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Multidrug resistance phenotype in leukaemic cells from patients with acute myelocytic leukaemia can be detected with $^{99}Tc^{m}$-MIBI

A Gruber[1,2], I Areström[2], D Xu[1], J Liliemark[2,3], SA Larsson[4] and H Jacobsson[5]

Departments of [1]Hematology and Infectious Diseases, [2]Clinical Pharmacology, [3]Oncology, [4]Nuclear Medicine and [5]Radiology, Karolinska Hospital, S-171 76 Stockholm, Sweden

Summary The aim of the study was to investigate whether $^{99}Tc^{m}$-MIBI (Cardiolite), recently shown to be a substrate for P-glycoprotein, has the potential to be used as a marker for mdr1 gene expression and whether cyclosporin A (CyA) can modify its accumulation in vivo. Leukaemic cells from ten patients with acute myelocytic leukaemia (AML) were used, five with undetectable mdr1 gene expression and five with mdr1 mRNA levels ranging from 1.0 to 3.8 mdr1 mRNA transcripts per cell. Cells were incubated with $^{99}Tc^{m}$-MIBI, or with daunorubicin (Dnr), with and without 3 μM CyA. The median $^{99}Tc^{m}$-MIBI accumulation (% of added radioactivity) in mdr1-negative cells was 0.89% and in the mdr1-positive cells 0.34%, $P = 0.01$. In mdr1-negative cells, the median increase in $^{99}Tc^{m}$-MIBI accumulation with CyA was 30% compared with the mdr1-positive cells with a median increase of 242%, $P = 0.009$. CyA had no significant effect on Dnr accumulation in four of the mdr1-negative samples. The median increase of Dnr accumulation in the mdr1-positive cells was 40%. The results show that $^{99}Tc^{m}$-MIBI with a high sensitivity can detect rather low levels of mdr1 gene expression in clinical samples. Consequently, $^{99}Tc^{m}$-MIBI scintigraphy has the potential to be used for monitoring the effect of resistance modifiers on the accumulation and retention of cytostatic drugs in human tumours in vivo.

Keywords: multidrug resistance; P-glycoprotein; $^{99}Tc^{m}$-MIBI; drug transport

P-glycoprotein, encoded by the mdr1 gene causes classical multidrug resistance (MDR). This is characterized by resistance of tumour cells to a wide variety of anti-cancer drugs. P-glycoprotein causes cellular efflux of such drugs, a process that can be reversed by so-called resistance modifiers, e.g. verapamil, cyclosporins and quinidine (Fojo, 1991).

A large proportion of human tumour types have been investigated for mdr1 gene expression, which was initially described in drug-selected cell lines. Acute leukaemias have been extensively studied, and in several studies mdr1 gene expression in acute myelocytic leukaemia (AML) was an adverse prognostic factor (Campos et al, 1992; Te Boekhorst et al, 1995; Leith et al, 1997). However, there are also studies that could not confirm this finding (Ino et al, 1994). Mdr1 gene expression has also been detected in lymphomas and in solid tumours, such as breast cancer, ovarian cancer and osteosarcomas, but its relationship to treatment results is less clear (Goldstein et al, 1992; Arao et al, 1994; Yuen and Sikic, 1994; Lee et al, 1996; Linn et al, 1996). In small clinical studies of AML and multiple myeloma, promising results have been reported when resistance modifiers have been added to chemotherapy, and trials are under way to investigate whether this can improve treatment results (Sonneveld et al, 1992; List et al, 1993). Clinical studies in solid tumours are, so far, mostly phase I or contain few patients and are therefore not conclusive (Raderer and Scheithauer, 1993).

More recently, it was shown that multidrug resistance can also be conferred by the transport protein multidrug resistance-associated protein (mrp) (Cole et al, 1992). Its clinical relevance is still unclear but there have been studies that have demonstrated an increase of mrp expression in relapsed AML (Hart et al, 1994; Schneider et al, 1995).

