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Research Article

Aloe emodin decreases the ERK-dependent anticancer activity of cisplatin

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Abstract. The present study describes the ability of an anthraquinone derivative aloe emodin (AE) to reduce the cytotoxic activity of the platinum(II)-based anticancer agent cisplatin toward murine L929 fibrosarcoma and C6 glioma cell lines. The protective effect of AE was demonstrated by MTT and crystal violet assays for cell viability, and involved supression of cisplatin-induced apoptosis and necrosis, as assessed by lactate dehydrogenase release and flow cytometric analysis of DNA fragmentation or phosphatidylserine exposure. Cell-based ELISA and Western blot analysis revealed that AE abolished cisplatin-triggered activation of extracellular signal-regulated kinase (ERK) in tumor cells, while activation of c-Jun N-terminal kinase was not significantly altered. A selective blockade of ERK activation with PD98059 mimicked the protective effect of AE treatment in both tumor cell lines. Moreover, AE failed to protect tumor cells against the ERK-independent toxicity of the Pt(IV)-based complex tetrachloro(O,O-dibutyl-ethylenediamine-N,N′-di-3-propanoate)platinum(IV). Taken together, these data indicate that herbal anthraquinone AE can downregulate the anticancer activity of cisplatin by blocking the activation of ERK in tumor cells.

Key words. Aloe emodin; cisplatin; ERK; apoptosis; necrosis; cancer.

Cisplatin (cis-diamine-dichloroplatinum) has been considered one of the most effective chemotherapeutic agents, displaying clinical activity against a wide variety of solid tumors [1, 2]. The cytotoxicity of cisplatin is primarily ascribed to its interaction with nucleophilic N7 sites of purine bases in DNA to form DNA-protein and DNA-DNA interstrand and intrastrand crosslinks [3]. The DNA adducts formed by the interaction of cisplatin with DNA activate several signal transduction pathways, including those controlled by mitogen-activated protein (MAP) kinases, which lead to induction of the apoptotic death of tumor cells [4]. DNA damage-mediated apoptotic signals, however, can be attenuated by various mechanisms, and the resistance that ensues is a major limitation in cisplatin-based chemotherapy [4]. In addition, the toxic side-effects of cisplatin therapy, particularly nephrotoxicity, ototoxicity and peripheral neuropathy, present a major disadvantage for cisplatin application [1, 4]. For these reasons, considerable interest is being shown in pharmacological adjuncts that would increase the efficiency and/or reduce the toxicity of cisplatin therapy.

The herbal anthraquinone derivatives emodin (3-methyl-1,6,8-trihydroxyanthraquninone) and aloe emodin (1,8-dihydroxy-3-hydroxymethyl-anthraquinone), which possess

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tyrosine kinase-inhibiting activity, have been shown efficient in limiting proliferation of various tumor cell lines in vitro [5–16], as well as in restricting tumor growth in vivo [10, 16]. Moreover, emodin was found to senzitize human breast and lung cancer cells to various chemotherapeutic drugs, including cisplatin [10, 11]. On the other hand, Chang et al. [17] have demonstrated the ability of emodin to increase the repair of UV- and cisplatin-induced DNA damage in normal human fibroblasts, through mechanisms presumably involving an increase in calcium influx and subsequent expression of the nucleotide excision repair complex subunit ERCC1. However, as a similar effect has not been demonstrated so far in tumor cells, the question remained open whether this protection from cisplatin toxicity could be selective for non-transformed cells.

In the present study, using murine fibrosarcoma L929 and rat glioma C6 cell lines, we show for the first time that AE can prevent cisplatin-induced death of cancer cells. The mechanisms underlying the observed action of AE probably involved interference with cisplatin-triggered activation of p44/p42 MAP kinase (or extracellular signal-regulated kinase, ERK1/2) in tumor cells.

Materials and methods

Cells and reagents

Cisplatin and the tetrachloro(O,O-dibutyl-ethylenediamine-N,N′-di-3-propanoate)platinum(IV) complex [Pt-(dbeddp)Cl_4] were prepared according to the previously described procedures [18, 19]. All chemicals used in experiments were purchased from Sigma (St. Louis, Mo.), unless specified otherwise. The rat glioma cell line C6 (subclone of the original CCL107 clone from ATCC) was a kind gift from Dr. P. Tranque (Universidad de Castilla-La Mancha, Albacete, Spain), while the murine fibrosarcoma cell line L929 was obtained from the European Collection of Animal Cell Cultures (Salisbury, UK). Cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2, in 25-cm^2 tissue culture flasks containing HEPES-buffered RPMI 1640 medium supplemented with 5% FCS, 2 mM glutamine, 0.01% sodium pyruvate, 5×10^{-5} M 2-mercaptoethanol and antibiotics (culture medium). Cells were used for experiments after a conventional trypsinization procedure. AE was stored at –20 °C at a concentration of 200 mM in DMSO, and diluted in culture medium immediately before use. Control cell cultures contained the amount of DMSO corresponding to its content in the solution with the highest concentration of AE used in the particular experiment.

