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Modulation of Excision Repair Cross Complementation Group 1 (ERCC-1) mRNA Expression by Pharmacological Agents in Human Ovarian Carcinoma Cells

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ABSTRACT. Excision repair cross complementation group 1 (*ERCC-1*) is a DNA repair gene that is essential for life, and it appears to be a marker gene for nucleotide excision repair activity. Overexpression of *ERCC-1* during cisplatin-based chemotherapy is associated with clinical and cellular drug resistance. We therefore began to assess the influence of various pharmacological agents on the induction of ERCC-1 mRNA in A2780/CP70 human ovarian carcinoma cells. Cisplatin exposure in culture resulted in a 4- to 6-fold induction for the steady-state level of ERCC-1 mRNA in A2780/CP70 cells. ERCC-1 mRNA induction was concentration and time dependent. Cyclosporin A and herbimycin A, which suppress c-*fos* and c-*jun* gene expressions, respectively, blocked the cisplatin-induced increase in ERCC-1 mRNA. This effect of cyclosporin A or herbimycin A on the down-regulation of *ERCC-1* correlates with enhanced cytotoxicity of cisplatin in this system. The products of c-*fos* and c-*jun* are components of the transcription factor AP-1 (activator protein 1). 12-*O*-Tetradecanoylphorbol 13-acetate (TPA), a known AP-1 agonist, induced ERCC-1 mRNA to the same extent as cisplatin, but did not synergize with cisplatin in this regard. The TPA effect was biphasic, with an initial increase during the first 1–6 hr, followed by decreasing mRNA levels at 24–72 hr. These data suggest that the effects of these pharmacological agents on *ERCC-1* gene expression may be mediated through the modulation of AP-1 activities. BIOCHEM PHARMACOL **57**;4:347–353, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. cisplatin; ovarian cancer; *ERCC-1*; phorbol ester; cyclosporine; herbimycin A

CDDP† (cisplatin) is one of the most widely used antineoplastic agents, and the drug is especially useful in the treatment of ovarian cancer and cancers of the head and neck, bladder, esophagus, and lung [1–4]. Cisplatin can react with DNA, forming both chemically stable intrastrand adducts and interstrand cross-links [3]. DNA adducts formed by cisplatin inhibit DNA replication and transcription and lead to breaks and miscoding [3, 5]. The ability of patients to form and sustain DNA-platinum adducts in cells has been correlated with response to treatment [6]. Although most ovarian cancers are initially sensitive to the cytolytic effect of cisplatin, "recurrent" cancers are often resistant to the drug. The emergence of drug-resistant cancer cells is one of the greatest limitations to curative cancer therapy [1–4, 7]. The causes of tumor cell resistance to cisplatin and its analogs are not understood completely. A number of factors influence cisplatin sensitivity in experimental cells, including intracellular drug accumulation [6–8], intracellular levels of glutathione and other sulfhydryls, such as metallothionein, that bind to and inactivate the drug [7–11], and rates of repair of DNA adducts [6–8, 12]. DNA repair is a complex, multistep process, involving recognition of DNA damage, incision of the damaged segment, synthesis of a new DNA strand, and ligation of the new strand to the parental DNA. The repair of cisplatin adducts occurs primarily by the NER pathway [13–17], wherein one key protein is ERCC-1 [13, 14, 18–22].

Several lines of evidence suggest that ERCC-1 may be important in the repair of cisplatin–DNA adducts and may contribute to reduced cytotoxicity of the drug. For example, the *ERCC-1* gene is overexpressed in cisplatin-resistant cells compared with control cell lines [13–16]. In contrast, the levels of expression of *ERCC-1* in cisplatin-hypersensitive, repair-deficient cells are 30- to 50-fold lower than in inherently resistant cells [22]. Furthermore, cells that lack a

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†Abbreviations: CDDP, cisplatin, *cis*-diamminedichloroplatinum (II); *ERCC-1*, excision repair cross complementation group 1; AP-1, activator protein 1; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; NER, nucleotide excision repair; CSA, cyclosporin A; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PTK, protein tyrosine kinase; and PKC, protein kinase C.

