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Attenuation by Glutathione of *hsp72* Gene Expression Induced by Cadmium in Cisplatin-Resistant Human Ovarian Cancer Cells

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ABSTRACT. Intracellular GSH has some effects on protecting cells against cadmium and is involved in the development of resistance to cisplatin (CDDP). To determine the effects of intracellular GSH on expression of the heat shock genes (*hsp*) induced by cadmium in CDDP-resistant cancer cells, we used two human ovarian cancer cell lines: CDDP-sensitive A2780 and its CDDP-resistant derivative A2780CP. The concentration of intracellular GSH was significantly higher in A2780CP than in A2780 cells. A2780CP cells were more resistant to $CdCl_2$ exposure than A2780 cells. The treatment of the two cell lines with 50 μM $CdCl_2$ induced hsp72, hsp32 and metallothionein (MT-II) mRNAs, and the induction level of each mRNA did not differ in the two cell lines. However, the treatment with 20 μM $CdCl_2$ induced the hsp72 and hsp32 mRNAs in A2780CP cells less than in A2780 cells, while the MT-II mRNA was induced to similar levels in the two cell lines. The DNA binding activity of the heat shock factor (HSF) in response to 20 μM $CdCl_2$ exposure was also significantly lower in A2780CP cells. The treatment of A2780 cells with *N*-acetyl-L-cysteine increased the intracellular GSH concentration, and profoundly suppressed hsp72 mRNA induction and HSF activation by $CdCl_2$. These results indicate that the regulation of the *hsp72* gene expression induced by $CdCl_2$ was more suppressive in A2780CP than in A2780 cells. Our findings suggest that increased GSH biosynthesis in CDDP-resistant cancer cells may be involved in the attenuation of HSF activation by $CdCl_2$. BIOCHEM PHARMACOL **58**;1:69–76, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. heat shock protein 72; glutathione; cadmium; cisplatin-resistant cells

CDDP† is a frequently used and very effective chemotherapeutic drug in treating various human cancers of the brain, ovary, testicle, bladder, head, and neck [1]. A lethal dose of CDDP kills cells primarily by forming DNA adducts, then causing G2 arrest in the cell cycle, and finally triggering apoptosis [2]. A sublethal dose induces resistance to CDDP by several mechanisms, including changes in drug uptake and efflux, GSH and MT levels, and DNA repair [2]. Chemotherapeutic agents are capable of inducing synthesis of a number of hsp [3]. The hsp70 protein is often involved in increased resistance to drugs such as doxorubicin, teniposide, actinomycin D, camptothecin, and etoposide [4–6]. However, there is little evidence that the hsp70 protein is involved in the development of resistance to CDDP.

GSH is present in almost all mammalian cells, and protects cells against various forms of stress such as oxidative stress and exposure to heavy metals [7]. CDDP cytotoxicity increases when intracellular GSH concentration is reduced, and cellular resistance to CDDP is associated with a marked increase in GSH synthesis in human ovarian cancer cell lines [8]. CDDP forms an adduct with GSH, and this adduct is exported out of the cells through an ATP-dependent system [9, 10]. GSH also contributes to cellular resistance indirectly by acting as a cofactor for DNA repair enzymes [11].

Cadmium is a heavy metal of high toxicity to most organs, including the liver, kidneys, lungs, bones, and reproductive organs [12]. Cadmium causes dose- and time-dependent increases in intracellular GSH content [13], while decreasing GSH content at toxic concentrations [14]. GSH acts as both an antioxidant and a metal-chelating agent, and plays an important role as a first line of cellular defense against cadmium [15]. Syntheses of hsp and MT are stimulated in response to cadmium exposure and are involved in protecting against toxicity [16]. The treatment of mammals or cultured cells with cadmium induces several kinds of hsp including hsp32, mitochondrial hsp60, hsp70, hsp90, and hsp110 [17–19]. The hsp70 protein is the best characterized among the hsp. Synthesis of hsp70 is increased by different types of stress, such as exposure to higher temperature, heavy metals, and oxidative stress,

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† *Abbreviations:* hsp, heat shock proteins; HSF, heat shock factor; HSE, heat shock responsive element; CDDP, cisplatin [*cis*-diamminedichloroplatinum (II)]; MT, metallothionein; NAC, *N*-acetyl-L-cysteine; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium; and OPT, *o*-phthalaldehyde.

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which in turn invoke thermotolerance and protection against a number of agents that would provoke cellular injury [20]. The hsp70 family is comprised mainly of two members: constitutively expressed HSC70 (hsp73) and stress-inducible hsp70 (hsp72). Transcription of the heat shock genes is regulated by HSF, which binds to the HSE located in the promoter regions of the heat shock genes [21]. Previously, we showed that $CdCl_2$ exposure activated HSF–binding capacity in human cultured cells, and this activation was enhanced by treatment with diethyl maleate, a GSH-depleting reagent [22].

