Modulation of Cisplatin Sensitivity and Accumulation by Amphotericin B in Cisplatin-resistant Human Lung Cancer Cell Lines

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ABSTRACT

To ascertain whether resistance to cis-diaminedichloroplatinum(II) (cisplatin) could be overcome, we determined the effects of amphotericin B (AmB), an antifungal agent, on cisplatin cytotoxicity, cisplatin-induced DNA interstrand cross-links formation, and cellular accumulation of cisplatin in human lung cancer cell lines, PC-9, PC-14, PC-7, and H69 and their corresponding respective cisplatin-resistant sublines PC-9/CDDP, PC-14/CDDP, PC-7/CDDP, and H69/CDDP in vitro. In PC-9/CDDP but not PC-9 cells, attenuation of cytotoxicity was observed when a nontoxic concentration (10 μg/ml) of AmB was combined with cisplatin, cis-diammine(1,1-cyclobutanedicarboxylato)platinum(II), and cis-diammine(glycolato)platinum(II). Sensitizing effects of AmB of varying magnitudes on cisplatin cytotoxicity also were observed in all the other cell lines except PC-14. AmB-induced increases in cisplatin-induced interstrand cross-links formation were observed, the magnitudes of which corresponded to the magnitudes of AmB-augmented cisplatin cytotoxicity. Increased intracellular cisplatin accumulation was observed in the presence of AmB in all the cells that were sensitized to cisplatin by AmB. Therefore, the increases in cisplatin accumulation were considered to be responsible, at least in part, for the mechanism of the sensitizing effect. Further experiments using other human lung cancer cell lines showed that cells that were more resistant to cisplatin were more sensitized to cisplatin by AmB than cells that were cisplatin-sensitive.

INTRODUCTION

CDDP is one of the most effective anticancer agents available at present. However, acquired resistance to cisplatin occurs frequently and some tumors are naturally resistant to the drug. Both types of cellular resistance to cisplatin are major obstacles to the successful treatment of solid tumors. The mechanism of cisplatin resistance is known to be multifactorial: elevation of cellular GSH, increased DNA repair ability, and reduced intracellular accumulation of the drug. Among the rest, the reduced accumulation of cisplatin is suggested to be a consistent finding in a variety of cisplatin-resistant cell lines (1), and a recent study indicates that it occurs at an early stage in the process of resistance development (2). In the light of these findings, we hypothesized that some drugs that alter the membrane transport of drugs may sensitize resistant cells to cisplatin. In our previous report, we demonstrated that only AmB, an antifungal agent, among the drugs we examined could reverse cisplatin resistance in PC-9/0.5 cells, a cisplatin-resistant subline from a non-small cell lung cancer cell line, and that AmB could increase cellular accumulation of cisplatin in the cells (3).

AmB has already been shown to reverse resistance to some chemotherapy agents (4-7). The effect of AmB on cells is characterized by a decrease in intracellular potassium ions resulting from the modification of membrane permeability, and it is generally believed that aqueous channels formed by AmB binding to sterol molecules are the cause of the increased membrane permeability (8, 9). Therefore, the mechanism by which AmB potentiates cytotoxicity of chemotherapeutic agents probably relates to the increased membrane permeability resulting in augmented cell uptake of the agents. However, only a few studies have demonstrated that AmB can increase cellular accumulation of actinomycin D (10, 11), Adriamycin (12), nitrogen mustard (13), and cisplatin (3, 7), and effects of AmB on the intracellular events caused by cisplatin have not been studied yet. The precise mechanism of AmB-augmented cytotoxicity of anticancer agents, especially cisplatin, remains unclear.

We have extended our previous study to determine whether the augmented AmB-induced sensitization to cisplatin is a widespread occurrence in cancer cells and whether AmB can sensitize cells to other platinum analogues. Furthermore, we have investigated the modulating effects of AmB on the extent of cisplatin-induced DNA-ICL formation and intracellular accumulation of cisplatin in several human lung cancer cell lines.