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functional ERCC-1 do not repair cisplatin–DNA damage [13]. Reports from several studies show that *ERCC-1* expression is increased in tumor specimens obtained from patients where the cancer is unresponsive to platinum [21, 23]. Therefore, the goal of the present study was to examine the effects of agents known to modulate cisplatin sensitivity on the induction of ERCC-1 mRNA expression in the human ovarian cancer cell line A2780/CP70.

MATERIALS AND METHODS

Cell Line and Cell Culture Conditions

The human ovarian cancer cell line A2780/CP70 has been described previously [24] and was used in all the experiments. Cells were cultured in monolayer using RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 0.2 U/mL of human insulin, 50 U/mL of penicillin, and 50 μg/mL of streptomycin (Gibco BRL). Cells were grown logarithmically at 37° in a humidified atmosphere consisting of 5% CO_2:95% air. Cells were tested routinely for mycoplasmal infection, using a commercial assay system (MycoTect; Gibco BRL), and new cultures were established monthly from frozen stocks. All medium and reagents contained < 0.1 ng/mL of endotoxin as determined by the *Limulus polyphemus* amebocyte lysate assay (Whittaker Bioproducts). Cell viability was determined in triplicate by trypan blue dye exclusion. Before starting the experiments, the cells were grown to ~90% confluence after subculturing. Cisplatin (Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute) was dissolved initially in PBS without Ca^{2+} or Mg^{2+} at 1.0 mg/mL (3.33 mM cisplatin), and dilutions from this solution were made in medium to obtain the desired drug treatment concentrations. The cisplatin concentration for A2780/CP70 cells was 40 μM unless otherwise indicated. Cisplatin treatments were for 1 hr. After drug treatments, cells were washed twice with PBS without Ca^{2+} or Mg^{2+}, given fresh drug-free medium, and incubated for 24–48 hr or the time indicated. Thereafter, the cells were harvested for use in the RNA isolation assays. CSA (Sigma Chemical Co.) was dissolved in water. TPA (Sigma) and herbimycin A (Gibco BRL) were dissolved in DMSO and diluted with the cell culture medium. The final concentration of DMSO in culture flasks was 0.005 to 0.05% (v/v). Cisplatin cytotoxicity was determined using the colony growth assay and the crystal violet staining assay, as described previously [25]. The concentrations of DMSO, TPA, and other drugs used in the studies were not toxic to the cells, as confirmed by cell recoveries, trypan blue dye exclusion, and cytotoxicity assay.

RNA Isolation and Northern Blot Analysis

Total RNA was isolated from cells by acid guanidinium thiocyanate-phenol-chloroform extraction [26], or by using a commercial total RNA isolation reagent kit (Gibco BRL) according to the manufacturer's instructions. Denatured RNA (30 μg/lane) was separated by electrophoresis (Gibco BRL) through 1% agarose-formaldehyde and transferred to nylon membrane (Zeta-Probe GT; Bio-Rad Laboratories) by electrophoretic transfer (Trans-Blot Cell; Bio-Rad Laboratories). Membranes were prehybridized in Quik-Hyb (Stratagene) for 15 min at 68° and then were hybridized for 1–2 hr at 68° in Quik-Hyb containing 0.67 μg/mL of denatured salmon testes DNA (Stratagene) and ^{32}P-labeled cDNA probe. After washings of increasing stringency, the membranes were air-dried, exposed to Kodak XAR-5 X-ray film with intensifying screens at −80°, analyzed by Collage Analysis (Fotodyne Inc.), and quantitated by densitometric scanning. Before hybridization with a second labeled cDNA probe, the first probe was removed by washing for 2 hr at 75° in 1 mM Tris · HCl (pH 8.0) containing 1 mM EDTA and 0.1x Denhardt's solution [27]. The entire sequence of experiments (including growth of A2780/CP70 cells, drug treatment, and northern blotting and hybridization) was performed, and the results were reproduced in two or more separate experiments. Equal RNA loading was determined by visualization of 18S and 28S ribosomal RNA bands in ethidium bromide-stained gels and quantification of GAPDH housekeeping gene transcript on northern blots.