The aim of this study was to better determine the effects of intracellular GSH on the response of the *hsp72* gene to cadmium exposure in CDDP-resistant cancer cells. The present results indicate that the regulation of *hsp72* gene expression in response to cadmium is more suppressive in CDDP-resistant cancer cells than in CDDP-sensitive cancer cells. Our findings suggest that increased biosynthesis of GSH has an important effect on attenuation of *hsp72* gene expression by $CdCl_2$ in CDDP-resistant cells.

MATERIALS AND METHODS

Reagents

CDDP was purchased from Nihon Kayaku Co. NAC, OPT, and glutathione (reduced form) were purchased from Sigma Chemical Co. Cadmium chloride (guaranteed grade) was obtained from Wako Pure Chemical Industries Ltd. Other chemicals were of the highest purity commercially available.

Cell Cultures

A2780, a human ovarian cancer cell line derived from an untreated patient, and A2780CP, a CDDP-resistant cell line, were kind gifts from Dr. T. C. Hamilton (Fox Chase Cancer Center, Philadelphia, PA, USA). The A2780CP cell line was established by exposure of A2780 cells to stepwise-increasing concentrations of the drug up to 70 μM [23]. Cells from the two lines were maintained in Eagle's minimal essential medium supplemented with 10% fetal bovine serum (GIBCO) and 2 mM glutamine at 37° in an atmosphere of 95% air and 5% CO_2. Cells were heated by incubating them in a temperature-regulated circulating water bath.

RNA Isolation and Northern Blot Analysis

Total RNA was extracted from cells by the guanidinium thiocyanate procedure [24]. Equal amounts of RNA (20 μg/lane) were separated by electrophoresis through 1% agarose gels and transferred onto nylon membrane (Hybond N, Amersham). The membrane was prehybridized with salmon sperm DNA (20 μg/mL) at 42° for 3 hr in 4 × standard saline citrate, 50% formamide, 5 × Denhardt's solution. The membrane was then hybridized with one of the following cDNA probes ^{32}P-labeled by nick translation: hsp70 (human), *Bam*HI-*Eco*RI fragment (0.8 kb) [25]; metallothionein-II (MT-IIA) (human), *Hind*III fragment (3.0 kb) [26]; hsp32 (mouse), *Eco*RI fragment (1.5 kb) [27]; and β-actin (human), *Hinf*I fragment (0.4 kb) [28]. Hybridization was carried out at 42° for 16–24 hr. The membrane was washed in 1 × standard saline citrate, 0.1% SDS several times at 42°, and analyzed using Bio-Analyzer BAS-2000 (FUJIX).

Cell Viability

Cells were trypsinized and plated at a density of 2×10^4 cells/well onto 96-well plates and allowed to attach overnight. Serial dilutions of CDDP or $CdCl_2$ were added to the wells. After incubation for 24 hr, cell viabilities were determined by measuring the conversion of MTS to formazan by dehydrogenases in metabolically active cells using an MTS assay kit (CellTiter 96 AQ Assay, Promega Corp.).

Glutathione Assay

The GSH (reduced form) levels were determined using a fluorometric method with OPT [29]. Briefly, cells were homogenized in 0.1 M sodium phosphate–EDTA buffer. Following the addition of 25% H_3PO_4, the mixture was centrifuged (10,000 *g*, for 30 min) at 4°. The supernatant (100 μL) was mixed with 100 μL of OPT (1 mg/mL in methanol) and 1800 μL of sodium phosphate–EDTA buffer. After incubation at room temperature for 15 min, intensity of fluorescence was read with a spectrofluorometer with excitation at 350 nm and emission at 420 nm.

Nuclear Protein Extraction

Nuclear protein extracts were prepared from treated cells grown to 80% confluence in dishes (FALCON 3003). All nuclear extraction procedures were performed on ice with ice-cold reagents. Cells were washed twice with PBS and harvested by scraping into 1 mL of PBS and pelleted at 1500 *g* for 5 min. The pellet was washed with PBS, resuspended in one packed cell volume of lysis buffer (10 mM HEPES–KOH, pH 7.8, 10 mM KCl, 1.5 mM $MgCl_2$, 0.2% v/v Nonidet P-40, and 0.5 mM dithiothreitol) incubated for 5 min with occasional vortexing. After centrifugation at 1500 *g*, one cell pellet volume of extraction buffer (20 mM HEPES–KOH, pH 7.8, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA–NaOH, pH 8.0, 25% v/v glycerol, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 2 μg/mL pepstatin A, and 2 μg/mL leupeptin) was added to the nuclear pellet and incubated on ice for 30 min with occasional vortexing. The nuclear proteins were isolated by centrifugation at 18,000 *g* for 15 min. Protein concentrations were determined by Bradford assay (Bio-Rad) and stored at −70° until used for the gel mobility shift assay.