MATERIALS AND METHODS

Chemicals. Cisplatin and cis-diammine(1,1-cyclobutanedicarboxylato)platinum(II) (carboplatin) were donated by Bristol-Myers Squibb Co. (Tokyo, Japan). Cis-diammine(glycolato)platinum(II) (NSC 375101, 254-S) and tetraethylammonium(1,1,3,3-tetraphenylcyclohexaneplatinum(IV) (ormaplatin) were obtained from Shionogi Pharmaceutical Co., Ltd. (Osaka, Japan) and Upjohn Pharmaceuticals, Ltd. (Tokyo, Japan). Solubilized AmB (amphotericin B, sodium deoxycholate, and sodium phosphate at the weight ratio of 57:35:22, respectively), sodium deoxycholate, and MTT were purchased from Sigma Chemical Co. (St. Louis, MO). [methyl-14C]Thymidine (57 μCi/mmol), proteinase K, and tetraphenylpropylammonium hydroxide were purchased from Amersham (Buckinghamshire, United Kingdom), Merck AG (Darmstadt, Germany), and Eastman Kodak Co. (Rochester, NY), respectively. [15SNP]CDDP (initial specific activity, 200 μCi/mg CDDP) was provided by Dr. M. Akaboshi, Research Reactor Institute of Kyoto University (Osaka, Japan).

Cell Lines. Three NSCLC cell lines, PC-7, PC-9, and PC-14, which were derived from untreated patients with pulmonary adenocarcinoma, were provided by Professor Y. Hayata, Tokyo Medical College. A small cell lung cancer cell line, H69, was established at the National Cancer Institute, Bethesda, MD. The cisplatin-resistant sublines, PC-7/CDDP (14), PC-9/CDDP (15), PC-14/CDDP (16), and H69/CDDP (17), were established by exposing PC-7/1.0, PC-9/0.5, PC-14/1.5, and H69/0.4 cells, respectively, to cisplatin for 1 year. These cell lines were established in our laboratory by exposing PC-7, PC-9, PC-14, and H69 cells continuously to gradually increasing concentrations of cisplatin and using the limiting dilution technique (18). The cisplatin resistance of each cell line was confirmed to be maintained at a constant level in cisplatin-free medium for at least 1 year (data not shown). All the cisplatin-resistant cells were cultured in cisplatin-free medium for at least 4 weeks before being used for the experiments. The following lung cancer cell lines were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan): adenocarcinoma (A549, PC-3, and RERF-LC-MS), squamous cell carcinoma (EBC-I, PC-10, and VMRC-LCP), large cell carcinoma (Lu66 and PC-13), and small cell lung cancer (SBC-3, SBC-5, and RERF-LC-MA). All cell lines were recovered from Mycoplasma infection-free cryopreserved seed stocks and cul-

Received 3/16/92; accepted 5/10/93.

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This research was supported in part by Grants-in-Aid from the Ministry of Health and Welfare for the Comprehensive 10-Year Strategy for Cancer Control, from the Ministry of Education, Science and Culture, Japan, and from the Foundation of Bristol-Myers Squibb Co.

Recipient of a research resident fellowship from the Foundation for Promotion of Cancer Research.