Preparation of cDNA Probes

A 1.05-kb cDNA probe for human *ERCC-1* was obtained from Dr. Aziz Sancar (University of North Carolina). A 0.8-kb cDNA for human GAPDH was obtained from Dr. Mitchell Olman (University of California, San Diego). A GAPDH probe was also obtained commercially from Oncogene Research Products. cDNA inserts were excised using appropriate restriction enzymes, isolated by electrophoresis through 1% agarose onto DEAE-membrane (NA-45; Schleicher & Scheull) [27], and purified by using the Geneclean II Kit (Bio 101 Inc.). cDNA was labeled with ^{32}P using a commercial random primer kit (Gibco BRL) according to the manufacturer's instructions.

RESULTS

Up-regulation of ERCC-1 *Gene Expression by Cisplatin in Human Ovarian Carcinoma A2780/CP70 Cells*

Previous studies from our laboratory have shown that cisplatin-resistant human ovarian cancer tissues show an increased expression of ERCC-1 mRNA [21, 23]. In the present study, we first determined whether cisplatin can directly stimulate *ERCC-1* gene expression in a human ovarian cancer cell line, A2780/CP70. The A2780/CP70 cell line was treated with 40 μM cisplatin for 1 hr, and the expression of ERCC-1 mRNA was measured at various time points following drug exposure. Cisplatin caused time- and concentration-dependent increases in ERCC-1 mRNA levels (Figs. 1 and 2, respectively). Northern analysis (Fig. 1) showed that ERCC-1 mRNA abundance was increased by more than 2-fold as early as 6 hr after incubation with 40

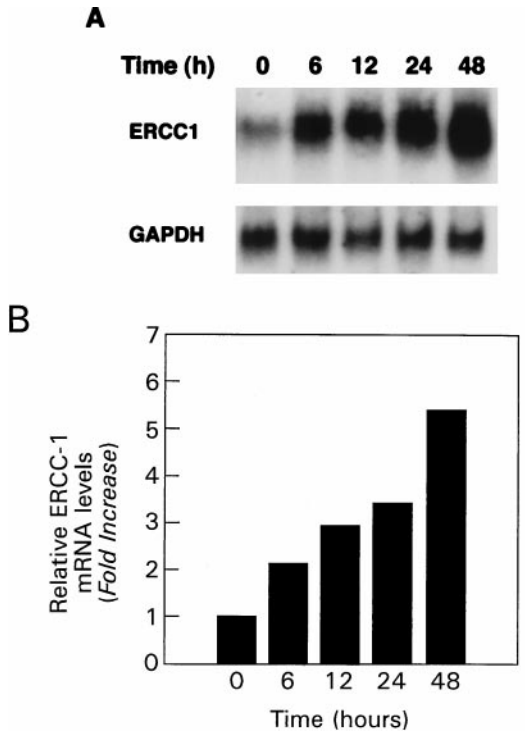

FIG. 1. Time course of cisplatin effect on ERCC-1 mRNA levels in A2780/CP70 cells. Cells were incubated in the presence of medium containing 40 μM cisplatin for 1 hr. Then the medium was removed and replaced with fresh medium, and RNA was isolated sequentially at different times after a 1-hr exposure to the drug, and analyzed by northern blotting as described in Materials and Methods. Total cellular RNA (30 μg) was blotted and hybridized with a ^{32}P-labeled ERCC-1 probe. Loading of RNA was monitored by rehybridization to labeled GAPDH probe. ERCC-1 band densities (upper) were quantified by densitometry and expressed as a ratio to GAPDH (lower), and these values are shown graphically in panel B. One representative experiment of four is shown.

