Sensitization of Human Renal Cell Carcinoma Cells to cis-Diaminedichloroplatinum(II) by Anti-Interleukin 6 Monoclonal Antibody or Anti-Interleukin 6 Receptor Monoclonal Antibody

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ABSTRACT

Cytotoxic chemotherapy has shown little antitumor activity against renal cell carcinoma (RCC). It has been demonstrated that RCC cells secrete interleukin 6 (IL-6) and express IL-6 receptors (IL-6Rs). IL-6 inhibits apoptosis and enhances manganese superoxide dismutase expression. Several anticancer chemotherapeutic agents exert their cytotoxic activity in part through the induction of apoptosis and the production of free radicals. Thus, the resistance of RCC cells to the anticancer agents might correlate with IL-6 expression. The present study tested this hypothesis by examining the effect of anti-IL-6 mAb and anti-IL-6R mAb on the sensitivity of human RCC cells to anticancer chemotherapeutic agents. Treatment of Caki-1 cells with anti-IL-6 mAb or anti-IL-6R mAb in combination with cis-diaminedichloroplatinum(II) (CDDP) or mitomycin C overcame their resistance to CDDP or mitomycin C. However, treatment of Caki-1 cells with anti-IL-6 mAb or anti-IL-6R mAb in combination with Adriamycin, vinblastine or 5-fluorouracil did not overcome their resistance to these anticancer agents. Treatment of CDDP-resistant Caki-1 cells (Caki-1/DDP), two other RCC cell lines (ACHN and A704), and three freshly derived RCC cells with CDDP in combination with anti-IL-6 mAb or anti-IL-6R mAb reversed the resistance to CDDP in all these tumors. We then studied the effectiveness of other platinum derivatives. Treatment of Caki-1 cells with anti-IL-6 mAb or anti-IL-6R mAb enhanced their sensitivity to carboplatin, but not to trans-diaminedichloroplatinum(II). Several experiments investigated the mechanism of the antibody-mediated sensitization of RCC cells to CDDP. Incubation of Caki-1 cells with anti-IL-6 mAb or anti-IL-6R mAb did not change the intracellular accumulation of CDDP. The expression of the multidrug resistant phenotype (gp170) and c-myc oncogene were not affected by the antibody-mediated sensitization. Treatment of Caki-1 cells with the anti-IL-6 mAb or anti-IL-6R mAb down-regulated the expression of glutathione S-transferase (GST) mRNA. This study demonstrates that treatment of RCC cells with CDDP in combination with anti-IL-6 mAb or anti-IL-6R mAb can overcome their CDDP-resistance and that the down-regulation of glutathione S-transferase expression by anti-IL-6 mAb or anti-IL-6R mAb might play a role in the enhanced cytotoxicity obtained. The synergistic effect obtained with established CDDP-resistant RCC cells and freshly isolated RCC cells suggests that treatment with CDDP in combination with anti-IL-6 mAb or anti-IL-6R mAb may be applicable in the treatment of CDDP-resistant RCC.

INTRODUCTION

Cytotoxic chemotherapy, an integral part of the therapeutic approach for many solid tumors, has shown little or no antitumor activity against RCC and has played no role in either an adjuvant or a neoadjuvant support therapy (1, 2). Due to the absence of any significant antitumor response with the use of single therapeutic agents, the use of multidrug combination regimens also proved ineffective when evaluated in prospective trials against a single drug (3, 4). Although P-glycoprotein has been described as a possible defensive factor against anticancer chemotherapeutic agents such as ADR and VBL in RCC, the precise mechanisms of the protective action of RCC cells against other anticancer agents such as CDDP are not yet fully understood.

IL-6 is a multifunctional cytokine produced by various types of lymphoid and nonlymphoid cells (5). Although the first detected function of IL-6 was the induction of B cell differentiation and the production of immunoglobulins, IL-6 is also involved in other activating processes (6, 7). IL-6 has been shown to stimulate liver cells, to synthesize acute phase proteins, to play a role in hematopoiesis and T cell activation, and to act as a potent growth factor for myeloma cells and plasmacytoma cells (10, 11). RCC cells secrete IL-6 and express IL-6Rs, and published reports showed that IL-6 plays a role in growth activation of RCC cells (12, 13). Furthermore, and elevated serum level of IL-6 has been reported to be associated with poor prognosis in patients with RCC (14, 15). However, little is known of whether there exists a correlation between the expression of IL-6 and the resistance of RCC cells to anticancer chemotherapeutic agents.