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The abbreviations used are: CDDP, di-diamminedichloroplatinum(II) (cisplatin); GSH, glutathione; AmB, amphotericin B; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NSCLC, non-small cell lung cancer; IC50, drug concentration that inhibited cell growth by 50%; PBS, phosphate-buffered saline; ICL, DNA interstrand cross-links.
CHEMOSENSITIVITY TEST. Chemosensitivity to cisplatin was determined using the tetrazolium dye (MTT) assay, as described previously (19). Briefly, single-cell suspensions were harvested during the exponential growth phase and seeded into 96-well tissue culture plates (MICROTEST III, Falcon 3072). The number of cells seeded per well was 1000 for PC-9, PC-14, and their respective corresponding cisplatin-resistant sublines, and 4000 for the other cell lines. The cells were preincubated at 37°C overnight, after which various concentrations of the required drugs were added and they were incubated at 37°C for 4 days (continuous exposure) or for 3 h followed by incubation in drug-free medium for an additional 4 days (3-h exposure). After incubation, 20 μl of 5 mg/ml MTT in PBS were added to each well and incubated at 37°C for 4 h, and the medium was aspirated as completely as possible without disturbing the formazan crystals and cells on the plastic surfaces. Dimethylsulfoxide (200 μl) was added to each well, the plates were agitated on a plate shaker for 5 min to solubilize the formazan crystals, and the absorbance was measured at 562 nm using an automated microplate reader model EL340 (Bio-Tek instruments, Inc., Winooski, VT). In all experiments, 6 replicate wells were used to determine each point. The IC50 value was defined as the drug concentration that reduced the absorbance by 50% and was determined graphically from the concentration-response curve.

Evaluation of DNA Intercal Cross-Links. The frequencies of cisplatin-induced ICL were determined by the filter elution method, as described previously (17). Briefly, exponentially growing cells were radiolabeled by incubation with [methy1-14C]thymidine (0.1 μCi/ml) overnight. The radiolabeled cells were washed 3 times with fresh medium, and 3 × 106 cells were incubated in 4 ml culture medium containing the required concentrations of cisplatin with or without AmB in 60-mm tissue culture plates at 37°C. After various times of incubation at 37°C, the dishes were plated on ice and immediately irradiated with 6 Gy by a CsCo γ-iradiator at a dose rate of 1.05 Gy/min to induce DNA single-strand breaks. Then the cells were layered onto a polycarbonate membrane (pore size, 2 μm), washed with cold PBS, and lysed with a lysis solution containing 2% dodecyl sulfate, 50 mM glycine, 25 mM disodium EDTA, 50 mM Tris (pH 10.0), and 0.5 mM mg/ml proteinase K. The lysis solution was drawn through the filters and DNA retained on the filters was washed with 0.02 M disodium EDTA (pH 10.0), then eluted at 0.5 ml/min with 30 ml of 20 mM EDTA (acid form) containing tetracyclammonium hydroxide for a pH of 12.1. A part of eluted solution was mixed with Aquasol 2 (New England Nuclear Research Products, Boston, MA) containing 0.5% acetic acid. After DNA elution, the filters were put into vials with 0.4 ml of 0.1 M NaOH and heated for 1 h at 70°C. One ml of 0.4 N HCl was added and mixed with Aquasol 2. The radioactivity was counted in a liquid scintillation counter (Beckman LS3801; Beckman Instruments, Inc., Irvine, CA). Each experiment was repeated at least 3 times. The frequency of cisplatin-induced ICL formation was presented as a cross-link index, which was calculated using the following formula:

\[ C_{IC50} = \left( \frac{1 - R_{RAD}}{1 - R_{CDDP}} \right)^{1/2} - 1 \]

where \( C_{IC50} \) is the cross-link index in cisplatin-treated cells, \( R_{RAD} \) is the relative retention of 6-Gy-irradiated control cells, and \( R_{CDDP} \) is that of cisplatin-treated cells before 6-Gy-irradiation.

CHEMOSENSITIVITY TO CISPLATIN BEING AMPHOTERICIN B. The cells were incubated in 10-cm tissue culture plates (Falcon 3003) at a density of 5 × 105 cells/ml in the culture medium containing 10 μg/ml [1Hmethyl]CDDP with or without 10 μg/ml AmB. After various times of incubation at 37°C, cells were harvested by scraping, transferred to 15-ml plastic tubes (Corning 25317), and washed 3 times with ice-cold PBS by centrifugation and resuspension. The radioactivities of the cells were measured using a gamma counter. All the results obtained were normalized according to the cellular protein content, which was measured using the BCA® protein assay kit (Pierce, Rockford, IL).