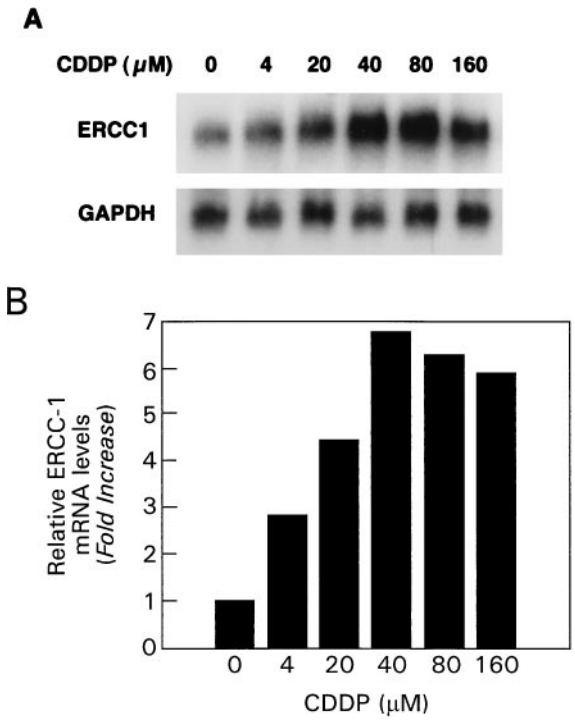

FIG. 2. Concentration response of cisplatin on ERCC-1 mRNA levels in A2780/CP70 ovarian cancer cells in culture. A2780/CP70 cells were exposed to cisplatin for 1 hr at the indicated concentrations. Then the medium was removed and replaced with fresh medium, and cells were incubated for an additional 48 hr. Total RNA was then isolated and analyzed by northern blotting. The same filters were probed with a GAPDH cDNA probe. ERCC-1 band densities (upper) were quantified by densitometry and expressed as a ratio to GAPDH (lower), and these values are shown graphically in panel B. One representative experiment of four is shown.

μM cisplatin and eventually attained a peak level of (6-fold increase) at 24–48 hr after cisplatin administration. Concentration–response experiments (Fig. 2) showed that the effect of cisplatin was maximal at 40–80 μM with more than a 6-fold increase in the ERCC-1 mRNA level. The ERCC-1 increase was not associated with changes in the steady-state levels of GAPDH mRNA in A2780/CP70 cells (Figs. 1 and 2). The toxicity of cisplatin concentrations from 0.001 to 500 μM was examined by a colony formation assay (data not shown); our results demonstrated that these cells had an IC_{50} of ~40 μM and an IC_{90} of ~100 μM. The concentration of cisplatin used in subsequent experiments to assess ERCC-1 mRNA was 40 μM. Cisplatin cytotoxicity was also examined by crystal violet staining assays. The IC_{50} of cisplatin by crystal violet staining assays was ~150 μM, and the IC_{90} was ~300 μM.

Effects of CSA, Herbimycin A, and Phorbol Ester on the Cisplatin-Related Increase in ERCC-1 *mRNA*

CSA, an immunosuppressant, has been demonstrated to enhance cisplatin chemotherapy *in vivo* [28] and to reverse resistance to cisplatin in a variety of tumor cells *in vitro* [29]. CSA also suppresses the expression of the c-*fos* gene in human ovarian cancer cells at a concentration of 5 μg/mL [29]. We therefore examined the effect of CSA at 5 μg/mL on cisplatin-induced *ERCC-1* expression in A2780/CP70 cells. As shown in Fig. 3 (lanes 1, 2, and 3), CSA reduced somewhat, the baseline ERCC-1 mRNA level in A2780/CP70 cells. CSA also blunted the cisplatin-induced increase of ERCC-1 (lanes 2, 4, 5, and 6). The effect of CSA on cisplatin-induced *ERCC-1* expression was sequence-dependent, which is in agreement with its effect on cisplatin cytotoxicity and on c-*fos* expression in ovarian cancer cells [29]. When the cells were pretreated with CSA for 18 hr, followed by immediate exposure to cisplatin for 1 hr, the ERCC-1 mRNA levels (Fig. 3, lane 5) were depressed when compared with both cisplatin treatment alone (lane 2) and the control, non-treated cells (lane 1) for ERCC-1. When cisplatin and CSA were given together for 1 hr, the decrease in ERCC-1 mRNA levels was small (lane 4). If cells were exposed to cisplatin first for 1 hr, followed by CSA for an additional 24 hr, the ERCC-1 mRNA levels were reduced moderately (lane 6).

