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SHORT COMMUNICATION

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Increased cation transport in *mdr1*-gene-expressing K562 cells

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Abstract Cation-transport properties were compared in a human leukemic cell line (K562) and its vincristine-selected, *mdr1*-gene-expressing sublines (K562/Vcr30 and K562/Vcr150) by the capacity of the cells to accumulate the potassium analogue thallium (^{201}Tl). Determination of the time course of thallium accumulation in the absence and presence of ouabain, an inhibitor of sodium-potassium adenosine triphosphatase (ATPase), showed that the initial (at 20 min) rate of ouabain-resistant uptake was about 70% higher in the K562/Vcr30 cells than in the parental line. The maximal rate (V_{max}) of ouabain-resistant uptake was 78 mmol/h for K562 cells and 115 mmol/h for K562/Vcr30 cells, and the Michaelis constant (K_m) was 0.37 and 0.18 mmol, respectively. Bumetanide (50 μ*M*), a specific inhibitor of ouabain-resistant Na-K-Cl cotransport, inhibited the elevated ^{201}Tl uptake in K562/Vcr150 cells but had no effect on cellular vincristine accumulation. Incubation with different multidrug resistance (MDR)-reversing agents (verapamil as well as cyclosporin A and its analogue PSC833) had no significant effect on ^{201}Tl uptake. Membrane depolarization by an elevation of the potassium concentration in the incubation medium did not affect vincristine accumulation in any cell line, which indicated that the changed drug-transport properties in *mdr1*-gene-expressing cells were not due to membrane hyperpolarization. It was concluded that P-glycoprotein-positive cells have a more efficient ouabain-resistant cation-transport mechanism than to cells without P-glycoprotein. A functional relationship between this phenomenon and MDR was not identified.

Key words Multidrug resistance · Cation transport Thallium · Bumetanide · Vincristine

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Introduction

Primary and secondary resistance to cytotoxic drugs are serious problems in the treatment of malignancies. Secondary resistance is readily reproduced in transplanted animal tumors and cultured cells. Multidrug resistance (MDR) has been intensively studied in cell lines and is characterized by cross-resistance to several antineoplastic drugs of natural origin but with different chemical structures and mechanisms of action [9]. In classic MDR, the resistant cells accumulate less drug due to the membrane-pump function of P-glycoprotein encoded by the *mdr1* gene. A number of noncytotoxic drugs of different chemical structures (e. g., verapamil, cyclosporins) reverse resistance by inhibiting the pump function of P-glycoprotein. Since the chemical structures of the drugs transported by the P-glycoprotein are very different [9], it is not easy to fit the drug P-glycoprotein interaction into a classic ligand-receptor mechanism.

The P-glycoprotein resembles the cystic fibrosis transmembrane regulator (CFTR) with regard to both primary sequence and predicted topological structure [2, 20]. CFTR functions as a chloride channel, and it has been reported that the expression of P-glycoprotein generates adenosine triphosphate (ATP)-dependent, chloride-selective ion channels [22]. To compare membrane-transport properties between P-glycoprotein-negative and -positive cells, we studied cation transport in the human leukemic cell line K562 [16] and two vincristine-selected sublines, K562/Vcr30 and K562/Vcr150 [12], by measuring the cellular accumulation of the potassium analogue thallium.

Materials and methods

Cell lines

Cell lines were grown in medium RPMI 1640 (Gibco, Paisly, Scotland) supplemented with 10% fetal serum, 1% L-glutamine, and antibiotics. The vincristine-resistant sublines were grown in medium containing 30 (K562/Vcr30) or 150 n*M* (K562/Vcr150) vincristine, respectively. The resistant cells were cultured in the absence of vincristine during one passage before all experiments. As determined by a previously described quantitative RNA-RNA solution hybridization technique, the resistant cell lines contained about 100 and 200 *mdr1* RNA transcripts/cell, respectively, whereas no *mdr1* RNA could be detected in the parental line [12]. The mean volume of the cells from the three lines was similar, being 1.00×10^{-12}, 1.00×10^{-12}, and 1.05×10^{-12} for K562, K562/Vcr30, and K562/Vcr150 cells, respectively, as measured by a Coulter multisizer (Coulter Counter, England).