STATISTICS. All values are expressed as the means ± SD, and were analyzed by Student’s t test if not otherwise mentioned. P values of less than 0.05 were considered to be significant.

RESULTS

Effect of Amphotericin B on Sensitivities to Platinum Analogues. The direct cytotoxicity of AmB on PC-9 and PC-9/CDDP cells was evaluated (Table 1). Cell proliferation was not affected by AmB at concentrations up to 10 μg/ml, but at 30 μg/ml AmB inhibited the growth of both cell lines slightly. The IC50 values of cisplatin were determined in PC-9 and PC-9/CDDP cells with and without AmB. The IC50 of cisplatin in PC-9/CDDP cells decreased markedly in the presence of AmB, although in PC-9 cells no significant change of the IC50 was observed when AmB was added. The resistance factor obtained using the formula: (IC50 in PC-9/CDDP)/(IC50 in PC-9) decreased from 22.3 to 5.1, 3.8, and 2.0 in the presence of 3, 10, and 30 μg/ml AmB, respectively. Therefore, partial reversal of cisplatin resistance was induced by AmB in this cisplatin-resistant cell line. Inasmuch as water-solubilized AmB is a mixture of AmB and deoxycholate, we determined the effect of deoxycholate on cisplatin sensitivity in both cell lines. Deoxycholate alone did not augment the cytotoxicity of cisplatin at concentrations present in solubilized AmB up to 30 μg/ml (data not shown).

Next, we ascertained whether AmB could sensitize PC-9/CDDP cells to other platinum analogues. The PC-9/CDDP cell line was highly resistant to carboplatin and 254-S (the resistant factors were 9.7 and 8.1, respectively), and slightly resistant to ormaplatin (the resistant factor was 2.5) (Table 2). AmB increased the cytotoxicity of carboplatin and 254-S, but not of ormaplatin, on PC-9/CDDP cells. No such sensitizing effect of AmB was observed in PC-9 with any of these platinum analogues.

Effect of AmB on Cisplatin Sensitivity in Other Cisplatin-resistant Cell Lines. The modulating effects of AmB on cisplatin sensitivity were studied in the cisplatin-resistant lung cancer cell lines, PC-14/CDDP, PC-7/CDDP, and H69/CDDP, and compared with its effect on the corresponding respective parental cell lines, PC-14, PC-7, and H69. No cytotoxicity was observed in any of these cell lines in the presence of 10 μg/ml AmB alone (data not shown). Table 3 shows the changes in the sensitivities to cisplatin of these cell lines induced by coinoculation with nontoxic concentrations of AmB. The IC50 of cisplatin in PC-14/CDDP cells was decreased markedly compared with that observed in the parental PC-14 cells by coinoculation with AmB, but no significant sensitizing effect of AmB was observed in PC-14 cells. Therefore, the cisplatin resistance of PC-14/CDDP cells was reversed completely by AmB. However, in PC-7 and H69 cells, AmB-induced sensitization was observed in both the cisplatin-resistant and parental cell lines. Therefore, the resistant factors of these cells did not change significantly in the presence of AmB.

Effect of AmB on Cisplatin-induced DNA-Interstrand Cross-Link Formation. Good correlations between cisplatin-induced ICL formation and cisplatin toxicity have been reported in several cell lines. The IC50 of cisplatin in PC-9/CDDP cells decreased markedly in the presence of AmB. The IC50 was defined as the drug concentration that reduced the absorbance by 50% and was determined graphically from the concentration-response curve.
Concentration of AmB used (Fig. 1C). On ICL formation in PC-9/CDDP cells was concentration-dependent, to 10 μg/ml cisplatin for 3, 6, and 9 h (Fig. 1B). The effect of AmB on cisplatin-induced ICL formation, which is expressed as a cross-link index, in PC-9 and PC-9/CDDP cells are illustrated in Fig. 1. After 6-h exposure to cisplatin, significantly less ICL were present in PC-9/CDDP than PC-9 cells, and coincubation with AmB increased the frequency of ICL in PC-9/CDDP markedly at each concentration of cisplatin used (Fig. 1A). In contrast, AmB had no effect on the frequency of ICL in PC-9 cells at any cisplatin concentration. Similar results were obtained when PC-9/CDDP and PC-9 cells were exposed to 10 μg/ml cisplatin for 3, 6, and 9 h (Fig. 1B). The effect of AmB on ICL formation in PC-9/CDDP cells was concentration-dependent, whereas AmB had no effect on ICL formation in PC-9 cells at any concentration of AmB used (Fig. 1C).