Gel Mobility Shift Assay

To assay for DNA-binding activity of HSF1, an oligonucleotide probe for HSE was prepared, consisting of the nucleotides from −109 to −85 of the human *hsp70* gene (5′-CTGGAATATTCCCGACCTGGCAGAA-3′) [30]. The probe was labeled with γ-[^{32}P]ATP with T4 polynucleotide kinase (Wako). Two μg of nuclear proteins was prepared and preincubated on ice for 10 min. A nucleotide probe mixture containing the radiolabeled oligonucleotide probe and 1.25 μg poly (dI-dC) (Pharmacia Biotech) was added. The binding reactions were incubated at room temperature for 20 min and then separated on 6% native polyacrylamide gels. Gels were run in 0.5× TBE buffer for 2 hr at 190 V. Gels were transferred to Whatman 3MM chromatograph paper (Whatman, Inc.), dried under a vacuum at 80° for 1 hr, and analyzed using Bio-Analyzer BAS-2000 (FUJIX).

Data Analysis

The data of GSH concentration and the cytotoxicity assay were expressed as means ± SD of at least three independent experiments. Statistical significance analysis was determined using the unpaired two-tailed Student's *t*-test.

RESULTS

Expression of Stress-Inducible Genes by $CdCl_2$ in A2780 and A2780CP

In the present study, we used the two human ovarian cancer cell lines CDDP-sensitive A2780 and its CDDP-resistant subclone, A2780CP. The cell viability of the two lines after CDDP exposure was determined by an MTS assay kit. After treatment with various concentrations (1–300 μM) of CDDP for 24 hr, cell viability was assayed. We found that A2780CP was more resistant to CDDP than A2780 (Fig. 1A). The intracellular concentration of GSH was significantly higher (2.7-fold) in A2780CP than in A2780 cells (Fig. 1B).

We assayed for cadmium toxicity on A2780 and A2780CP cells. Cells were treated with serial dilutions (10–300 μM) of $CdCl_2$ for 24 hr, and cell viability was then measured by an MTS assay kit. The treatment with 20 μM $CdCl_2$ was not cytotoxic to either cell line (Fig. 2). There were significant differences in cell viability between A2780 and A2780CP cells at the higher concentrations of $CdCl_2$. CDDP-resistant A2780CP cells were more resistant to $CdCl_2$ than were CDDP-sensitive A2780 cells at 50 and 100 μM. Interestingly, $CdCl_2$ at the lower concentrations (10 and 20 μM) significantly increased the viability of A2780 cells. Then, we investigated the effects of $CdCl_2$ on the stress-inducible gene expressions in the two cell lines. mRNA induction of MT-II and hsp72 and hsp32 were determined by Northern blot analysis after exposure to $CdCl_2$ (20 μM or 50 μM) for 4 hr. All three mRNAs (hsp72, hsp32, and MT-II) were induced by 50 μM $CdCl_2$ in both cell lines (Fig. 3). *MT-II* gene expression was also induced by 20 μM $CdCl_2$ to a similar level in both lines. However, there were significant differences in the induction level of the hsp72 and hsp32 mRNAs between A2780 and A2780CP when they were treated with 20 μM $CdCl_2$. The hsp72 and hsp32 mRNAs induced by 20 μM $CdCl_2$ were significantly lower in A2780CP than in A2780.