Cation-transport experiments

The cellular uptake of ^{201}Tl (thallous chloride; Dupont Scandinavia AB, Stockholm, Sweden) was measured after various periods of incubation (5–120 min) at 22 °C or 37 °C in cell-culture medium (as described above) or in buffered salt solutions (155 m*M* NaCl, 2.0 m*M* $CaCl_2$, 10.0 m*M* HEPES titrated with NaOH to pH 7.4) and in the presence of different potassium or nonradioactive thallium concentrations. The effect of cation-transport inhibitors (ouabain and bumetanide) and MDR-reversing agents (verapamil, cyclosporin A, and PSC833) was analyzed. A detailed description of the method is given in the report on a recent study of human lymphocytes [5]. The experiments were performed in parallel on 10 aliquots each of sensitive and resistant cell lines (1–2 million cells/test tube) suspended in 5 ml solution. Approximately 0.5 MBq of ^{201}Tl (as TlCl in 0.1 ml isotonic NaCl) was added to each tube. The incubations were terminated by three steps of centrifugation and resuspension in 5 ml cold (7 °C) potassium-free buffered salt solution. The cellular accumulation of ^{201}Tl was calculated from the radioactivity of the cell pellet and of the incubation medium and was expressed as the ratio between intracellular and extracellular concentrations.

Vincristine accumulation

Cells were incubated at 37 °C in RPMI 1640 medium with [^{3}H]-vincristine (10 n*M*; 5.6 Ci/mmol, Amersham, England). After 2 h 5-ml samples (1.5×10^6 cells) were withdrawn in duplicate and washed twice, and the pellets were kept at –20 °C until the day of analysis. The cell pellets were lysed by the addition of 2 *M* NaOH and then neutralized with HCl. The radioactivity was determined in a liquid scintillation counter after the addition of 10 ml scintillation liquid (Picofluor, Packard, USA).

Results

The time course of ^{201}Tl accumulation in K562 and K562/Vcr30 cells is shown in Fig. 1. After incubation for 120 min the accumulation ratio (intracellular versus extracellular concentration) was 45±7 in K562 cells and 50±8 in K562/Vcr30 cells (P = 0.24, not significant). Inhibition of the Na, K-ATPase activity with ouabain (1.0 m*M*) reduced the ^{201}Tl accumulation by 78% in both cell types. Comparison of the uptake curves generated in the absence/presence of ouabain at incubation periods of 10–60 min demonstrated a higher uptake in K562/Vcr30 cells that was due to an increase in the ouabain-resistant fraction of ^{201}Tl

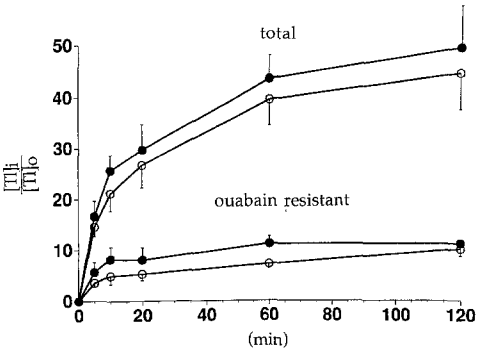

Fig. 1 Time course of ^{201}Tl uptake in K562 (*open circles*) and K562/Vcr30 cells (*filled circles*). Accumulation is given as the ratio between the intracellular and the medium concentrations. Incubation was carried out at 37 °C in the absence and presence of ouabain (1 m*M*). Data represent mean values ± SD (n = 7)

accumulation. The difference was most pronounced during the initial part of the incubation period (10–20 min), when K562/Vcr30 cells had 70% higher ^{201}Tl uptake than did K562 cells.

Previous studies of human erythrocytes in solutions with low potassium concentrations have shown that thallium stimulates the Na/K pump with maximal effect at 0.1 m*M* [6]. At lower concentrations the pump is inhibited, which is explained by the cooperativity of two potassium or thallium ions on the transport site. A similar effect has been observed in human lymphocytes incubated in solutions with different thallium concentrations [5].

The ^{201}Tl uptake was measured in the absence and presence of ouabain (1 m*M*), when the cells had been incubated for 20 min at 37 °C in potassium-free medium with different thallium (nonradioactive) concentrations. Figure 2 demonstrates that ouabain-sensitive uptake was 27% higher in K562/Vcr30 cells at 0.01 m*M* thallium (P = 0.011) but was not significantly different at lower or higher thallium concentrations, and it showed a similar dependence on thallium concentration in both cell types, with a peak occurring at 0.1 m*M*. A larger difference was seen in ouabain-resistant ^{201}Tl uptake at low thallium concentrations. It was 73% higher in the absence of thallium (P = 0.0003) and 50% higher at 0.01 m*M* thallium (P = 0.0014) in K562/Vcr30 cells as compared with the parental line. In a preliminary series of experiments performed in the absence of ouabain with 120 min of incubation at 22 °C in potassium- and thallium-free solutions (except for ^{201}Tl) the ^{201}Tl accumulation ratio was 244±18 (mean±SEM) in K562 cells, 257±22 in K562/Vcr30 cells, and 302±20 in K562/Vcr150 cells (n = 13), suggesting that the increase in ^{201}Tl accumulation was related to the degree of vincristine resistance.

