obtained in GLC\(_C\). In addition, evaluation of the enhancing effects of \(^{99m}\text{Tc-MIBI}\) on vincristine cytotoxicity with isobologram analysis according to Steel and Peckman (1979) demonstrated that the blocking effects of \(^{99m}\text{Tc-MIBI}\) on vincristine cytotoxicity in GLC/ADR\(_{150}\) is synergistic. Cytotoxicity testing revealed cross-resistance for \(^{99m}\text{Tc-MIBI}\) between GLC/ADR\(_{150}\) and GLC\(_C\). The increased \(^{99m}\text{Tc-MIBI}\) efflux, the effects of glutathione depletion on cellular \(^{99m}\text{Tc-MIBI}\) levels and the cross-resistance for \(^{99m}\text{Tc-MIBI}\) as well as increased cytotoxicity of vincristine induced by \(^{99m}\text{Tc-MIBI}\) in MRP-overexpressing cells indicate that TC-MIBI is a substrate for MPR. TC-MIBI seems to behave in a way similar to that of the chemotherapeutic drugs involved in MRP-mediated MDR. Glutathione S-conjugates are transported by MRP (Ishikawa, 1992) and, based on experiments with inside-out vesicles, it is suggested that only substrates with a hydrophobic part and at least two negative charges can be transported by MRP. There is still much debate in the literature on how doxorubicin and vincristine are transported by MRP (Versantvoort et al., 1995b; Zaman et al., 1995). For these chemotherapeutic drugs, no glutathione S-conjugates have been identified. One possibility might be that these conjugates are unstable and therefore not detectable. Another hypothesis is that the chemotherapeutic drugs are co-transported with glutathione by the MRP pump (Jedlitschky et al., 1994; Leier et al., 1994; Müller et al., 1994; Zaman et al., 1995).

\(^{99m}\text{Tc-MIBI}\) is a synthetic \(\gamma\)-ray-emitting organotechnetium complex. In vitro, it is a substrate for both P-gp and MRP. Therefore, it might be used in vivo for functional efflux imaging in tumours. Efflux of \(^{99m}\text{Tc-MIBI}\) could then be extrapolated to the efflux of chemotherapeutic agents. This is of potential interest as it is known that in drug-resistant cells both P-gp and MRP can be overexpressed at the same time (Brock et al., 1995; Schuurhuis et al., 1995). For P-gp, several blockers are known, such as verapamil and cyclosporin A, which enables the study of the functional efflux inhibition of \(^{99m}\text{Tc-MIBI}\) by these blockers from P-gp-positive tumours. We are aware of the fact that verapamil and cyclosporin A are not specific inhibitors for P-gp and affect to some extent MRP (Twentyman et al., 1996). For MRP, it is suggested that probenecid and sulfisoxazone may be useful as specific reversal agents for MRP-mediated drug resistance (Evers et al., 1996). Therefore, clinical studies focusing on inhibition of \(^{99m}\text{Tc-MIBI}\) efflux with such compounds from MRP-positive tumours could be a topic of further investigation.

**ABBREVIATIONS**

ATP, adenosine triphosphate; BSO, d,l-buthionine S,R-sulphoximine; DMEM, Dulbecco’s modified Eagle medium; DMP, dose-modifying factor; FCS, fetal calf serum; IC\(_{10}\), drug concentration inhibiting survival by 10%; IC\(_{50}\), drug concentration inhibiting survival by 25%; MDR, multidrug resistance; MRP, multidrug resistance-associated protein; MTA, microculture tetrazolium assay; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; P-gp, P-glycoprotein; PBS, phosphate-buffered saline (0.14 M sodium chloride, 2.7 mM potassium chloride, 6.4 mM disodium hydrogen phosphate, 1.5 mM potassium dihydrogen phosphate, pH 7.4); RPMI 1640, Roswell Park Memorial Institute 1640; Tc, Technetium; \(^{99m}\text{Tc-MIBI}\), \(^{99m}\text{Tc-sestamibi}; \(^{99m}\text{Tc-MIBI}\), \(^{99m}\text{Tc-sestamibi}; \(^{99m}\text{Tc-MIBI}\), \(^{99m}\text{Tc-sestamibi}.

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