The radiopharmaceutical $^{99}Tc^{m}$-hexaxis-2-methoxyisobutyl isonitrile ($^{99}Tc^{m}$-MIBI, $^{99}Tc^{m}$-Sestamibi, Cardiolite), originally developed for myocardial scintigraphy, has been shown to be a substrate for P-glycoprotein (Piwnica-Worms et al, 1993). In cell lines with different levels of P-glycoprotein expression, the accumulation of $^{99}Tc^{m}$-MIBI and the effect of resistance modifiers were proportional to the level of P-glycoprotein expression (Ballinger et al, 1995; Piwnica-Worms et al, 1995).

The level of mdr1 gene expression in tumours is, however, lower than in drug-selected cell lines. Using a quantitative RNAase protection assay, we found the median level of mdr1 mRNA in positive cell samples from patients with AML to be 0.7 mdr1 mRNA transcripts per cell (Gruber et al, 1992). In two vincristine-selected K562 cell lines, the levels were approximately 100 and 200 transcripts per cell.

The aim of the present study was to investigate whether $^{99}Tc^{m}$-MIBI can be used to detect the rather low mdr1 gene expression found in human tumours. We therefore compared the accumulation of $^{99}Tc^{m}$-MIBI and the effect of cyclosporin A (CyA) in leukaemic cell samples with and without mdr1 gene expression. We also investigated whether the effect of CyA on cellular $^{99}Tc^{m}$-MIBI accumulation was similar to the effect on daunorubicin (Dnr).

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Correspondence to: A Gruber, Department of Hematology and Infectious Diseases, Karolinska Hospital, S-171 76 Stockholm, Sweden

MATERIALS AND METHODS

Cell lines

The human leukaemic cell line K562, two vincristine-selected sublines grown in 30 and 150 nM vincristine (K562/Vcr30, K562/Vcr150) and a mitoxantrone-resistant subline grown in mitoxantrone 100 ng ml^{-1} (K562/Mxn) were used. K562/Vcr30 and K562/Vcr150 expressed approximately 100 and 200 mdr1 mRNA transcripts per cell, respectively, as determined by a quantitative RNAase protection assay (Gruber et al, 1992). Mdr1 mRNA was detected in solution with a [^{35}S]UTP-labelled 403-nucleotides antisense probe and quantification was performed by comparison with a standard curve, generated by hybridizations with increasing amounts of in vitro transcribed sense RNA. The maternal line and K562/Mxn had no detectable mdr1 mRNA.

Leukaemic cells

Peripheral leukaemic cells from ten patients with AML were used. Cells isolated on Lymphoprep (Nycomed, Pharma, AS, Norway) were frozen in a programmed freezer and kept in liquid nitrogen. The patients, peripheral white blood cell counts ranged from 32 to 363×10^9 l^{-1} (median 80×10^9 l^{-1}), and the percentage of leukaemic cells was between 70% and 100% (median 90%). The viability of the cells after thawing was controlled with trypan blue exclusion and was 70% and 73% in two samples; in the remaining, the viability was over 85%. The samples were chosen according to their mdr1 mRNA expression, which was determined earlier with a quantitative RNAase protection assay (Gruber et al, 1992). Cells from five of the patients had no detectable mdr1 mRNA levels, and cells from five patients had mdr1 mRNA levels ranging from 1.0 to 3.8 mdr1 mRNA transcripts per cell. That the function of P-glycoprotein in thawed leukaemic cells is comparable to that in fresh cells has been demonstrated by Broxterman and co-workers (Broxterman et al, 1996).

The mdr1 mRNA expression and also the mrp mRNA expression was reanalysed with a quantitative reverse transcription polymerase chain reaction (RT-PCR) method with some modifications (Xu et al, 1996). By this method, the median mdr1 mRNA expression in the samples found to be negative with the RNAase protection assay was 0.04 transcripts per cell (range 0.01–0.28) and in the positive samples 8.8 transcripts per cell (range 1.4–15.8). Mrp expression was positive in all samples, median 2.3 transcripts per cell (range 0.5–4.0) in mdr1 mRNA-negative samples and 3.1 transcripts per cell (range 1.5–4.2) in the mdr1 mRNA-positive samples, $P = 0.2$.