Table 3 Effect of amphotericin B on sensitivity to platinum analogues in PC-9 and PC-9/CDDP

<table>
<thead>
<tr>
<th>AmB (μg/ml)</th>
<th>IC50 of cisplatin (μg/ml)</th>
<th>Resistance factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-9/CDDP</td>
<td>PC-9/CDDP</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4.7 ± 1.6 (1)</td>
<td>4.4</td>
</tr>
<tr>
<td>5</td>
<td>3.8 ± 2.1 (1.2)</td>
<td>1.2</td>
</tr>
<tr>
<td>10</td>
<td>3.2 ± 2.3 (1.5)</td>
<td>0.9</td>
</tr>
<tr>
<td>PC-7</td>
<td>PC-7/CDDP</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7.0 ± 2.0 (1)</td>
<td>4.1</td>
</tr>
<tr>
<td>5</td>
<td>2.9 ± 0.5 (2.4)</td>
<td>3.4</td>
</tr>
<tr>
<td>10</td>
<td>1.8 ± 0.5 (3.9)</td>
<td>3.5</td>
</tr>
<tr>
<td>H69</td>
<td>H69/CDDP</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.5 ± 0.3 (1)</td>
<td>7.4</td>
</tr>
<tr>
<td>5</td>
<td>1.3 ± 0.5 (1.2)</td>
<td>6.0</td>
</tr>
<tr>
<td>10</td>
<td>0.8 ± 0.3 (1.9)</td>
<td>5.1</td>
</tr>
</tbody>
</table>

* Cells were exposed to cisplatin with the indicated concentration of AmB for 3 h followed by subsequent incubation in drug-free media for 4 days. Results are presented as means ± SD of 3 independent experiments.

Values in parentheses, degree of increase in cross-link index induced by AmB.

Table 2 Effects of amphotericin B on sensitivity to platinum analogues in PC-9 and PC-9/CDDP

<table>
<thead>
<tr>
<th>IC50 (μg/ml)</th>
<th>AmB (+)</th>
<th>AmB (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-9/CDDP</td>
<td>0.54 ± 0.27</td>
<td>1.4 ± 0.5 (2.5)</td>
</tr>
<tr>
<td>PC-9</td>
<td>0.43 ± 0.14</td>
<td>1.6 ± 1.1 (3.6)</td>
</tr>
</tbody>
</table>

Values in parentheses, modulation factors, which were calculated by the formula: Modulation factor = IC50 of cisplatin without AmB/IC50 of cisplatin with AmB.

The results suggest that the AmB-augmented cytotoxicity of cisplatin may be dependent upon the modulation of ICL formation by AmB.
ouabain has been reported to inhibit cisplatin accumulation in ovarian carcinoma cells (24), and a similar result was obtained with PC-14 cells (16). Therefore, we investigated the effects of AmB on cisplatin accumulation in ouabain-treated PC-14 cells. The effect of AmB on cisplatin accumulation after 4-h exposure to 15 μg/ml cisplatin in PC-14 and PC-14/CDDP cells pretreated with 1 nM ouabain for 1 h are shown in Fig. 2. Ouabain pretreatment reduced cisplatin accumulation in PC-14 cells by approximately 50%, but coincubation with AmB potentiated cisplatin accumulation significantly, which reached the same level as that of the untreated control cells. In PC-14/CDDP cells, in which cisplatin accumulation is reduced, pretreatment with ouabain had no effect on cisplatin accumulation, nor on its modulation by AmB.