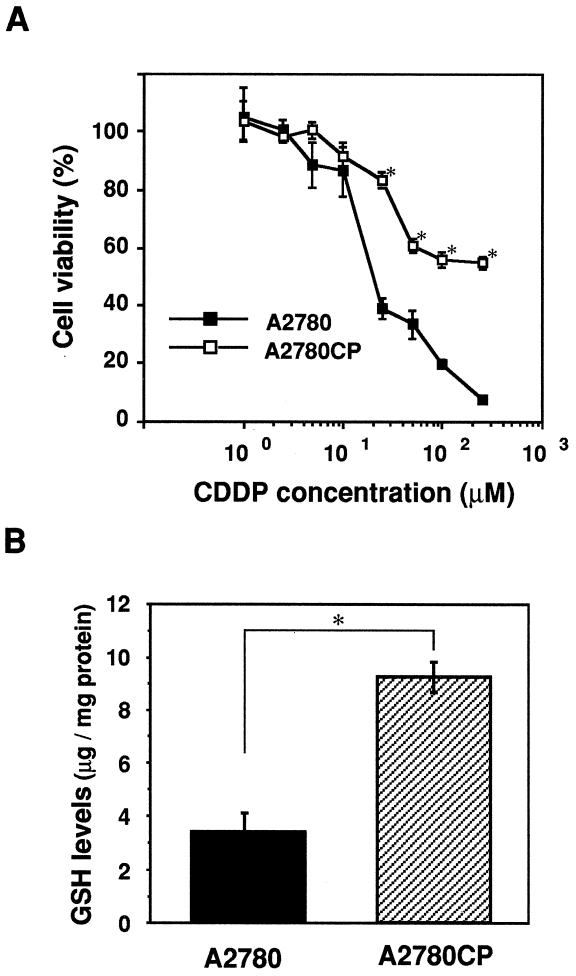

FIG. 1. Cell viability following CDDP treatments and intracellular GSH levels in A2780 and A2780CP cells. (A) Cells of the two lines were incubated with serial dilutions (1–300 μM) of CDDP for 24 hr. After CDDP treatment, cell viabilities were determined by an MTS assay. Values are means ± SD of four separate cultures. *$P < 0.001$ versus A2780 cells by Student's *t*-test. (B) The GSH (reduced form) concentration in cells was measured as described in Materials and Methods. Values are means ± SD of three separate cultures. *$P < 0.001$.

HSF-Binding Activity Induced by $CdCl_2$ in A2780 and A2780CP

We investigated the transcriptional regulation of the *hsp72* gene in the two cell lines exposed to $CdCl_2$. The binding activity of the HSF to the HSE in the promoter region of the *hsp72* gene was determined by a gel mobility shift assay using an HSE-specific oligonucleotide probe (Fig. 4). A2780 and A2780CP cells were treated with $CdCl_2$ (20 or

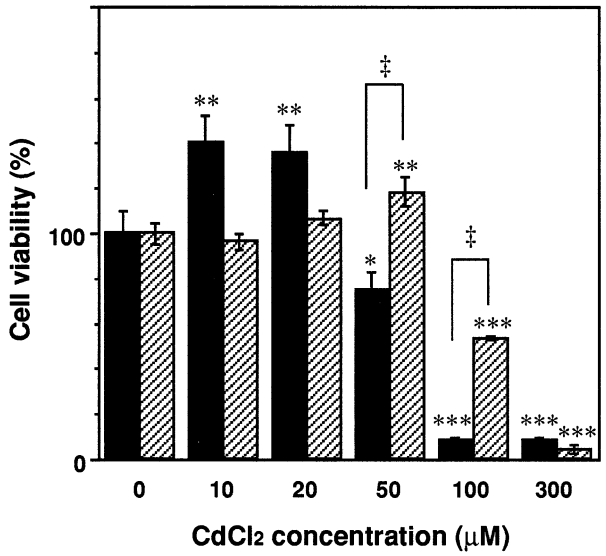

FIG. 2. Cell viability following $CdCl_2$ treatments in A2780 and A2780CP cells. Cells were exposed to serial dilutions of $CdCl_2$ (10–300 μM). After incubation with $CdCl_2$ for 24 hr, cell viabilities of A2780 (solid bars) and A2780CP (hatched bars) were measured by an MTS assay. Values are means ± SD of 4 separate cultures. *$P < 0.01$, **$P < 0.005$, ***$P < 0.001$ compared with the untreated control (0 μM $CdCl_2$). ‡$P < 0.001$.