Herbimycin A is a PTK inhibitor that has been demon-

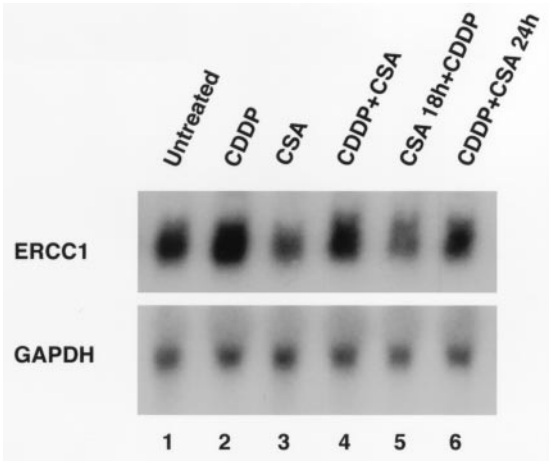

FIG. 3. Effect of CSA on cisplatin-mediated increase in ERCC-1 mRNA abundance in A2780/CP70 cells. Total RNA (30 μg/lane) was obtained from untreated A2780/CP70 cells (lane 1), cells treated for 1 hr with 40 μM cisplatin (lane 2), cells treated for 24 hr with 5 μg/mL of CSA (lane 3), cells exposed to cisplatin and CSA concurrently for 1 hr (lane 4), cells pretreated for 18 hr with CSA (5 μg/mL), then cisplatin (1 hr, 40 μM, lane 5), or cells exposed to cisplatin for 1 hr, then CSA (24 hr, 5 μg/mL, lane 6). RNA was extracted from 24-hr cultures after a 1-hr exposure to cisplatin and analyzed for ERCC-1 mRNA by northern blotting (upper). Equal loading was confirmed by rehybridization to labeled GAPDH probe (lower). One representative experiment of three is shown.

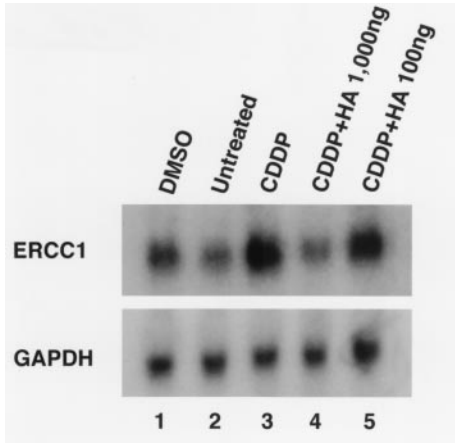

FIG. 4. Effect of herbimycin A (HA) on cisplatin-mediated increase in ERCC-1 mRNA abundance in A2780/CP70 cells. A2780/CP70 cells were treated with DMSO (lane 1), or cultured with medium alone (lane 2), or stimulated with cisplatin at 40 μM for 1 hr (lane 3). A2780/CP70 cells were preincubated for 3 hr with HA and then incubated with cisplatin and HA together for 1 hr; then the cells were washed and replaced with fresh medium containing the same concentrations of inhibitor (lanes 4 and 5). RNA (15 μg/lane) was extracted 24 hr later from the cells and analyzed for *ERCC-1* expression (upper). Equal loading was confirmed by rehybridization to the labeled GAPDH probe (lower). One representative experiment of three is shown.

strated to suppress cisplatin-induced c-jun mRNA expression in some cancer cells [30]. Cells were preincubated for 3 hr with herbimycin A at different concentrations prior to treatment with cisplatin for 1 hr, and then washed with PBS; next fresh medium containing the inhibitor was re-applied. As shown in Fig. 4, herbimycin A was found to down-regulate cisplatin-induced ERCC-1 mRNA expressions in a concentration-dependent manner (lanes 4 and 5). In contrast, when the herbimycin A was not re-applied after cisplatin exposure, there was little effect on decreasing ERCC-1 mRNA levels (data not shown), suggesting that the inhibitory effect of herbimycin A on ERCC-1 induction needs continuous exposure of cells to the inhibitor.

We next conducted studies to determine the effect of CSA or herbimycin A on cisplatin cytotoxicity in A2780/CP70 cells. In one set of studies, cells were pretreated with 5 or 10 μg/mL of CSA for 18 hr, followed by immediate exposure to cisplatin for 1 hr. Cytotoxicity was determined using the crystal violet staining assay. As shown in Fig. 5, CSA had little effect on cellular growth at concentrations of 5 or 10 μg/mL, when no cisplatin was present. A cisplatin concentration of 120 μM was associated with an approximate 20% reduction in cellular growth. CSA at 5 μg/mL increased this reduction in growth to 60%, and at 10 μg/mL, the reduction was further increased to 75% (Fig. 5). In a second set of studies, herbimycin A at concentrations of 100 or 1000 ng/mL had minimal effects on cellular growth when cisplatin was not present. At a cisplatin concentration of 100 μM, herbimycin A increased the cisplatin reduction in cellular growth from 30 to 60 or to 75%, as the herbimycin A concentration increased (Fig. 6). These results indicate that down-regulation of *ERCC-1* expression by CSA or herbimycin A is associated with enhanced cytotoxicity of cisplatin in A2780/CP70 ovarian tumor cells.