Cell viability determination by MTT, crystal violet and lactate dehydrogenase release assay

Mitochondrial-dependent reduction of 3-4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT) to formazan reflects the mitochondrial activity of cultured cells [20], intensity of crystal violet staining is directly proportional to the number of adherent cells [21], while the release of cytosolic lactate dehydrogenase (LDH) indicates the loss of membrane integrity that occurs in necrotic cells [22]. The cells were seeded in flat-bottomed 96-well plates (3×10^4 cells/well) in a final volume of 200 µl culture medium containing different agents, and the assays were performed after 24 h incubation exactly as previously described [20–22]. Mitochondrial-dependent production of formazan and crystal violet absorption by adherent cells were assessed by an automated microplate reader at 570 nm, while the pyruvate-mediated conversion of 2,4-dinitrophenylhydrazine into visible hydrazone precipitate in the LDH assay was measured at 492 nm. The results of the MTT and crystal violet assay are presented as percentage of control values obtained in untreated cell cultures. The percentage of LDH release was determined using the following formula: $(E – C)/(T – C) \times 100$, where E is the experimental absorbance of cell cultures, C is the control absorbance of cell-free culture medium and T is the absorbance corresponding to the maximal (100%) LDH release of Triton-lysed cells.

Apoptosis analysis

Apoptotic cell death was assessed by flow cytometry analysis of DNA fragmentation in cells stained with the DNA-binding dye propidium iodide (PI). Following a 24-h incubation in 60-mm Petri dishes (1×10^6 cells), cells were detached by scraping in cold PBS-EDTA and fixed in 70% ethanol at 4°C for 30 min. After removing the ethanol by repeated washing in PBS, cells were resuspended in PBS containing 1 mg/ml RNase and PI (40 µg/ml) and kept at 37 °C in the dark for 30 min. Red fluorescence was analyzed with a FACSCalibur flow cytometer (BD, Heidelberg, Germany), using a peak fluorescence gate to exclude cell aggregates. Cell distribution among cell cycle phases was determined with Cell Quest Pro software and hypodiploid cells in the sub-G0/G1 compartment were considered apoptotic. Alternatively, apoptosis was analyzed by double staining with annexin V-FITC and PI (both from BD Pharmingen, San Diego, Calif.), in which annexin V bound to the apoptotic cells with exposed phosphatidylserine, while PI labeled the necrotic cells with a damaged membrane. Staining was performed according to the manufacturer's instructions, and flow cytometry was conducted on a FACSCalibur flow cytometer. The percentage of apoptotic (annexin$^+$/PI$^–$) and necrotic (annexin$^+$/PI$^+$) cells was determined using Cell Quest Pro software.

Cell-based ELISA and Western blot analysis of MAP kinase activation

A slightly modified method for cell-based ELISA [23] was used to measure the level of active, phosphorylated

forms of the MAP kinase family members ERK and c-Jun N-terminal kinase (JNK). After incubation with various agents in 96-well flat-bottomed plates (3×10^4 cells/well), cells were fixed in 4% paraformaldehyde, endogenous peroxidase was quenched with 1% H_2O_2 in PBS containing 0.1% Triton X-100 (PBST), and unspecific binding of antibodies blocked with PBST solution containing 10% FCS. Primary mouse monoclonal antibodies specific for rat/mouse phospho-ERK and phospho-JNK (1:200; both from Santa Cruz Biotechnology, Santa Cruz, Calif.), were applied in PBST supplemented with 2% bovine serum albumine (PBSTB), followed by secondary peroxidase-conjugated goat anti-mouse IgG (1:2500 in PBSTB; USB Corporation, Cleveland, Ohio). Both incubations were undertaken at 37 °C for 1 h. After incubation with the peroxidase substrate TMB, the reaction was stopped with 0.1 M HCl and the absorbance was measured in an automated microplate reader at 450 nm. The obtained absorbances were corrected for the cell number that was determined by crystal violet staining, as described in the original protocol. The results are presented as relative expression in comparison with the control value, which was arbitrarily set to 1. The phosphorylation status of ERK1 and ERK2 was also analyzed by Western blotting followed by chemiluminiscent detection, using the PhosphoPlus p44/42 MAP kinase (Thr202/Tyr204) Antibody Kit (Cell Signaling Technology, Beverly, Mass.) according to the manufacturer's instructions.

Statistical analysis

The significance of the differences between various treatments was analyzed by t test or ANOVA followed by a Student-Newman-Keuls test for multiple comparisons. A p value less than 0.05 was considered significant.

Results

AE reduces the anticancer activity of cisplatin

In accordance with the well-known anticancer activity of cisplatin, the crystal violet test confirmed the ability of cisplatin to reduce the numbers of cultured C6 glioma and L929 fibrosarcoma cells in a dose-dependent manner (fig. 1A, B). While AE alone had a much less pronounced toxic effect, its presence markedly impaired the toxic action of cisplatin toward the tumor cell lines (fig. 1A, B). The protective effect of AE was also observed in an MTT assay for mitochondrial respiration, which was reduced to 20–30% in cell cultures treated with 20 µM cisplatin, but partly restored in the presence of AE (fig. 1C). The protective action of AE was dose dependent, with 20 µM AE being most potent (fig. 1C). As higher doses of AE (40 and 80 µM) did not exert significantly better protection (fig. 1A and data not shown), the concentration of 20 µM was chosen for further experiments.

