Properties of Amino Acid Transport Systems in K562 Cells Sensitive and Resistant to cis-Diaminedichloroplatinum(II)

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ABSTRACT

When K562 cells were made resistant to cisplatin their neutral amino acid transport systems changed. K562 cells cloned in cisplatin developed a 6.7-fold resistance to the drug. The generation times for K562 cells sensitive (S) and resistant (DDP) to cisplatin were similar. The initial uptake and rapid efflux of cisplatin were similar in both cell lines. However, they differed in their sodium dependent neutral amino acid transport properties. In K562S cells the sodium-dependent uptake for methylaminoisobutyric acid was significantly inhibited by threonine (KI = 10.3 mM), but in K562DDP the uptake of neutral amino acids was slightly lower, and methylaminoisobutyric acid uptake was minimally inhibited by threonine. When K562DDP cells were grown in the absence of cisplatin for 8 weeks, their amino acid transport properties reverted to those of K562S cells. In K562 cells, changes in plasma membrane essential amino acid transport systems were concomitant to both the development of resistance and the redevelopment of sensitivity to cisplatin. Therefore, human malignant cell sensitivity and resistance to cisplatin may be related to membrane nutrient transport systems.

INTRODUCTION

Cisplatin [cis-diaminedichloroplatinum(II)] has been a potent anticancer chemotherapeutic agent, both as a single agent and in combination regimens (1). Its antitumor activities may be explained in part by the cross-linking reactions involving DNA and protein (2–4) and its effects with cell surface nucleic acids (5) or the plasma membrane (6). Cisplatin has been shown previously to inhibit the uptake of neutral amino acids into the L1210 cells via the ASC-like transport system, while in the L1210 resistant cell line cisplatin was a poor inhibitor of amino acid uptake (6, 7). This permanent alteration of ASC-like transport system in L1210 cells was also associated with a stable resistance to cisplatin (7). In contrast to these mouse cell studies, there are few reports of human cell lines with a stable resistance to only cisplatin (8). Thus, the definitive mechanisms of resistance of human cells to cisplatin remain to be solved. Although the resistance of K562 cells to cisplatin was not stable, data presented in this study suggest that changes in the neutral amino acid transport systems might be correlated to cisplatin cytotoxicity. These interactions with the plasma membrane might account for part of the antitumor activities of cisplatin in human tumors.

MATERIALS AND METHODS

Chemicals. L-[3H]Threonine (20 Ci/mmol) were purchased from Amersham, Arlington Heights, IL. MeAIB (51.6 mCi/mmol) was purchased from New England Nuclear, Boston, MA. [153mPt]Cisplatin (145 mCi/mmol) was obtained from Oak Ridge National Laboratories, Oak Ridge, TN (6). Cisplatin (NSF no. 119875-1) was obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD.

Cell Culture. K562 cells (K562S) (9) were obtained from Dr. Bertino at Yale University and maintained in Fischer’s medium (Grand Island Biological Co., Grand Island, NY) supplemented with penicillin (62.9 mg/l), streptomycin (100 mg/ml), and 10% swine serum (Hyclone, Ogden, UT) (10). Cisplatin-resistant cells (K562DDP) were obtained by cloning in soft agar with 16.5 μg/ml cisplatin and 15% swine serum. The K562DDP cells were maintained either by continuous exposure to 3.3 μM cisplatin in suspension culture or in the absence of drug. Cell growth studies were determined by soft agar cloning of K562 cells in the continuous presence of cisplatin, and colonies were scored at day 12–14 (7).

Uptake and Efflux of Radioactive Cisplatin. The uptake and efflux of [153mPt]cisplatin into K562S and K562DDP cells were determined with 0.6–1.0 x 10⁶ cells/ml in Earle’s balanced salt solution as described previously (6). The cell pellet was dissolved in 6.5 ml of Scinti-Vers (Fisher Scientific, Fair Lawn, NJ) and counted by Liquid Scintillation System LS3801 (Beckman Instruments, Inc., Somerset, NJ).