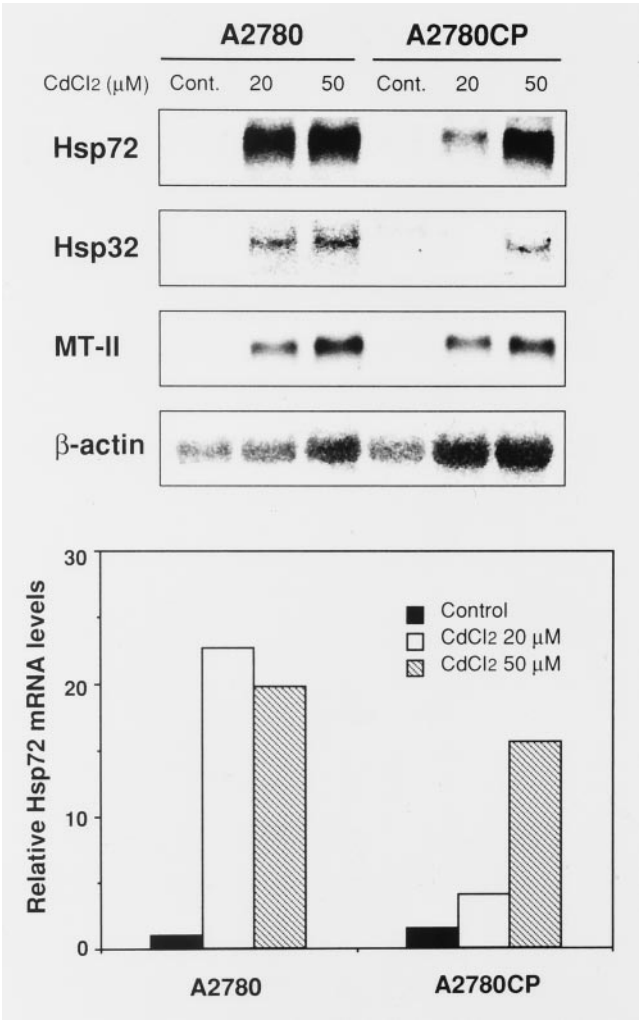

FIG. 3. hsp72, hsp32, and MT-II mRNA induction by $CdCl_2$ in A2780 and A2780CP. (A) Cells were exposed to $CdCl_2$ (20 or 50 μM) for 4 hr, and then the total RNA was prepared. The induction level of each mRNA was determined by Northern blot analysis. Expression of β-actin mRNA is shown to control for equal amounts of RNA. Relative mRNA levels of hsp72 are plotted below (solid bar, untreated control; open bar, 20 μM $CdCl_2$; hatched bar, 50 μM $CdCl_2$). All experiments were repeated at least twice with similar results.

50 μM, for 4 hr), and nuclear proteins were then extracted. The HSF activities of the two lines were extensively and equally increased after treatment with 50 μM $CdCl_2$. There was a significant difference in the HSF-binding activity of the two cell lines after exposure to 20 μM $CdCl_2$; this activity was extensively suppressed in A2780CP but not in A2780 cells. The induction of HSF-binding activity in response to $CdCl_2$ exposure in the two cell lines was parallel to the induction of hsp72 mRNA.

Effects of NAC on Induction by $CdCl_2$ of hsp72 mRNA and HSF-Binding Activity

To determine the effects of intracellular GSH on *hsp72* gene expression induced by $CdCl_2$, A2780 and A2780CP cells were exposed to 30 mM NAC for 2 hr prior to exposure to 50 μM $CdCl_2$ for 4 hr. Treatment of A2780 with 30 mM NAC for 2 hr increased intracellular GSH concentration 9-fold (Fig. 5A). In both cell lines, pretreatment of NAC (30 mM, for 2 hr) profoundly suppressed the induction of hsp72 mRNA by $CdCl_2$ (50 μM, for 4 hr) (Fig. 5B). NAC pretreatment also suppressed hsp32 mRNA induction by $CdCl_2$ in the two cell lines (data not shown). Therefore, we determined the effects of NAC on the $CdCl_2$-induced HSF-binding activity in the two lines. Cells were exposed to 30 mM NAC for 2 hr prior to exposure to 50 μM $CdCl_2$ for 4 hr, and HSF-binding activity was then determined by a gel mobility shift assay. Pretreatment with NAC inhibited $CdCl_2$-induced HSF activation as well as hsp72 mRNA induction in both cell lines (Fig. 5C). NAC pretreatment suppressed cadmium-induced HSF activation more extensively in A2780CP than in A2780 cells (Fig. 5C, lanes 2 and 4). These results suggest that the increase in GSH concentration is involved in the attenuation of HSF activation by $CdCl_2$. Furthermore, we investigated the effects of NAC on suppression of cadmium cytotoxicity (Fig. 6). Following pretreatment of the two cell lines with 30 mM NAC for 2 hr, cells were exposed for 4 hr to $CdCl_2$ in serial dilutions (50 μM–10 mM). Twenty-four hours later, cell viabilities were determined by the MTS assay. Pretreatment with NAC efficiently suppressed the cytotoxic effects of cadmium on the two cell lines. Interestingly, the viability of A2780 cells exposed to $CdCl_2$ at 50–500 μM was increased more by NAC pretreatment than was that of A2780CP cells.