Several anticancer chemotherapeutic agents such as CDDP and MMC induce apoptosis, and IL-6 suppressed the induction of apoptosis by the cytokotic agents (16, 17). Some anticancer chemotherapeutic agents such as CDDP and MMC exert their cytotoxic activity in part through free radical generation (18, 19). It has been reported that IL-6 enhances the expression of MnSOD, a free radical scavenger, and anti-IL-6 antibody abrogates the enhanced expression of MnSOD (20, 21). Since RCC cells produce IL-6, it is possible that IL-6 might be one factor regulating sensitivity and resistance of RCC cells to anticancer agents. To determine the contribution of IL-6 production to drug resistance, this study investigated whether treatment of RCC cells with anti-IL-6 mAb or anti-IL-6R mAb enhances their sensitivity to anticancer chemotherapeutic agents. Furthermore, this study explored possible underlying mechanisms involved in reversal of drug resistance by anti-IL-6/IL-6R mAbs.

MATERIALS AND METHODS

Tumor Cells. The Caki-1, ACHN, and A704 human RCC cell lines were maintained in monolayers on plastic dishes in RPMI 1640 (Gibco Bio-cult, Glasgow, United Kingdom) supplemented with 25 mm HEPES (Gibco), 2 mm L-glutamine (Gibco), 1% nonessential amino acid (Gibco), 100 units/ml penicillin (Gibco), 100 /μg/ml streptomycin (Gibco), and 10% heat-inactivated fetal bovine serum (Gibco). 

Received 8/23/94; accepted 11/17/94.

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The abbreviations used are: RCC, renal cell carcinoma; ADR, Adriamycin; CDDP, cis-diaminedichloroplatinum(II); 5-FUra, 5-fluorouracil; GST-ω, glutathione S-transferase; IL-6, interleukin 6; IL-6R, interleukin 6 receptor; MDR, multidrug resistance; MMC, mitomycin C; MnSOD, manganese superoxide dismutase; MT, microculeulor tetrazolium dye; TDDP, trans-diaminedichloroplatinum(II); VBL, vinblastine.

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Patient 2, T2N0M0, Grade 2; Patient 3, T3N0M1, Grade 2. Briefly, cell suspensions were prepared by treating finely minced tumor tissues with collagenase (Sigma Chemical Co., St. Louis, MO). The cell suspensions were layered on discontinuous gradients consisting of 100, 80, and 50% Ficoll-Hypaque and centrifuged. Tumor cells were collected from the 100% interface. Cell suspensions enriched with tumor cells were sometimes contaminated by macrophages, mesothelial cells, or lymphocytes. To eliminate further contamination of host cells, we layered the cell suspensions on a discontinuous gradient containing 25, 15, and 10% Percoll and centrifuged them. Tumor cells depleted of lymphoid cells were collected from the bottom, washed, and suspended in complete medium. Cells having less than 5% contamination with nonmalignant cells were accepted for use as tumor cells.

Reagents. Anti-human IL-6 mAb (MH166), anti-human IL-6R mAb (PM-1), anti-human IL-6 polyclonal antibody and isotype-matched control antibody (MOPC31C) were gifts from Chugai Pharmaceutical Co., Ltd., Tokyo, Japan (26, 27). The isotype of the antibodies is IgGl. Anti-IL-6 mAb by Kyowa Hakkou Co., Ltd., Tokyo, Japan. VBL (Lot NI Y7400) was obtained from Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan. MMC (Lot 845ABF), ADR (Lot 659AA5) and 5-FUra (Lot 471ABJ) were kindly supplied by Kyowa Hakko Co., Ltd., Tokyo, Japan. VBL (Lot N1Y7400) was obtained from Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan. GST-π cDNA was used in making probes for Northern blot analysis was gifts from Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan.