Figure 3 shows a double reciprocal plot of ouabain-resistant Tl uptake versus Tl concentrations in K562 and K562/Vcr30 cells. The V_{max} was 78 mmol/h for the parental line and 115 mmol/h for the resistant line, and the K_m was 0.37 and 0.18 mmol, respectively. Human lymphocytes have an ouabain-resistant mechanism for Tl uptake that is carried by Na-K(Tl)-Cl cotransport [4]. This mechanism

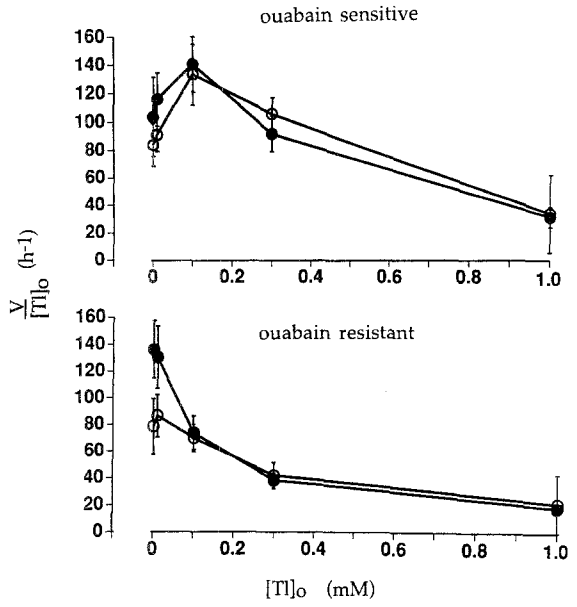

Fig. 2 Effect of thallium (nonradioactive) concentration on the rate of ouabain-sensitive (*top*) and ouabain-resistant (*bottom*) ^{201}Tl accumulation in K562 (*open circles*) and K562/Vcr30 cells (*filled circles*). The accumulation rate (V) is given as a ratio relative to the thallium concentration of the medium. Measurements were made after 20 min of incubation at 37 °C in a potassium-free salt solution in the absence and presence of ouabain (1 m*M*). The ouabain-sensitive uptake was calculated from the difference in ^{201}Tl accumulation observed between cells incubated in the absence and presence of ouabain. Data represent mean values ± SD (n = 7)

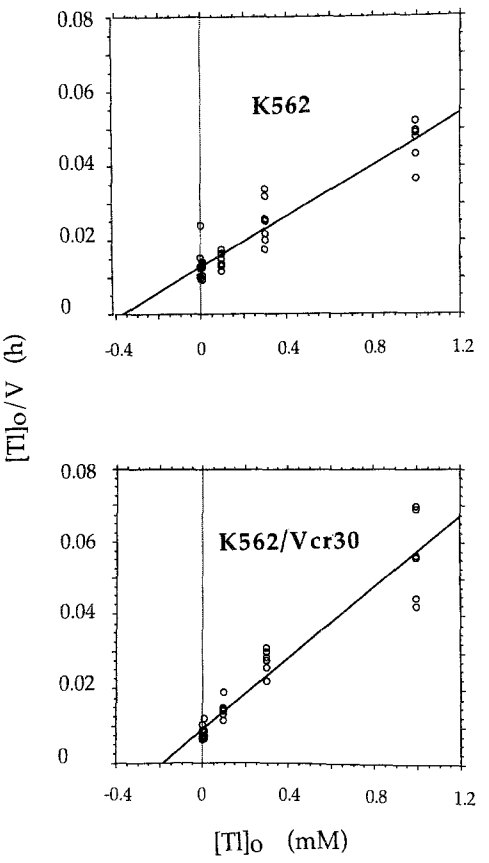

Fig. 3 Reciprocal plot of the rate of ouabain-resistant ^{201}Tl accumulation (V) versus the thallium (nonradioactive) concentration of the incubation medium in K562 (*top*) and K562/Vcr30 cells (*bottom*). Data were taken from Fig. 2. The *straight lines* represent $[Tl]_o/V = K_m/V_{max} + [Tl]_o/V_{max}$ where V_{max} is the maximal rate and K_m is the $[Tl]_o$ at which $V = \frac{1}{2}V_{max}$. Regression analysis indicated that V_{max} was 78 mmol/h for K562 cells and 115 mmol/h for K562/Vcr30 cells and that the K_m was 0.37 and 0.18 mmol, respectively ($P < 0.0001$)

has been identified in many types of cells and is inhibited by loop diuretics such as bumetanide (see [11]). In 50 μ*M* bumetanide the ^{201}Tl uptake decreased by 19% in K562/Vcr150 cells and by 2.5% in K562 cells (mean of triplicate samples).