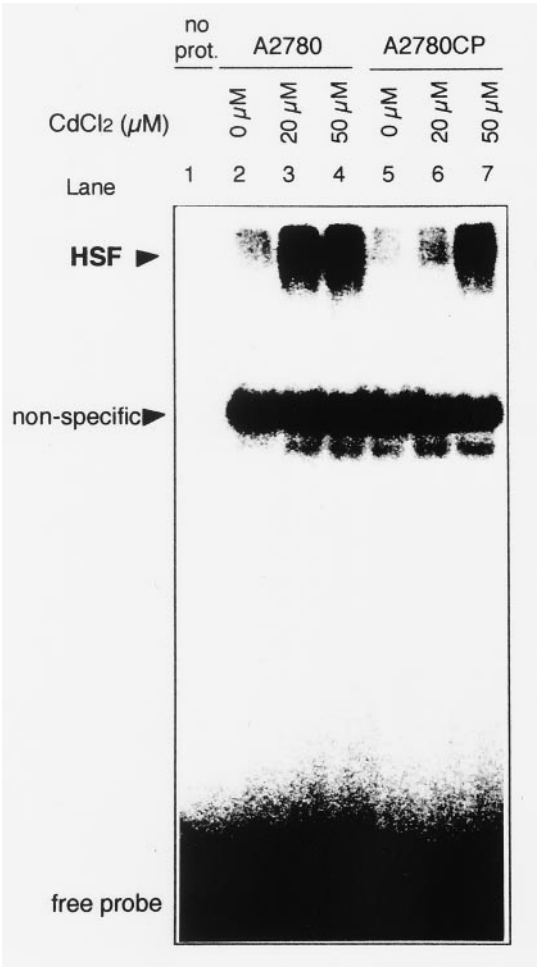

FIG. 4. $CdCl_2$-induced HSF-binding activity in A2780 and A2780CP cells. Cells were exposed to $CdCl_2$ (20 or 50 μM) for 4 hr. After the treatment, nuclear protein extracts were prepared and HSF-binding activity was analyzed by a gel mobility shift assay using an HSE-specific oligonucleotide probe as described in Materials and Methods. Lane 1, no nuclear protein extract; lanes 2 and 5, untreated control; lanes 3 and 6, 20 μM $CdCl_2$ treatment; lanes 4 and 7, 50 μM $CdCl_2$ treatment. Lanes 2–4, A2780; lanes 5–7, A2780CP. All experiments were repeated at least twice with similar results.

DISCUSSION

Cadmium enhances expression of several classes of genes; it stimulates expression of the immediately early genes (c-*fos*, c-*jun*, and c-*myc*), of the tumor suppressor gene *p53*, and of genes coding for syntheses of protective molecules, including MT, GSH, and hsp [31]. hsp may play a role in repairing metal-induced cell damage, while MT seem to participate directly in detoxification [32–34]. The present study focuses on the analysis of intracellular GSH functions in regulating expression of the *hsp* and *MT* genes in response to cadmium in a CDDP-resistant human ovarian cancer cell line.

CDDP-resistant A2780CP cells were cross-resistant to $CdCl_2$. As shown in Fig. 2, A2780CP cells were more resistant to $CdCl_2$ at 50–100 μM than were A2780 cells. Furthermore, the GSH level, which had been increased by NAC pretreatment, sufficiently suppressed the cytotoxic effects of cadmium on both cell lines (Fig. 6). These results suggest that increased GSH biosynthesis is involved in cross-resistance to $CdCl_2$ in CDDP-resistant A2780CP cells.

Unexpectedly, the lower concentrations (10 and 20 μM) of $CdCl_2$ significantly increased cell viability more than 100% in A2780 cells, but not in A2780CP cells (Fig. 2). This apparently paradoxical finding is open to question. In the present study, we estimated cell viability by assaying activity of an enzyme in mitochondria. One possibility is that cadmium at lower concentrations has no general cytotoxic activity, but displays, via an unknown mechanism, stimulatory effects on metabolic activities, especially in mitochondria. It might be that CDDP-sensitive A2780 cells respond more sensitively than CDDP-resistant A2780CP cells to cadmium both at higher cytotoxic and lower stimulatory concentrations. Alternatively, the higher cell viability of A2780 cells might be ascribed to a greater increase in cellular GSH. It has been reported that lower concentrations of cadmium increase the cellular GSH level and cysteine uptake in some cultured mammalian cells [13, 35], and that this increase in GSH and cysteine levels precedes an increase in DNA synthesis [36]. Under the present experimental conditions, the transient induction of cellular GSH in A2780 cells by cadmium at the lower concentrations might be greater than in A2780CP cells, whereas the constitutive basal level of cellular GSH is higher in A2780CP cells.

In Fig. 6, we also found intriguing the following somewhat paradoxical phenomena; A2780 cells showed better cell viability (above 100%) than A2780CP cells when pretreated with NAC at the fixed concentration and then treated with $CdCl_2$ over a wide range of concentrations. We cannot at present provide a definitive explanation for this finding, and it remains to be experimentally elucidated in future investigations. One can, however, hypothesize that NAC treatment may increase the cellular GSH level in A2780 cells with a lower basal level of GSH more than in A2780CP cells containing a higher constitutive level of GSH, and a steeper rise in the concentration of GSH in A2780 cells could induce higher cell viability. Still another possibility is that the steeper rise in the cellular GSH level in A2780 cells might provoke higher activation of mitochondrial enzymes, including dehydrogenases.

In the present study, we found that similar levels of MT-II mRNA were induced in response to exposure to $CdCl_2$ (20 and 50 μM) in A2780 and A2780CP cells, while expression of the *hsp72* and *hsp32* genes was significantly lower in A2780CP than in A2780 cells (Fig. 3). An increase in GSH biosynthesis in A2780CP cells may contribute to suppression of expression of the *hsp* genes, but not of the *MT-II* gene. Although MT seems to be involved in the development of CDDP resistance [2], Schilder *et al.* reported that there is no causal relationship between MT expression and CDDP resistance in human ovarian cancer cell lines [37]. The hsp70 protein family, including hsp72 and hsp73, acts as 'molecular chaperone' to assist in the