Cytotoxicity Assay. The MTT assay was used to determine tumor cell lysis as described previously (28, 29). Briefly, 100 μl of target cell suspension (2 X 10^4 cells) were added to each well of 96-well flat-bottomed microtiter plates (Corning Glass Works, Corning, NY), and each plate was incubated for 24 h at 37°C in a humidified 5% CO2 atmosphere. After incubation, the supernatant was aspirated, tumor cells were washed three times with RPMI, 200 μl of drug solution were distributed in the 96-well plates, and each plate was incubated for 24 h at 37°C. Following incubation, 20 μl of MTT working solution (5 mg/ml; Sigma) were added to each culture well and the cultures were incubated for 4 h at 37°C. The cell medium was removed from the wells and replaced with 100 μl of isopropyl alcohol supplemented with 0.05 M HCl. The absorbance of each well was measured with a microculture plate reader (Immunoreader; Japan Intermed Co., Ltd., Tokyo, Japan) at 540 nm. The percentage of cytotoxicity was calculated as:

% of cytotoxicity = \left[1 - \frac{\text{absorbance of experimental well}}{\text{absorbance of control well}}\right] \times 100

IL-6 ELISA. IL-6 in the supernatant of tumor cells was quantitated by ELISA. Wells of 96-well ELISA plates were coated with anti-IL-6 mAb. To set up the assay, coated plates were washed and blocked with ELISA PBS containing 1% BSA. Plates were washed and 100 μl of tumor supernatants and IL-6 standard were added to the wells. After 1 h of incubation, plates were washed and 100 μl of anti-IL-6 polyclonal antibody were added to each well. After 1 h incubation, alkaline phosphatase-conjugated goat anti-rabbit IgG was added to each well and incubated for an additional 1 h. Finally, plates were washed and incubated with substrate (p-nitrophenyl) phosphate, disodium, hexahydrate. Plates were read 2 h later at 405 nm using an ELISA reader (Immunoreader).

Northern Blotting. Cytoplasmic RNA from tumor tissues was prepared as described in detail elsewhere (28, 29). Briefly, 10 μg/lane of tumor cell RNA were electrophoresed in 1.2% agarose-2.2 μM HCHO gels in 1 X 3-[N-morpholino]propanesulfonic acid buffer [200 mM 3-[N-morpholino]propanesulfonic acid-50 mM sodium acetate-10 mM sodium EDTA]. The RNA was transferred to Biodyne A membranes (Poli, East Hills, NY) in 20X SSC (3 M NaCl-0.3 M sodium citrate, pH 7.0). Fifty to 100 ng of cDNA probe were labeled with [α-32P]dCTP (NEN, MA) by random oligoprimer extension. The nylon membranes were UV-cross-linked and hybridized.

Flow Cytometric Examination. After Caki-1 cells were fixed with the FACS Lysing Solution (Becton Dickinson), the expression of P-glycoprotein and c-myc gene product on the cell surface was quantitated by flow cytometry (Becton Dickinson Immunocytochemistry Systems) using mouse anti-human MDR mAb (Lot PC 03; Oncogene Science, Inc.) and mouse anti-human c-myc mAb (Lot OPA 02/1; Medac).

CDDP Determination. The CDDP content in Caki-1 cells was determined by flameless atomic absorption spectrophotometry using a Zeeman Z-8000 spectrophotometer (Hitachi Co., Ltd., Tokyo, Japan) as described in detail elsewhere (30, 31).

5-FUra Determination. Determinations of 5-FUra in Caki-1 cells were made by gas-liquid chromatography-mass fragmentography and gas-liquid chromatography-mass spectrometry using a JMS-D 300 mass spectrometer with a JGC-20KP gas chromatograph (JEOL, Tokyo, Japan) as described in detail elsewhere (32).

Statistical Analysis. All determinations were made in triplicate, and the results were expressed as the mean ± SD. Statistical significance was determined by Student’s t test. P = 0.05 or less was considered significant.

RESULTS

Tumor cells synthesize and secrete various cytokines and growth factors. Several of these cytokines can act in an autocrine/paracrine fashion in stimulation of tumor cell proliferation. Under these conditions, antibodies to the cytokines and/or the cytokine receptors, therefore, should inhibit the cytokine-mediated proliferation and may sometimes stimulate cell death and/or apoptosis. However, it is not clear whether blocking the cytokines/receptors by antibodies sensitizes the cells to the cytotoxic effect of chemotherapeutic agents. RCC lines and freshly derived RCC tumors used in this study secrete IL-6. We examined whether anti-IL-6 mAb or anti-IL-6R mAb sensitize the RCC cells to cytotoxic drugs.