Incubation of cells with $^{99}Tc^{m}$-MIBI and daunorubicin

From cell lines and patients, 0.5 and 1.0×10^6 cells, respectively, were incubated in triplicates for 1 h at 37°C in 1 ml of RPMI 1640 medium (Gibco, Glasgow, UK), supplemented with 10% newborn calf serum and 2 mM L-glutamine, with $3–5 \times 10^6$ c.p.m. $^{99}Tc^{m}$-MIBI (Du Pont, Stevenage, UK). The incubations were performed with and without CyA 3 μM (Sandoz, Basle, Switzerland). Cell line cells were also incubated with higher concentrations of CyA (10 and 20 μM). The incubations were stopped by centrifugation at 4°C for 5 min. After two washes with ice-cooled phosphate-buffered saline (PBS), the activity of the cells was assessed with a well-type gamma-counter (1282 Compugamma, LKB-Wallac,

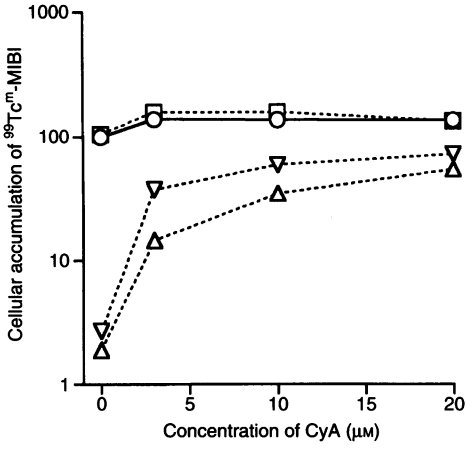

Figure 1 Accumulation of $^{99}Tc^{m}$-MIBI in K562 (—○—), K562/Mxn (.....□.....), K562/Vcr30 (.....▽.....) and K562/Vcr150 (....△....) and effect of CyA 3, 10 and 20 μM. The cellular $^{99}Tc^{m}$-MIBI accumulation is expressed as a percentage of the accumulation in K562 without CyA

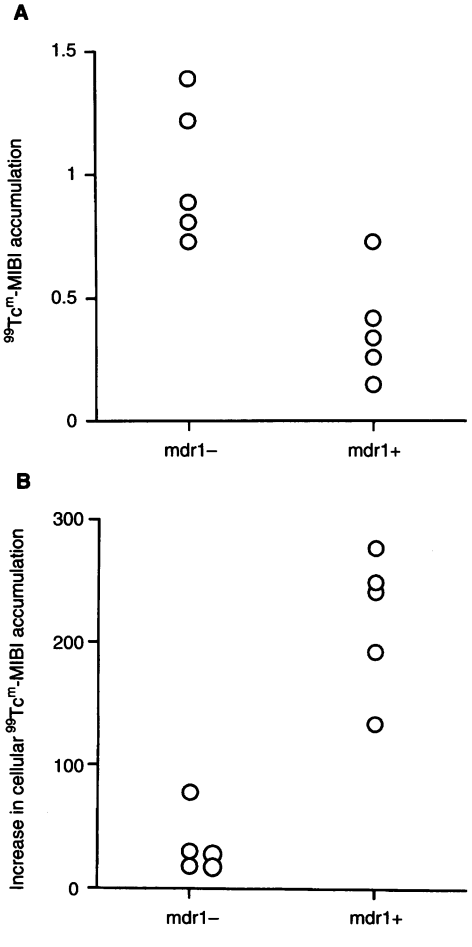

Figure 2 (**A**) Accumulation of $^{99}Tc^{m}$-MIBI in five mdr1 mRNA-negative leukaemic cell samples (median 0.89%) and in five with mdr1 RNA levels ranging from 1.0 to 3.8 transcripts per cell (median 0.34%), $P = 0.012$. The cellular $^{99}Tc^{m}$-MIBI accumulation is expressed as a percentage of added radioactivity. (**B**) Per cent increase of $^{99}Tc^{m}$-MIBI accumulation in mdr1 mRNA-negative (median 30%) and -positive (median 242%) samples with 3 μM CyA, $P = 0.009$