Amino Acid Transport Studies. The net sodium-dependent amino acid transport was determined by subtracting the sodium-independent transport from the total transport in the presence of sodium (6). Incubation medium for determining sodium-independent transport was Earle’s balanced salt solution in which 116 mM choline chloride was substituted for 116 mM NaCl. Uptake of labeled amino acids (threonine and MeAIB) was determined by measuring the incorporation of radioactivity into the cell pellet. Log-phase cells were resuspended in the incubation medium to a density of 2.5 x 10⁶ cells/ml, and 200-μl aliquots were withdrawn at timed intervals after adding labeled amino acids (6). The cell pellets were separated immediately by centrifugation through a gradient of oil (2 parts silicone: 1 part dinonylphthalate) (11) into 12% perchloric acid, dissolved in 6.5 ml of Scinti-Vers, and counted by Liquid Scintillation System LS3801. The initial velocity was determined by linear-least squares fit of the uptake versus time. The kinetic parameters (Km and Vmax) were determined by an iterative non-linear least-squares program to the direct Michaelis-Menten equation. The data were not weighed (12). Ki was defined as the concentration of the amino acid substrate required for one-half saturation of a mediated-transport system (13).

RESULTS

Tumor Cell Growth in the Presence of Cisplatin. Cisplatin inhibited the colony formation of K562S and K562DDP cells with an ED₅₀ (concentration which induces 50% inhibition of cell proliferation) of 0.67 and 4.5 μM, respectively (Fig. 1). The K562DDP cells were 6.7-fold resistant to cisplatin, and the resistance was unstable. Within 4 weeks of being grown in drug-free medium, the K562DDP cells were again equally as sensitive to cisplatin as were the parent cells (Fig. 2). The generation time for K562S and K562DDP cells in culture was 22.2 ± 0.8 SD) and 25.4 ± 2.3 h, respectively.

Initial Uptake and Efflux of [153mPt]Cisplatin. The uptake of cisplatin (1 and 5 μM) during the first 7.5 min was similar in both K562S and K562DDP cells (Fig. 3). The uptake of cisplatin (25 μM) into K562S and K562DDP cells increased with time over 75 min (Figs. 4 and 5). In the resistant cells, the initial binding of cisplatin to the cell was 13% less when compared to the sensitive cells (Figs. 4 and 5). The net uptake...
MEMBRANE PROPERTIES OF K562 CELLS

Fig. 1. Colony formation in the presence of cisplatin. K562S (○) and K562DDP (●) cells were grown in the presence of six concentrations of cisplatin, and the number of K562 colonies was determined at 12–14 days in soft agar. See “Materials and Methods” for details. Bars, SE.

Fig. 2. Cisplatin resistance in K562 cells. K562 cells were grown in the presence of six concentrations of cisplatin in soft agar, and colonies were counted at 12–14 days. This measurement was made in K562S and K562DDP cells when grown in the absence of cisplatin for 1 to 12 weeks. These data were obtained from three separate experiments in duplicate. Bars, SE.

Fig. 3. The initial uptake of cisplatin (1 and 5 μM) was measured over 7.5 min. Uptake in K562S (○) and K562DDP (●) cells was measured as described in “Materials and Methods.” Bars, SE.

The efflux of cisplatin at 37°C and 24°C was rapid and complete in 2–5 min (Figs. 4 and 5). The efflux was measured by diluting cells 20-fold into drug free medium and measuring the amount of radioactivity associated with the cells over 20 min. With increasing time, there was an increased association of cisplatin with the K562 cells that could not be effluxed from the cells. The rate of cellular retention for radioactively labeled cisplatin between sensitive and cisplatin resistant cells appeared to be similar. There was significantly less uptake and cellular retention of cisplatin at 24°C; similar results were obtained for the K562DDP cells.

Sodium-dependent Amino Acid Transport. The sodium-dependent uptake of the amino acids was significantly lower in K562DDP cells than in K562S cells. MeAIB uptake was exclusively sodium-dependent. V_{max} and K_{i} values in S-cells were 0.42 ± 0.02 pmol/10^6 cells/30 s and 355.8 ± 34.6 μM, and those in DDP cells were 0.17 ± 0.005 pmol/10^6 cells/30 s and 150.3 ± 11.8 μM (Fig. 6). K_{i} values for threonine were 171 ± 16 μM in S and 332 ± 49 μM in DDP for sodium-dependent uptake (Fig. 7).