IL-6 Secretion by RCC Cells. Four RCC lines (Caki-1, Caki-I- DDP, ACHN, A704) and three freshly isolated RCC cells constitutively produced IL-6 (Table 1). The RCC lines also expressed IL-6R (data not shown). Flow cytometric analysis demonstrated that the levels of IL-6R expression on Caki-1 cells, A704 cells, and ACHN cells were almost the same.

Sensitization of RCC Cells to CDDP and MMC by Antibodies to IL-6 and IL-6R. We first examined the effect of anti-IL-6/IL-6R mAbs on the sensitivity of Caki-1 cells to CDDP, MMC, ADR, VBL, or 5-FUra. Various concentrations of each agent were used alone and in combination and cytotoxicity was measured by a 1-day MTT assay. Clearly, there was significant augmentation of cytotoxicity of CDDP (Fig. 1, A and B) and MMC (Fig. 2) by the addition of the antibodies, but the sensitizing effect to MMC was modest. Treatment of Caki-1 cells with isotype-matched control antibody had no effect on their sensitivity to CDDP and MMC (data not shown). The results also demonstrate that synergy was achieved by the combined use of low concentrations of each agent alone and that these concentrations were 10-100-fold less than those required to achieve similar cytotoxicity by either agent alone. In contrast, treatment of Caki-1 cells with anti-IL-6 mAb or anti-IL-6R mAb did not change their sensitivity to ADR, VBL, or 5-FUra (data not shown). Anti-IL-6/IL-6R mAbs by themselves did not inhibit cell proliferation in a 1-day or a 3-day MTT assay (data not shown).

We examined the sensitivity of a CDDP resistant subline, Caki-1/ DDP, to the synergistic effect of CDDP in combination with anti-IL-6.
Fig. 1. Enhanced sensitivity of RCC cell lines to CDDP by anti-IL-6 mAb or anti-IL-6R mAb. The cytotoxic effect of CDDP in combination with anti-IL-6 mAb (A, C, E, G) or anti-IL-6R mAb (B, D, F, H) on Caki-1 cells (A, B), Caki-1/DDP cells (C, D), ACHN cells (E, F), and A704 cells (G, H) was assessed in a 1-day MTT assay. The results are expressed as the mean of 3 different experiments. SD ranged from 0.4 to 8.9. *, P< 0.05 versus CDDP alone. ▲, CDDP alone; ■, CDDP with anti-IL-6 mAb or anti-IL-6R mAb at 0.1 ng/ml; △, CDDP with anti-IL-6 mAb or anti-IL-6R mAb at 1 μg/ml; ●, CDDP with anti-IL-6 mAb or anti-IL-6R mAb at 10 μg/ml.

Fig. 2. Enhanced sensitivity of Caki-1 cells to MMC by anti-IL-6 mAb or anti-IL-6R mAb. The cytotoxic effect of MMC in combination with anti-IL-6 mAb (A) or anti-IL-6R mAb (B) on Caki-1 cells was assessed in a 1-day MTT assay. The results are expressed as the mean of 3 different experiments. SD ranged from 0.4 to 3.8. *, P < 0.05 versus CDDP alone. ●, CDDP alone; △, CDDP with anti-IL-6 mAb or anti-IL-6R mAb at 0.1 μg/ml; △, CDDP with anti-IL-6 mAb or anti-IL-6R mAb at 1 μg/ml; ●, CDDP with anti-IL-6 mAb or anti-IL-6R mAb at 10 μg/ml.
anti-IL-6/IL-6R mAbs, but higher cytotoxicity was obtained irrespective of the sequence of treatment.

We then asked whether anti-IL-6 mAb or anti-IL-6R mAb affect the intracellular accumulation of CDDP. The findings in Table 3 demonstrate that the antibodies had no effect on intracellular accumulation of CDDP.

Caki-1 cells express the MDR phenotype. It was possible that treatment with anti-IL-6 mAb or anti-IL-6R mAb modified MDR gp170 expression. The expression of P-glycoprotein by flow cytometry was not modified by the antibody treatment, suggesting that the MDR phenotype is not directly involved in sensitzation (Table 4).