Bromma, Sweden). Correction was made for decay of $^{99}Tc^m$ during the measuring time to obtain a maximal statistical uncertainty of 3%, indicated as one standard deviation.

Approximately 2.0×10^6 of the patient and cell line cells were incubated in duplicates for 1.5 h at 37°C in 2 ml of medium containing 1 μM Dnr with and without CyA 3 μM. Dnr incubations were stopped by addition of 5 ml of ice-cooled PBS to the tubes and centrifugation at 4°C for 5 min. After two washing steps with PBS, the cellular Dnr content was analysed with high-performance liquid chromatography (Baurin et al, 1978).

Statistical analyses

The differences in $^{99}Tc^m$-MIBI and Dnr accumulation, and effects of CyA in mdr1-negative and -positive patient samples were analysed using the Mann-Whitney test. Correlations between the effect of CyA on cellular $^{99}Tc^m$-MIBI and Dnr accumulation were analysed with linear regression analysis. A P-value of < 0.05 was set as significant.

RESULTS

Accumulation of $^{99}Tc^m$-MIBI and daunorubicin in cell line cells

K562 cells accumulated 3.1% of added $^{99}Tc^m$-MIBI compared with K562/Vcr150, which accumulated only 0.06%. The accumulation of $^{99}Tc^m$-MIBI in K562/Vcr150 cells was only 1.9% of that in K562 and in K562/Vcr30, 2.7% of that in K562. The accumulation of $^{99}Tc^m$-MIBI in the mitoxantrone-resistant cell line was equal to that in the maternal line (Figure 1).

CyA 3 μM increased $^{99}Tc^m$-MIBI accumulation in K562/Vcr150 with 662% from 1.9% to 14.5% of that in K562 without CyA, and in K562/Vcr30 with 1342% from 2.7% to 37.3% of that in K562. CyA, 10 and 20 μM, further increased the $^{99}Tc^m$-MIBI accumulation in the resistant cell lines but not reaching the same level as in K562. In the maternal line and in K562/Mxn, the increase caused by CyA was the same at all three concentrations, approximately 40% and 50% respectively (Figure 1).

The accumulation of Dnr in K562/Vcr150 was 29% of that in K562. CyA 3 μM increased Dnr accumulation in the resistant line to the same level as that in K562 without CyA. CyA 3 μM increased Dnr accumulation with 13% in K562 cells (not shown).

Accumulation of $^{99}Tc^m$-MIBI and daunorubicin in patient leukaemic cells

The median accumulation of $^{99}Tc^m$-MIBI in the five cell samples from patients with undetectable mdr1 mRNA expression was 0.89% of the input (range 0.73–1.39), compared with the five samples positive for mdr1 mRNA, which accumulated 0.34% (range 0.15–0.73; $P = 0.012$) (Figure 2A).

The median increase in $^{99}Tc^m$-MIBI accumulation caused by CyA 3 μM in mdr1 mRNA-negative samples was 30% (range 17–78) compared with the mdr1 mRNA-positive samples, in which the median increase was 242% (range 134–278; $P = 0.009$) (Figure 2B).

The accumulation of Dnr in 2×10^6 patient cells varied between 0.21 and 0.97 nmol. There was a trend towards lower Dnr accumulation in mdr1-positive samples compared with mdr1-negative samples [mean 0.39 nmol (s.d. 0.215) vs 0.56 nmol (s.d. 0.285); $P = 0.25$]. The increase in Dnr accumulation with CyA 3 μM in mdr1-negative cells was 74% in one sample. In the remaining four it was 0, 4, 4 and 10%. The median increase of Dnr in the mdr1-positive cells was 40% (range 19–68%; $P = 0.11$) (Figure 3).