Effects of AmB on Cisplatin Sensitivity and Intracellular Accumulation in Other Human Lung Cancer Cell Lines. We studied the effects of AmB on cisplatin sensitivity and intracellular accumulation in other lung cancer cell lines to ascertain whether the modulating effect of AmB was observed in the naturally resistant cells. All the cell lines used in this experiment were derived from patients who had not received cisplatin therapy. As the proliferation of these cell lines was inhibited slightly by continuous exposure to 10 μg/ml AmB, 3 μg/ml AmB, which was not cytotoxic in any of cell lines, were used in these experiments. The IC50 values of cisplatin after 4 days of continuous exposure, the cellular accumulation of cisplatin after 3 h exposure to 10 μg/ml cisplatin, and the effects of AmB on both these parameters are shown in Fig. 3. The cisplatin IC50 values and the amounts of cisplatin accumulated by NSCLC cell lines presented in Fig. 3 and those obtained with PC-9, PC-14, and PC-7 cells are shown in Fig. 4. An inverse correlation between the cisplatin IC50 values and its cellular accumulation was observed with the NSCLC cell lines, which suggests that defective cisplatin accumulation may be an important mechanism of the naturally occurring resistance to cisplatin in NSCLC cells. These cells were sensitized to the cytotoxic effects of cisplatin by AmB to varying extents. The sensitivities of highly sensitive cell lines, such as PC-10 and SBC-3, were not affected by AmB, whereas the more resistant cells, such as PC-3 and EBC-1, appeared to be more strongly sensitized to cisplatin by AmB (Fig. 3A). In addition, the AmB-induced increases in cisplatin accumulation in these cell lines corresponded to the AmB-induced decreases of the cisplatin IC50 values (Fig. 3B).

**DISCUSSION**

The mechanism of cisplatin resistance is believed to be multifactorial (1). In fact, the cisplatin-resistant cell lines used in this study have different resistance mechanisms as follows: decreased accumulation and increased cellular GSH content in PC-9/CDDP (15); decreased accumulation in PC-14/CDDP (16) and PC-7/CDDP; and increased cellular metallothionein content in H69/CDDP (17). We have reported that AmB, at nontoxic concentrations, can sensitize PC-9/0.5, a cisplatin-resistant subline, to cisplatin-induced cytotoxicity (3). In the present study, we have demonstrated that AmB can sensitize cells to other platinum analogues, such as carboplatin and

![Table 5 Effect of amphotericin B on cellular accumulation of cisplatin](cancerres.aacrjournals.org)

![Fig. 2. Effects of AmB on cisplatin accumulation in ouabain-treated cells. The cells were incubated for 1 h in medium alone or with 1 nM ouabain, then exposed to 15 μg/ml [195mPt]CDDP with (dotted column) or without (open column) 10 μg/ml AmB. Columns, mean of 3 determinations; bars, SD; values in parentheses, -fold increases in IC50 values induced by AmB. B. cisplatin accumulation. The cells were exposed to 10 μg/ml [195mPt]CDDP with (dotted column) or without (open column) 3 μg/ml AmB for 3 h. Columns, mean of 3 determinations; bars, SD; values in brackets, -fold increases in cisplatin accumulation induced by AmB.