It has been reported that the c-myc oncogene increases the resistance of tumor cells to CDDP (33, 34). The role of c-myc expression in reversing CDDP resistance was examined. Treatment with anti-IL-6 mAb or anti-IL-6R mAb had no effect on the expression of p62 c-myc gene product (Table 4).

We then analyzed the expression of GST-π gene which has been reported to be overexpressed in CDDP-resistant cells (35, 36). Caki-1 cells constitutively expressed mRNA for GST-π (Fig. 5, A and B). While treatment of Caki-1 cells with CDDP had no effect on GST-π mRNA expression, treatment with anti-IL-6 mAb or anti-IL-6R mAb down-regulated GST-π mRNA expression. Kinetics studies demonstrated that the down-regulation of GST-π mRNA expression became noticeable by 1 h and continued until 24 h (Fig. 5, C and D). These results suggest that the down-regulation of GST-π mRNA expression may be in part responsible for the observed sensitization of RCC to CDDP by anti-IL-6 mAb or anti-IL-6R mAb.

**DISCUSSION**

RCC cells are resistant to anticancer chemotherapeutic agents including CDDP (1, 2). RCC cells secrete IL-6 and IL-6 can act as a resistance promoting factor (13, 17, 20, 21, 37). We reasoned that agents that can inhibit IL-6 secretion may render the cells more sensitive to drugs. The present study tested this hypothesis and provides experimental evidence that down-regulation of IL-6 sensitizes RCC cells to the cytotoxic effect of CDDP.

The present study demonstrates that treatment of RCC cells with anti-IL-6 mAb or anti-IL-6R mAb enhanced their sensitivity to CDDP. The enhanced sensitivity was obtained in the parental Caki-1 cell line, the CDDP-resistant Caki-1/DDP line, two other RCC lines and three freshly isolated RCC cells. The mechanism of overcoming CDDP resistance by CDDP in combination with anti-IL-6 mAb or anti-IL-6R mAb was examined. There was no observed effect on CDDP accumulation in the cells. The level of GST-π expression in tumor cells correlates with their resistance to CDDP (35, 36). Treatment of Caki-1 cells with anti-IL-6 mAb or anti-IL-6R mAb down-regulated the expression of GST-π mRNA, thus rendering the cells sensitive to CDDP.

The fresh RCC cells from Patient 2 appeared to produce the lowest level of IL-6 concentration and were highly sensitive to CDDP but demonstrated the highest synergy between anti-IL-6/IL-6R antibodies and CDDP. The concentration of 10 μg/ml antibodies may be high enough to abrogate IL-6 secretion by the fresh RCC cells in a 24-h MTT assay completely. This may result in the highest synergy between the antibodies and CDDP in the fresh RCC cells from Patient 2.
ENHANCED SENSITIVITY OF RCC TO CDDP BY ANTI-IL-6/IL-6R mAbs

**Table 2** Effect of sequence of treatment with CDDP in combination with anti-IL-6 mAb or anti-IL-6R mAb on cytotoxic activity against Caki-1 cells

<table>
<thead>
<tr>
<th>First treatment</th>
<th>Second treatment</th>
<th>% of cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 h</td>
<td>20 h</td>
<td>Anti-IL-6 mAb</td>
</tr>
<tr>
<td>Medium</td>
<td>mAb</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>Medium</td>
<td>CDDP</td>
<td>25.1 ± 2.4</td>
</tr>
<tr>
<td>Medium</td>
<td>CDDP plus mAb</td>
<td>46.1 ± 2.3*</td>
</tr>
<tr>
<td>mAb</td>
<td>CDDP</td>
<td>34.1 ± 6.4</td>
</tr>
<tr>
<td>CDDP</td>
<td>mAb</td>
<td>35.8 ± 4.4</td>
</tr>
</tbody>
</table>

* Caki-1 cells were pretreated with medium only, CDDP (1 μg/ml), anti-IL-6 mAb (10 μg/ml), or anti-IL-6R mAb (10 μg/ml) for 4 h (first treatment). The medium was aspirated and Caki-1 cells were washed twice with RPMI. The cells were then incubated with CDDP (1 μg/ml), anti-IL-6 mAb (10 μg/ml), and/or anti-IL-6R mAb (10 μg/ml) for 20 h (second treatment). Cytotoxicity was assessed in a 1-day MTT assay.