Inhibition of MeAIB Uptake by Threonine. Threonine was a potent inhibitor in MeAIB uptake in K562S cells with a K_{i} of 10.3 ± 0.5 mM (Fig. 8), whereas in K562DDP cells its inhibitory effect was significantly reduced (Fig. 9). When K562DDP cells were maintained in the drug-free medium for 8 weeks, MeAIB uptake reverted to about 70% of that of S-cells, and threonine again strongly inhibited MeAIB uptake (Fig. 9). Membrane transport changes could be seen as early as 1 week, and by 10 weeks sodium dependent transport of MeAIB closely resembled the parent K562S cells. When K562S cells were incubated with
cisplatin (10 μM) for 15 min at 37°C there was a decrease in the sodium dependent uptake of threonine, MeAlB, and methionine by 57.6, 33.1, and 28.3%, respectively (Table 1). Neutral amino acid uptake in K562DDP cells was not significantly influenced by cisplatin (Table 1).

DISCUSSION

In this study, cisplatin cytotoxicity in K562 cells did not correlate with drug permeability but with the expression of the essential sodium dependent amino acid transport systems. K562DDP cells were established by soft agar cloning in the presence of cisplatin. However, these cells could revert to the drug sensitive parental phenotype within 4–8 weeks if they were maintained in the drug-free medium. This finding contrasts with L1210 murine leukemia cells, which had a stable resistance
Table 1  Influence of cisplatin on amino acid uptake in K562 cells

<table>
<thead>
<tr>
<th>Amino acid (500 μM)</th>
<th>Cisplatin (μM)</th>
<th>% inhibition of sodium-dependent uptake</th>
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<tbody>
<tr>
<td></td>
<td>K562S</td>
<td>K562DDP</td>
</tr>
<tr>
<td>Threonine</td>
<td>10</td>
<td>56.7 ± 14*</td>
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<td></td>
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<td>&lt;5</td>
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<tr>
<td>MeAIB</td>
<td>10</td>
<td>33.1 ± 8</td>
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<td></td>
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<td>Methionine</td>
<td>10</td>
<td>28.3 ± 10</td>
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*Pretreatment for 15 min at 37°C.  
± SE.

to cisplatin and a permanently changed ASC amino acid transport system (7). Uptake of cisplatin was temperature sensitive, with the maximum uptake at 37°C. The initial uptake and efflux of cisplatin were similar between sensitive and resistant K562 cells (Fig. 3), as also obtained for murine leukemia cells (7). These data would suggest that cisplatin permeability does not play a significant role in the initial step of resistance in K562 cells. Neither verapamil nor DL-butanilne-S,R-sulfonimine play a role in reversing cisplatin resistance in K562 cells (data not shown).

Exogenous methionine is an absolute requirement for K562 cell growth (14), and cisplatin inhibits methionine uptake both in L1210 cells and in K562 cells. If the membrane transport system is damaged, then the cell must endogenously synthesize more methionine temporarily for growth. The potential elevation of the methylenetetrahydrofolate pool for methionine biosynthesis may play a role in the synergism noted clinically between cisplatin and fluorouracil (15). This is currently under investigation.

In L1210 cells, cisplatin has caused changes in both methionine transport and metabolism (6, 7). Methionine is primarily transported into tumor cells by the ASC amino acid transport system (16). The specificity of ASC amino acid transport systems has been characterized by threonine inhibition (K_i = 16 mM) of MeAIB uptake in rat HTC tumor cells (14). However, the corresponding normal rat hepatocytes lacked this overlapping transport system for MeAIB and threonine. Threonine was also a potent inhibitor (K_i = 8.3 mM) of MeAIB uptake in L1210 cells, while the cisplatin resistant cells lacked this property (6, 7). In K562S cells, threonine was an inhibitor (K_i = 10.3 mM) of MeAIB uptake, while the resistant cells lacked the ability of threonine to inhibit MeAIB uptake. These data would suggest that some types of tumor cells have different substrate specificity for the ASC amino acid transport system when compared to normal cells or drug resistant cells (6, 7). This could be the basis for new drug design or a new treatment strategy. Cisplatin’s damage to the cell membrane and the amino acid transport systems was transient and could be repaired in 4–8 weeks, as noted by the cells reverting to the parental K562S phenotype. It would be an oversimplification to suggest that cisplatin cytotoxicity and resistance is due solely to a single membrane effect. Cisplatin damage to the DNA and its subsequent repair of DNA may also account for this phenomenon, since it has been well documented that cisplatin binds to DNA (2–5).

In summary, there appeared to be a temporal relationship between the expression of the sodium-dependent amino acid transport systems and cisplatin cytotoxicity in sensitive and resistant K562 cells.

REFERENCES

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