![Fig. 3. Modulation of cisplatin sensitivity and accumulation by AmB in various lung cancer cell lines. A, cisplatin sensitivity. The cells were exposed to cisplatin continuously with (dotted column) or without (open column) 3 μg/ml AmB for 4 days. Columns, mean of 3 independent experiments; bars, SD; values in parentheses, -fold decreases in IC50 values induced by AmB. B, cisplatin accumulation. The cells were exposed to 10 μg/ml [195mPt]CDDP with (dotted column) or without (open column) 3 μg/ml AmB for 3 h. Columns, mean of 3 determinations; bars, SD; values in brackets, -fold increases in cisplatin accumulation induced by AmB.](cancerres.aacrjournals.org)
CDDP but not in PC-9 cells, although the magnitude of the reduction was lower in the former. However, the synergism was observed in PC-9/CDDP cells, but not in PC-9 cells, although the magnitude of the reduction of the cisplatin IC_{50} induced by AmB was comparable to that of the increases in cisplatin-induced ICL formation observed with AmB in our study. Therefore, we believe that the augmented cytotoxicity of the combination is due mainly to the potentiation of cisplatin-induced cytotoxicity by AmB and not to the potentiation of AmB-induced cytotoxicity by cisplatin, and concluded that AmB sensitized cells to cisplatin-induced cytotoxicity.

We have demonstrated in this study that increase in the cisplatin accumulation was induced by AmB in the cells that were sensitized to cisplatin by AmB. However, the magnitude of the AmB-induced increases in cisplatin accumulation were too small to account for the sensitizing effect of AmB. However, a significant AmB-induced increase in cisplatin accumulation was observed in PC-14 cells after the treatment with ouabain, an Na^{+}, K^{+}-ATPase inhibitor. We speculated that the effect of AmB on cisplatin accumulation might be exerted only in cells in which cisplatin accumulation was reduced, and that AmB could not increase cisplatin accumulation further in the sensitive cells because the drug fully accumulated in the absence of AmB. On the other hand, an AmB-induced increase in cisplatin accumulation was observed in PC-7 cells, which showed relatively low drug accumulation compared with that in PC-9 and PC-14 cells. It was suggested that PC-7 cells had naturally a reduced cisplatin accumulation mechanism. The results of the experiment using other lung cancer cell lines indicated that there might be a correlation between the sensitivity of the cells to cisplatin and the amount of the drug accumulated. The more sensitive cells of the NSCLC cell lines tended to be sensitized to cisplatin to a lesser extent than the more resistant cells by AmB. These findings are consistent with our hypothesis.

AmB could sensitize PC-9/CDDP also to carboplatin and 254-S, to which the cells were cross-resistant, but not to ormaplatin. Ormaplatin was well accumulated in PC-9/CDDP cells as well as PC-9 cells, and the accumulation was virtually the same in both cell lines (data not shown). For this reason, the sensitization by AmB might not occur for ormaplatin in PC-9 and PC-9/CDDP cells, inasmuch as AmB did not sensitize PC-9 to cisplatin. We have not obtained consistent data of accumulation of ormaplatin and sensitization to ormaplatin by AmB in other cisplatin-resistant cell lines such as PC-14/CDDP, PC-7/CDDP, and H69/CDDP. Therefore, such findings may be specific only for PC-9/CDDP and may not be true in other cell lines. Further examinations in the other cisplatin-resistant cell lines and ormaplatin-resistant cell lines should be required.

In conclusion, AmB sensitized both acquired and natural cisplatin-resistant human lung cancer cells we examined to the cytotoxic effects of cisplatin. Although a pilot study using the combination of AmB and multidrug chemotherapy has been conducted in patients with cancer (5), more precise preclinical and clinical evaluations of the efficacy of the combination and determination of the optimal administration timing, dosage, and host toxicity are essential. It is particularly important to find ways of preventing renal injury as both cisplatin and AmB are nephrotoxic. Several reports have indicated that higher blood levels of AmB and a greater antifungal effect can be achieved using AmB encapsulated in liposomes rather than solubilized AmB (27-29). Now, we are evaluating the antitumor effects of the combination of cisplatin and liposome-encapsulated AmB on a cisplatin-resistant tumor transplanted into athymic mice in vivo.

ACKNOWLEDGMENTS

The authors wish to thank Dr. K. Komiya (Department of Radiopharmacy, Hoshi University, Tokyo, Japan) for his assistance and advice on the accumulation study using {\textsuperscript{195}}mChCDDP.
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