**Table 3** Effect of treatment of Caki-1 cells with anti-IL-6 mAb or anti-IL-6R mAb on the intracellular accumulation of CDDP or 5-FUra

<table>
<thead>
<tr>
<th>Drug</th>
<th>Control (Medium)</th>
<th>Control Ab</th>
<th>Anti-IL-6 mAb</th>
<th>Anti-IL-6R mAb</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDDP</td>
<td>0.28 ± 0.05</td>
<td>0.27 ± 0.02</td>
<td>0.26 ± 0.05</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>5-FUra</td>
<td>1.48 ± 0.32</td>
<td>1.57 ± 0.45</td>
<td>1.50 ± 0.19</td>
<td>1.63 ± 0.31</td>
</tr>
</tbody>
</table>

* Caki-1 cells were treated with CDDP (10 μg/ml) or 5-FUra (100 μg/ml) in combination with medium, control antibody (10 μg/ml), anti-IL-6 mAb (10 μg/ml), or anti-IL-6R mAb (10 μg/ml) for 24 h. The medium was aspirated and Caki-1 cells were washed three times with RPMI. The intracellular concentration of CDDP was measured by flameless atomic absorption spectrometry and the intracellular concentration of 5-FUra was measured by a gas chromatographic-mass fragmentographic method as described in "Materials and Methods." The results are expressed as the mean ± SD of 3 different experiments.

The mechanisms responsible for cellular resistance to CDDP are believed to be multifactorial and to include alterations in the transmembrane transport of CDDP, the cytosolic quenching of CDDP due to increased levels of sulfhydryl compounds, the enhanced DNA adduct repair capability, and activation of oncogenes such as c-myc oncogene (33, 34). Alterations in the transmembrane transport of CDDP in tumor cells result in reduced intracellular accumulation of CDDP and resistance to CDDP, inasmuch as a correlation exists between intracellular accumulation of CDDP and CDDP resistance (38, 39). Evidence for cytosolic quenching of CDDP by either glutathione- or sulfhydryl-containing proteins has been obtained in tumor cell lines made resistant to CDDP in vitro. Some CDDP-resistant cells have higher levels of intracellular glutathione or metallothionein (40, 41). CDDP is also known to inhibit DNA synthesis by binding to DNA and forming intrastrand and interstrand cross-links in DNA (42, 43). Cell-mediated augmentation of DNA repair capability plays a major role in CDDP resistance in several mammalian cell lines studied (44).

Glutathione is a tripeptide thiol and is the most abundant nonprotein sulfhydryl compound in mammalian cells; it plays an important role in detoxification of alkylating agents and cross-linking agents such as CDDP and in repair of cellular injury by these drugs (28, 45, 46). One of the function of GST-π is to conjugate these drugs to glutathione. It has been reported that overexpression of GST-π is associated with the acquisition of resistance to CDDP (35, 36). We have demonstrated in this study that the expression of GST-π mRNA in Caki-1 cells was reduced following treatment of the tumor cells with anti-IL-6 mAb or anti-IL-6R mAb. This finding indicates that one of the mechanisms responsible for the sensitizing effect of anti-IL-6 mAb or anti-IL-6R mAb may be down-regulation of GST-π gene expression.