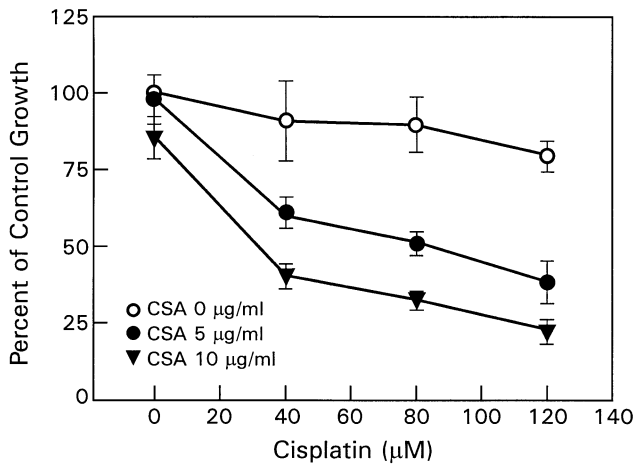

FIG. 5. Effect of CSA on *in vitro* cell toxicity by cisplatin in A2780/CP70 ovarian carcinoma cells. Microplates (96-well) were pretreated for 18 hr with CSA (0, 5, or 10 μg/mL), and then were exposed to cisplatin (0, 40, 80, or 120 μM) for 1 hr; 2750 cells were plated per well. Medium was changed, and cells were incubated for a further 24 hr with identical CSA concentrations. Cytotoxicity was measured by the crystal violet staining assay and expressed as a percentage of control (0 μg/mL CSA; 0 μM cisplatin). Each point represents the mean ± SD (N = 3).

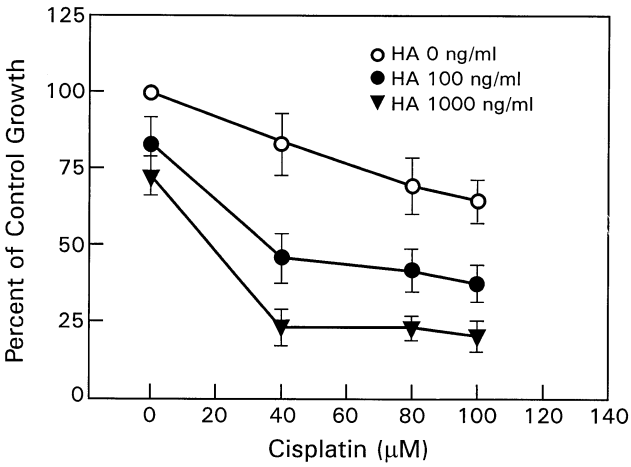

FIG. 6. Effect of herbimycin A (HA) on *in vitro* cell toxicity by cisplatin in A2780/CP70 ovarian tumor cells. Cells evenly distributed in 96-well microplates were preincubated for 3 hr with HA (0, 100, or 1000 ng/mL), and then were exposed to cisplatin (0, 40, 80, or 100 μM) for 1 hr; 2750 cells were plated per well. Medium was changed, and cells were incubated for a further 24 hr with identical HA concentrations. Cytotoxicity was determined by the crystal violet staining assay and expressed as a percentage of control (0 ng/mL of HA; 0 μM cisplatin). Results are expressed as the means ± SD of three different experiments.