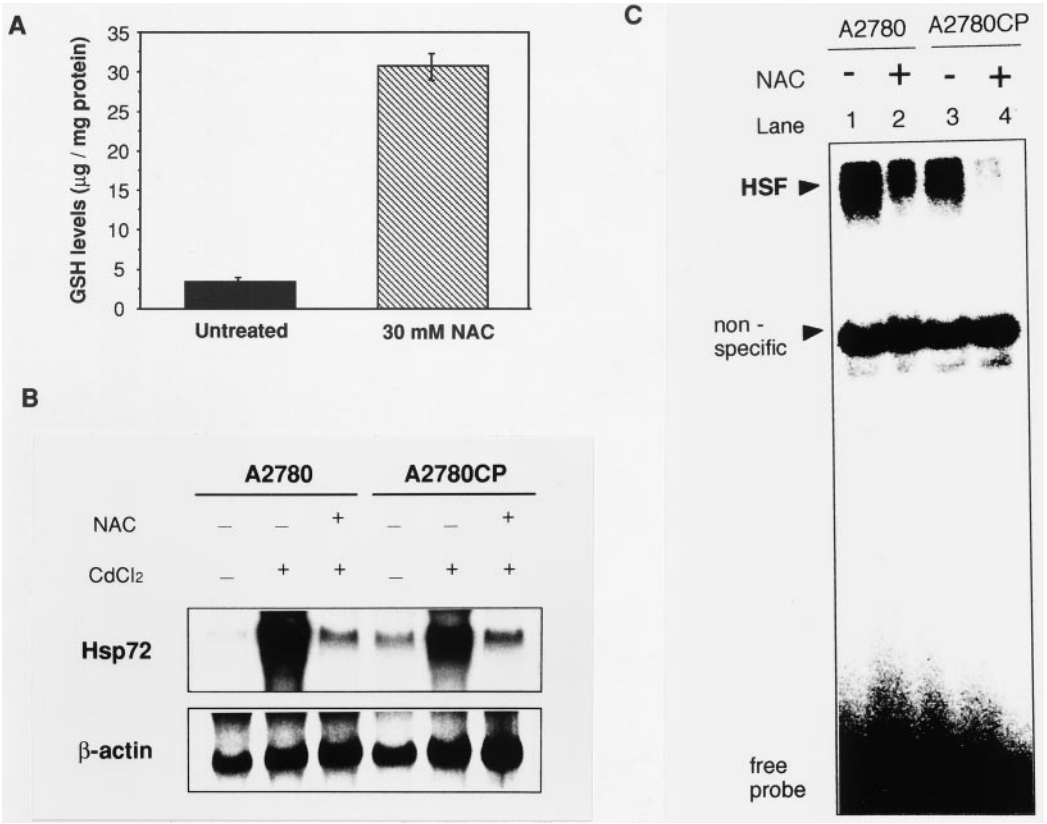

FIG. 5. Effects of NAC on $CdCl_2$-induced *hsp72* gene expression and HSF-binding activity in A2780 and A2780CP. (A) A2780 cells were incubated with 30 mM NAC for 2 hr, and then the GSH levels were measured. Values are means ± SD of 3 separate cultures. (B) Following pretreatment of the two cell lines with 30 mM NAC for 2 hr, cells were exposed to 50 μM $CdCl_2$ for 4 hr. After the treatment, the total RNA was prepared and the hsp72 mRNA induction was determined by Northern blot analysis. Lanes 1 and 4, untreated control; lanes 2 and 5, $CdCl_2$ treatment; lanes 3 and 6, NAC + $CdCl_2$ treatment. Lanes 1–3, A2780; lanes 4–6, A2780CP. Expression of β-actin mRNA is shown in the lower panel to control for equal amounts of RNA. (C) Following pretreatment of the two cell lines with 30 mM NAC for 2 hr, cells were exposed to 50 μM $CdCl_2$ for 4 hr. After the treatment, nuclear extracts were prepared and HSF-binding activity was analyzed by a gel mobility shift assay using an HSE-specific oligonucleotide probe. Lanes 1 and 3, 50 μM $CdCl_2$ treatment; lanes 2 and 4, NAC + 50 μM $CdCl_2$ treatment. Lanes 1 and 2, A2780; lanes 3 and 4, A2780CP. All experiments were repeated at least twice with similar results.

folding of nascent proteins and in their transport to various intracellular organella [38]. Induction of hsp72 mRNA may be very important in the suppression of cadmium-induced cell death through refolding of abnormal proteins provoked in A2780 cells exposed to 20 μM $CdCl_2$. On the contrary, a higher GSH concentration in A2780CP cells may prevent induction of abnormal proteins, and hsp72 may therefore not be necessary in these cells when exposed to 20 μM $CdCl_2$.