CDDP mediates a significant inhibitory effect on DNA synthesis. The effect is mediated through binding of CDDP to DNA and the formation of intrastrand or interstrand cross-links in DNA (42, 43). Carboplatin, a closely related platinum analogue, possesses antitumor activity similar to that of CDDP (47, 48). However, CDDP has much lower activity, since the chloride and ammonium groups are in the trans position and TDDP cannot bind tightly to DNA (49, 50). Treatment of Caki-1 cells with carboplatin in combination with anti-IL-6 mAb or anti-IL-6R mAb resulted in a synergistic cytotoxicity, while treatment with TDDP in combination with anti-IL-6 mAb or anti-IL-6R mAb did not. These results suggest that IL-6 may closely
Fig. 5. Effect of treatment of Caki-1 cells with CDDP, anti-IL-6 mAb, or anti-IL-6R mAb on the level of GST-\(\tau\) mRNA. Caki-1 cells were treated with medium (10 \(\mu\)g/ml), anti-IL-6 mAb (10 \(\mu\)g/ml), or anti-IL-6R mAb (10 \(\mu\)g/ml) for 4 h. Total RNA was then separated, Northern blotted, and probed for GST-\(\tau\) as described in "Materials and Methods." Each lane is GST-\(\tau\) mRNA of Caki-1 cells after the following treatment (A): Lane 1, medium only; Lane 2, CDDP; Lane 3, anti-IL-6 mAb; Lane 4, anti-IL-6R mAb. B, ethidium bromide staining of the same gel as control of the amount of RNA present in each lane. Caki-1 cells were treated with medium or anti-IL-6R mAb (10 \(\mu\)g/ml) for 1–24 h. Total RNA was then separated, Northern blotted, and probed for GST-\(\tau\) as described in "Materials and Methods." Each lane is GST-\(\tau\) mRNA of Caki-1 cells after the following treatment (C): Lane 1, medium only; Lane 2, 1-h treatment; Lane 3, 4-h treatment; Lane 4, 24-h treatment. D, ethidium bromide staining of the same gel as control of the amount of RNA present in each lane.

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regulate or participate directly in DNA repair processes that affect the type of DNA cross-linking damage caused by CDDP. Although the down-regulation of GST-\(\tau\) mRNA expression by anti-IL-6 mAb or anti-IL-6R mAb is suggestive for CDDP sensitization, the precise mechanism of overcoming resistance of Caki-1 cells to CDDP by treatment with CDDP in combination with anti-IL-6 mAb or anti-IL-6R mAb is not fully understood. CDDP shows its cytotoxicity partly through the induction of apoptosis (16, 51). Previous studies demonstrated that IL-6 suppressed the induction of apoptosis by anticancer chemotherapeutic agents (17, 37). Our findings suggest that treatment with anti-IL-6 mAb or anti-IL-6R mAb might enhance the sensitivity of RCC cells to CDDP by blocking IL-6-mediated inhibition of the induction of apoptosis. CDDP shows its cytotoxic activity in part through the generation of free radicals (16). It has been reported that IL-6 enhances the expression of MnSOD, a free radical scavenger, and anti-IL-6 antibody abrogates the increased expression of MnSOD (20, 21). Thus, treatment of RCC cells with anti-IL-6 mAb or anti-IL-6R mAb might enhance their sensitivity to CDDP by decreasing MnSOD expression. Since the effects of IL-6 on apoptosis and MnSOD expression may vary with cell types, further studies are needed to corroborate these hypotheses. As with GST-\(\tau\) expression, metallothionein has been associated with resistance to CDDP (35, 41). Metallothionein is highly regulated and the blocking of metallothionein action or production might be another mechanism of interaction. The mechanisms of CDDP sensitization by anti-IL-6/IL-6R mAbs await further investigations.

Treatment of Caki-1 cells with anti-IL-6 mAb or anti-IL-6R mAb enhanced their sensitivity to MMC, but the effect was modest. This finding is in agreement with the findings of other study (52). Like CDDP, MMC exerts its cytotoxic activity in part through free radical generation and in part through induction of apoptosis. Also, there is a correlation between MMC resistance and GST-\(\tau\) expression (17, 53, 54). Therefore, treatment of Caki-1 cells with MMC in combination with anti-IL-6 mAb or anti-IL-6R mAb may overcome their resistance to MMC by down-regulation of the expression of GST-\(\tau\) and MnSOD and suppressing the blocking of apoptosis.

The current studies showing that anti-IL-6 mAb or anti-IL-6R mAb sensitized both acquired and natural CDDP-resistant RCC cells to CDDP was not restricted to established cell lines but was also observed in freshly derived tumors. Therefore, the therapeutic use of CDDP in combination with anti-IL-6 mAb or anti-IL-6R mAb might be useful in the treatment of patients with CDDP-resistant RCC. We reported that a humanized anti-IL-6R mAb was developed (55), and its use in vivo might circumvent the induction of anti-mouse antibodies with murine mAbs.

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