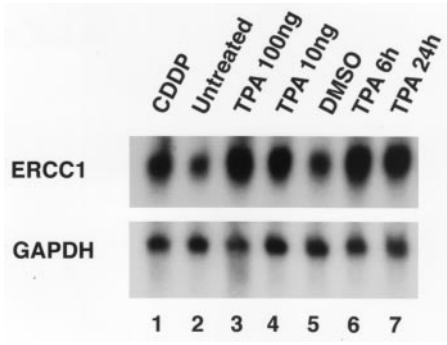

FIG. 7. Effect of TPA on ERCC-1 mRNA abundance in A2780/CP70 human ovarian cancer cells in culture. Northern blot analysis for A2780/CP70 cells exposed to cisplatin at 40 μM for 1 hr (lane 1), or cells cultured with medium alone (lane 2), or cells stimulated with TPA at 100 or 10 ng/mL for 6 hr, then washed, replaced in fresh medium, and incubated for an additional 18 hr (lanes 3 and 4), or cells treated with DMSO as control (lane 5), or cells treated with 100 ng/mL of TPA for 6 hr and incubated with fresh medium for 18 hr (lane 6), or cells incubated in the presence of 100 ng/mL of TPA for 24 hr (lane 7). RNA (30 μg/lane) was isolated from the cells at the total 24-hr incubation time and analyzed for ERCC-1 mRNA expression (upper). Equal loading was confirmed by rehybridization to the labeled GAPDH probe (lower). One representative experiment of three is shown.

We also investigated the effect of TPA, an AP-1 activator [31] and a tumor-promoting phorbol ester, on the induction of ERCC-1 mRNA expression in A2780/CP70 cells. Cells were stimulated for 6 hr with various concentrations of TPA (1–100 ng/mL), medium was then changed, total cellular RNA was isolated 18 hr after removal of the TPA, and ERCC-1 mRNA expression was assessed. TPA was found to induce ERCC-1 mRNA expression in a concentration-dependent fashion (Fig. 7, lanes 3 and 4) with maximal effect at 100 ng/mL. In addition, the effect of TPA on ERCC-1 induction was found to be time dependent. ERCC-1 mRNA levels started to increase when the cells were treated with 100 ng/mL of TPA for 1 hr (data not shown) and peaked at 6 hr (Fig. 7, lane 6).

In separate experiments, ERCC-1 mRNA levels were followed over time, with continuous exposure to TPA (Fig. 8). ERCC-1 levels peaked at 6 hr, and then decreased gradually as the incubation time with TPA increased. Long-term exposure of A2780/CP70 cells to TPA (100 ng/mL) for up to 48–72 hr caused further down-regulation of *ERCC-1* mRNA to levels comparable to those of the untreated cells in this system (Fig. 8, lanes 4 and 5). TPA did not synergize with cisplatin in the induction of ERCC-1 expression in this system (data not shown).

DISCUSSION

NER is the principal DNA repair pathway by which cisplatin damage, as well as other types of bulky DNA lesions, is removed from cellular DNA [13–17]. *ERCC-1* is one of the critical genes within NER [32–34] and may be responsible for the incision 5′ to the site of DNA damage [35]. *ERCC-1* gene expression is necessary for the repair of cisplatin-induced DNA damage [13], and enhanced expression is associated with clinical resistance to cisplatin [21, 23]. Thus, understanding the modulation of *ERCC-1* overexpression may provide new targets for therapeutic intervention aimed at overcoming or preventing DNA repair-related chemoresistance. In the present study, we demonstrated that cisplatin induces *ERCC-1* up-regulation in the A2780/CP70 human ovarian cancer cell line. Our data also

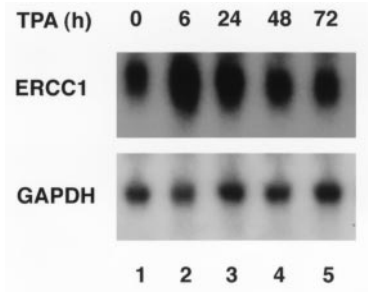

FIG. 8. Effect of long-term incubation of A2780/CP70 cells with TPA on ERCC-1 mRNA accumulation. A2780/CP70 cells were stimulated with TPA at 100 ng/mL for 6 hr (lane 2), then the cells were washed, medium was replaced with fresh medium, and incubation was continued for an additional 18 hr; or cells were incubated in the presence of 100 ng/mL of TPA for 24, 48, or 72 hr (lanes 3, 4, and 5, respectively). The cells were harvested at the end of each time point. Cells without treatment of TPA were controls (lane 1). Total RNA was isolated, and ERCC-1 mRNA (upper) was analyzed by northern blotting as described in Materials and Methods. Equal loading was confirmed by probing the same filters with a GAPDH cDNA probe (lower). One representative experiment of two is shown.

showed that this induction of ERCC-1 mRNA by cisplatin can be influenced by several pharmacological agents, where the common feature is that each agent influences one or both components of AP-1.