In CDDP-resistant human leukemia cells, heavy metals such as arsenite, cadmium, and zinc could increase the level of multidrug resistance-associated protein (MRP) mRNA [9]. MRP encodes a human GS-X pump which actively exports the glutathione–platinum complex from cells [10]. CDDP-resistant leukemia cells exposed to CDDP increased the intracellular GSH level and were cross-resistant to cadmium and arsenite [9]. The increased level of GSH may be an important factor in dealing with cadmium in CDDP-resistant A2780CP cells.

The hsp32 protein, a peculiar heat shock protein, like the enzyme heme oxygenase degrades heme to biliverdin, which is subsequently converted to bilirubin by another enzyme. In human cells, hsp32/heme oxygenase is induced by several agents, such as UVA radiation, hydrogen peroxide, cadmium, and sodium arsenite [17, 39]. Induction of heme oxygenase augments cellular resistance to oxidative injury, probably because bilirubin can suppress lipid peroxidation [40]. Reactive oxygen species (ROS) enhance the extent of induction of the *hsp32* gene by cadmium [41]. Our present finding of the higher expression of the *hsp32* gene in A2780 cells (Fig. 3) suggests more generation of ROS in A2780 than in A2780CP cells. This difference in the amount of ROS may also have affected the differential *hsp72* expression between the cell lines.

Previously, we found that NAC, which increases the intracellular GSH level, has at least two concentration-dependent functions in inducing *hsp70* and *MT-II* gene expression in human cultured amniotic cells exposed to $CdCl_2$ [17]. A low concentration of NAC can suppress the induction of hsp70 mRNA as well as the increase in lipid peroxidation, while a high concentration of NAC suppresses MT-II mRNA induction as well as cadmium-

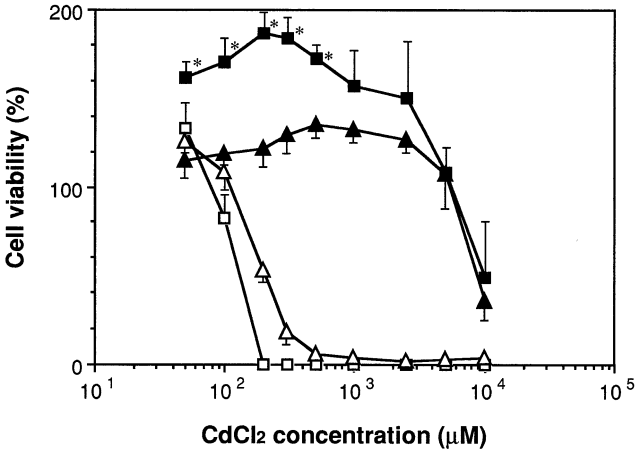

FIG. 6. Effects of NAC on suppression of cadmium-induced cytotoxic effects. Following pretreatment of the two cell lines with 30 mM NAC for 2 hr, cells were exposed to $CdCl_2$ for 4 hr in serial dilutions. Twenty-four hours later, cell viabilities were determined by the MTS assay. Values are means ± SD of 4 separate cultures. Open squares and triangles represent A2780 and A2780CP cells treated with $CdCl_2$, respectively. Closed squares and triangles represent A2780 and A2780CP cells treated with NAC + $CdCl_2$, respectively. *P < 0.001 compared with A2780CP cells (NAC + $CdCl_2$).

induced cell death. An increase in the intracellular GSH level by NAC may have more serious effects on the induction of hsp70 mRNA than that of MT-II mRNA. NAC can inhibit activity of several signal transduction components such as nuclear factor kappa B, activator protein 1, and c-*jun* N-terminal kinase (JNK) by redox modulation [42, 43]. In the present study, GSH biosynthesis increased by NAC significantly suppressed HSF-binding activity as well as the cytotoxicity of cadmium in the two cell lines (Figs. 5 and 6). Interestingly, NAC inhibited HSF-binding activity more extensively in A2780CP cells (Fig. 5C). Our findings suggest that activation of HSF-binding activity by cadmium may depend on intracellular GSH concentration. HSF contains cysteine residues within its DNA-binding domain [44]. HSF-binding activity is affected by redox modulation [45]. We previously demonstrated that pretreatment with a GSH-depleting reagent enhanced activation by $CdCl_2$ of HSF-binding capacity in a human amniotic cell line [22]. Thiol-reducing agents such as dithiothreitol and 2-mercaptoethanol inhibited the heat-initiated activation process of HSF, i.e. its trimerization, phosphorylation, and nuclear translocation [46]. The intracellular redox status, as reflected by GSH concentration, may play an important role in HSF activation by $CdCl_2$.

Our present study demonstrated that the regulation of *hsp72* gene expression induced by $CdCl_2$ was more suppressive in A2780CP than in A2780. The different induction level of the hsp72 mRNA in the two cell lines may primarily depend on the different GSH concentrations they contain. Our findings provide evidence for the first time that increased GSH biosynthesis is involved in the attenuation of *hsp72* gene expression induced by $CdCl_2$ in CDDP-resistant cells.

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