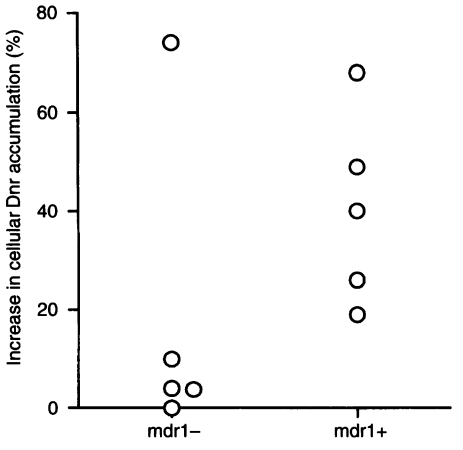

Figure 3 Per cent increase of Dnr accumulation in mdr1 mRNA-negative (median 4%) and -positive (median 40%) samples with 3 μM CyA, $P = 0.12$

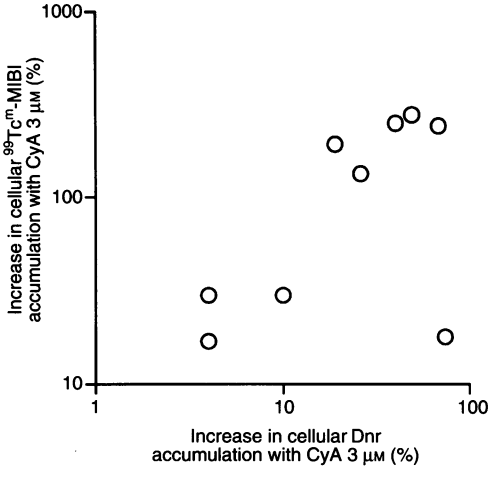

Figure 4 Relationship between the effect of 3 μM CyA on cellular accumulation of Dnr and $^{99}Tc^m$-MIBI in ten leukaemic cell samples, $r = 0.45$, $P = 0.19$

In nine of the samples, there was a strong correlation between the increase caused by CyA 3 μM on $^{99}Tc^m$-MIBI and Dnr accumulation ($r = 0.87$, $P = 0.0023$). Because of a large effect on Dnr accumulation (74% increase) in one mdr1-negative sample, the correlation was not statistically significant for all ten samples, $P = 0.19$ (Figure 4).

DISCUSSION

The results of this study show that the difference in cellular $^{99}Tc^m$-MIBI accumulation between K562 and the mdr1 gene-expressing K562/Vcr150 was much larger than the difference in Dnr accumulation. $^{99}Tc^m$-MIBI accumulation in K562/Vcr150 was only 1.9% of that in K562 compared with Dnr accumulation, which was 29%. CyA 3 μM restored Dnr accumulation in K562/Vcr150 to the same

level as that in the maternal line, while $^{99}Tc^{m}$-MIBI by the same CyA concentration was increased to only 14.5% of that in K562. Consistent with the results of Piwnica-Worms and co-workers (1993), the increase in $^{99}Tc^{m}$-MIBI accumulation caused by CyA 3 μM was larger in K562/Vcr30, with a lower degree of resistance than in K562/Vcr150. The results confirm the very high affinity of $^{99}Tc^{m}$-MIBI to P-glycoprotein and its high sensitivity to detect P-glycoprotein expression.