The transcriptional activator that may be responsible for the elevated levels of *ERCC-1* gene expression has not been definitively established. However, several lines of evidence suggest that the enhanced expression of *ERCC-1* in the A2780/CP70 ovarian cancer cell line appears to be mediated through AP-1. The literature shows that cisplatin induces increases in expressions of c-*fos*/c-*jun* [29, 30] and in AP-1 binding activity [30] in ovarian cancer cells and other tumor cells. It has been established that the AP-1 family is a group of general and cell-specific transcription factors responsible for the activation of a wide variety of genes in different cell types and tissues. If AP-1 is indeed mediating the transcription of the *ERCC-1* gene, then suppression of AP-1 activity might result in repression of an appropriate increase in ERCC-1 mRNA. Consistent with this hypothesis, the cisplatin-mediated increase in ERCC-1 mRNA level was reduced when the cells were treated with CSA or herbimycin A. These two agents have been demonstrated previously to block c-fos and c-jun mRNA expression, respectively [29, 30].

Precedent exists that AP-1 activity is regulated by two mechanisms: (a) levels of AP-1 family members (*fos*/*jun*) may be transcriptionally up-regulated, and (b) their relative activity may be increased by protein phosphorylation. TPA has been shown to induce the transcription factor AP-1 activity [31] via the PKC pathway [36–39]. Consistent with this notion, prolonged incubation of the cells with phorbol ester, which down-regulates PKC activity, caused a reduction in ERCC-1 mRNA levels. In addition, TPA has been reported to induce AP-1 binding activity by a mechanism independent of *de novo* protein synthesis [40] that is thought to be mediated by c-Jun phosphorylation. Herbimycin A, an inhibitor of PTK, may block the Ras pathway, which leads to phosphorylation of pre-existing c-Jun, and therefore decrease AP-1 activity and inhibit further c-*jun* transcription [41]. The CSA effect may be more complex.

CSA, used primarily as an immunosuppressant in organ transplantation, has been shown to reverse resistance to a variety of chemotherapeutic drugs, including cisplatin [28, 29]. CSA increases cisplatin toxicity in cisplatin-resistant human ovarian carcinoma cells and other tumor cells [29]. However, the mechanism underlying the sensitization to cisplatin by CSA is not fully elucidated. It has been suggested that CSA can increase drug accumulation by interactions with either plasma membrane potentials or calcium–calmodulin pathways. This agent could also act on signal transduction pathways in the cells and modulate the expression of nuclear oncogenes [29, 42]. It was reported that CSA suppressed cisplatin-induced expression of c-*fos* and c-H-*ras* oncogenes in cisplatin-resistant human ovarian cancer cells, decreasing the levels of resistance to cisplatin [29]. In this work, we show that CSA diminished the cisplatin-induced ERCC-1 mRNA level at concentrations that increased the cytotoxicity of cisplatin. This appears to suggest that CSA may decrease drug resistance by decreasing the ability to up-regulate DNA repair, as reflected in the changes observed on *ERCC-1*. Our data also suggest that the suppressive effect of CSA on *ERCC-1* expression may be through its inhibitory effect on c-*fos* as a component of AP-1.

The molecular mechanism accounting for the CSA-related down-regulation of *ERCC-1* expression requires further study. CSA could induce expression (mRNA and protein) of the transcriptional repressors of c-*fos*, or an induction in their inhibitory capacity against c-*fos* [41]. Alternatively, CSA could interfere with Ca^{2+}-dependent pathways that regulate the transcription factor AP-1 [43]. A third possibility is that CSA could form an inhibitory complex with peptidyl-prolyl *cis-trans* isomerase, which may block transcription factors [44, 45]. Precisely how CSA may affect *ERCC-1* expression via one or more of these mechanisms in ovarian tumor cells, thereby modulating cisplatin sensitivity, remains to be experimentally determined.

Investigations are in progress to further characterize these AP-1 components in response to cisplatin or TPA activation and elucidate the signal transduction pathway involved in the process. The relationship between the up-regulation of genes like *ERCC-1* and the generation of a cisplatin-resistance phenotype in ovarian tumors is complex. However, further understanding of this relationship may provide new insights into cancer carcinogenesis and into novel rational approaches for augmenting the effect of platinum-based chemotherapy.

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