The relationship between the increase in ^{201}Tl accumulation in K562/Vcr cells and the phenomenon of vincristine resistance was studied with different MDR-reversing agents. Incubations with cyclosporin A (3 μ*M*), verapamil (10 μ*M*), or PSC 833 (3 μ*M*) for 20 min at 37 °C prior to the ^{201}Tl incubation had no significant effect on the ^{201}Tl uptake in either K562 or K562/Vcr150 cells as measured after 20 min (n = 9, each determination carried out in duplicate or triplicate). Longer periods of incubation (90 min) with cyclosporin A (3 μ*M*) before ^{201}Tl incubation (for 90 min at 22 °C; n = 13, each determination performed in triplicate) decreased the uptake by 23% ± 7% (mean ± SEM) in K562/Vcr150 cells and by 2% ± 9% in K562 cells.

The cellular accumulation of vincristine measured in the K562/Vcr30 and K562/Vcr150 lines was 31% and 24%, respectively, of that detected in the parental line. The effect of membrane depolarization on vincristine accumulation in the cell lines was tested by increasing the potassium concentration in the medium from 5 to 10 m*M*, which corresponds to a depolarization of the membrane by approximately 15 mV [14]. However, this did not affect vincristine accumulation in any of the cell lines. Bumetanide (50 μ*M*) had no significant effect on vincristine accumulation in either K562 or K562/Vcr150 cells.

Discussion

The present study demonstrates that the rate of ^{201}Tl uptake is elevated in vincristine-selected, *mdr1*-gene-expressing cells as compared with parental cells with undetectable *mdr1* gene expression. Inhibition of the Na,K-ATPase-dependent transport system with ouabain indicated that the difference in cation transport was largely ouabain-resistant. The elevated ^{201}Tl uptake in K562/Vcr150 cells was inhibited by bumetanide, which is a specific inhibitor of Na-K-Cl cotransport.

Previously, a decreased rate of ouabain-sensitive ^{86}Rb uptake has been reported in a doxorubicin-resistant murine leukemia cell line (P388), indicating a decrease in the Na,K-ATPase activity in resistant cells [4]. The authors also found that either inhibition of Na,K-ATPase activity or depolarization *stimulated* the uptake of doxorubicin in sensitive cells but did not do so in resistant cells. Hasmann and co-workers [13] used fluorescent probes to measure membrane potential and found that doxorubicin-resistant cells accumulated only one-fourth as much of the probe as

did the sensitive parental cells. This large difference suggested a near-complete depolarization of the membrane in the resistant cells. Evidence from patch-clamp analysis of increased activity of the volume-regulated Cl^- channels, related to the level of expression of the *mdr-1* gene [22] has not been confirmed [8, 19], and a functional relationship between the volume-regulated Cl currents and P-glycoprotein-mediated transport has not been identified [1].

Several studies have reported a higher intracellular pH in resistant cells as compared with sensitive ones [3, 15, 17]. Although the amiloride-sensitive Na/H antiport was more efficient in mammalian MDR cells, blocking of the antiport with amiloride did not increase the drug sensitivity and reversal of MDR with amiloride did not affect the Na/H antiport activity [3]. Clearly, the present observation of elevated ^{201}Tl uptake, indicative of an elevated inward transport of potassium, may be related to other abnormalities in these cells such as changes in pH and in the Na/H antiport.

Studies in human lymphocytes have demonstrated that ouabain-resistant transport of potassium or its analogues (thallium and rubidium) is due to Na-K-Cl cotransport [5, 10]. This transporter seems to be important for volume regulation in lymphocytes as in many types of cells, e. g., muscle and epithelial cells [7, 10, 18]. However, blocking of this transport with bumetanide did not normalize the vincristine accumulation in K562/Vcr cells, nor was the high ^{201}Tl uptake in K562/Vcr cells normalized by MDR-reversing agents (cyclosporin A, verapamil, and PSC 833 [21]) as measured at short incubation periods. It is therefore concluded that the increase in ouabain-resistant cation transport and in drug transport are separate phenomena in these cells.

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