The high affinity of $^{99}Tc^{m}$-MIBI to P-glycoprotein is confirmed by the fact that the cellular accumulation of $^{99}Tc^{m}$-MIBI was much lower in the mdr1 gene-expressing human leukaemic cell samples than in the samples with undetectable mdr1 expression. In contrast, for Dnr, there was only a non-significant trend towards lower accumulation in mdr1-positive than in mdr1-negative samples. In parallel, the increase in cellular accumulation caused by CyA in mdr1-positive samples was much larger for $^{99}Tc^{m}$-MIBI than for Dnr (median 242% vs 40%). Although the increase caused by CyA 3 μM was much larger for $^{99}Tc^{m}$-MIBI than for Dnr, in nine of the samples there was a correlation between the effect on the two, which is a prerequisite for the use of $^{99}Tc^{m}$-MIBI in vivo for functional monitoring of P-glycoprotein activity. In one mdr1 mRNA-negative sample, CyA 3 μM increased the cellular Dnr accumulation by 74%, while the increase for $^{99}Tc^{m}$-MIBI was only 18%. One explanation for this discrepancy could be the existence of other transport proteins for which $^{99}Tc^{m}$-MIBI is not a substrate. Dnr is also transported by mrp. Whether $^{99}Tc^{m}$-MIBI is a substrate for mrp is unknown. However, all our samples were positive for mrp expression, in the case in question four transcripts per cell. Moreover, CyA was shown to be a rather poor modifier of reduced Dnr accumulation in mrp-positive cells (Barrand et al, 1993). Consequently, it seems likely that the increase in Dnr accumulation caused by CyA in this sample is a result of mechanisms other than mrp expression.

The potential use of $^{99}Tc^{m}$-MIBI as a marker for P-glycoprotein and the effect of resistance modifiers in vivo has recently been demonstrated by Luker and co-workers (1997). $^{99}Tc^{m}$-MIBI scintigraphy with and without the resistance modifier PSC-833, a cyclosporin D analogue, was performed on three patients. With administration of the modifier, $^{99}Tc^{m}$-MIBI was selectively retained in the liver and kidneys, two organs with high expression of P-glycoprotein. In a study of patients with untreated breast cancer, the efflux rate of $^{99}Tc^{m}$-MIBI was faster from tumours with high than from those with low P-glycoprotein expression (Vecchio et al, 1997).

The results of $^{99}Tc^{m}$-MIBI scintigraphy before chemotherapy have also been related to treatment response in a few patients with breast cancer (Moretti et al, 1996) and malignant lymphomas. Kapucu and co-workers (1997) found, in a study of 24 children with untreated malignant lymphomas, that children with positive scans responded better to chemotherapy than those with negative scans.

The results of trials in solid tumours when resistance modifiers were added to chemotherapy are often difficult to interpret. Only some patients seem to respond (Raderer and Scheithauer, 1993). An assay that can monitor P-glycoprotein function and the effect of resistance modifiers would be a tool to select, for example, patients with malignant lymphoma who may benefit from addition of resistance modifiers to chemotherapy (Miller et al, 1991; Sarris et al, 1996).

In summary, our results show that $^{99}Tc^{m}$-MIBI is very sensitive in detecting mdr1 gene expression at the low, but probably clinically relevant, levels that are present in human tumour cells. Secondly, the effect of CyA 3 μM on cellular $^{99}Tc^{m}$-MIBI accumulation seems to reflect the effect on cytostatic drugs (at least daunorubicin).

$^{99}Tc^{m}$-MIBI is a well-established radiopharmaceutical for myocardial scintigraphy used routinely world-wide since about 1990. As is the case for most radiopharmaceuticals, the amount of chemical substrate administered is very low, and adverse reactions are rare. The effective dose equivalent of a typical administered activity of 500 MBq is 7 mSv compared with approximately 5 mSv for an abdominal computerized tomography examination.

Consequently, $^{99}Tc^{m}$-MIBI scintigraphy has the potential of being used to monitor the effect of resistance modifiers on the accumulation and retention of cytostatic drugs in human tumours, e.g. lymphomas, in vivo. The use of $^{99}Tc^{m}$-MIBI scintigraphy in clinical trials in which resistance modifiers are added to chemotherapy will answer whether it can be used to predict the efficacy of such treatment.

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