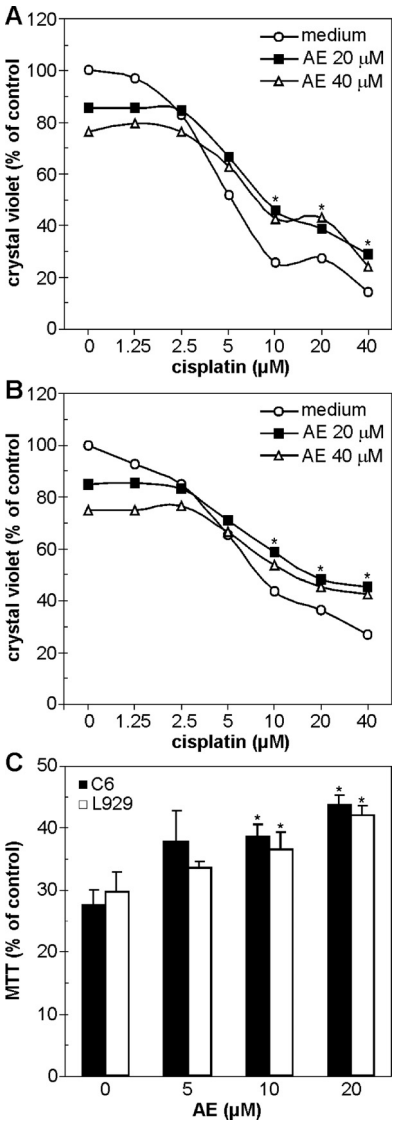

Figure 1. AE protects tumor cell lines against from cisplatin toxicity. C6 (*A*) or L929 cells (*B*) (3×10^4/well) were incubated with different doses of cisplatin in the presence or absence of 20 or 40 µM AE, and cell viability was determined after 24 h by crystal violet assay. Tumor cell lines were also treated with 20 µM cisplatin in the presence or absence of different AE concentrations, and an MTT assay was performed after 24 h (*C*). The data are presented as mean values (*A*, *B*; SD was within 15% of the mean) or mean ± SD (*C*) from three independent experiments (*$p < 0.05$ represents significantly higher viability of AE-treated vs. cisplatin alone-treated cells; t test).

AE interferes with cisplatin-induced apoptosis and necrosis of tumor cells

Cisplatin toxicity was associated with DNA fragmentation, a hallmark of apoptosis, as demonstrated by flow cytometric analysis of PI-stained DNA in L929 cells (fig. 2A). The release of intracellular LDH was also markedly increased in cisplatin-treated L929 and C6 cell cultures, indicating the ability of the drug to cause cell membrane damage characteristic of necrosis (fig. 2B). On the other hand,

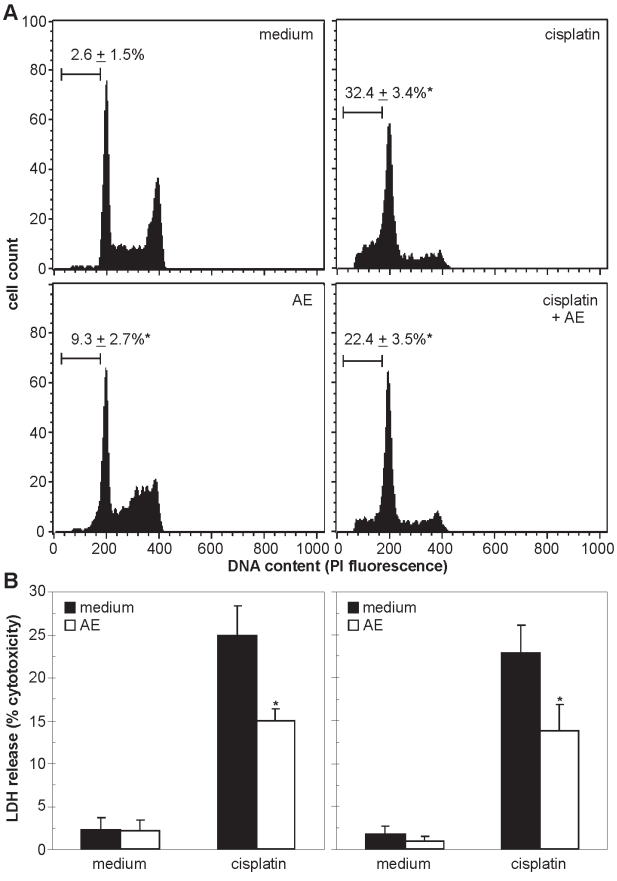

Figure 2. AE reduces cisplatin-induced DNA fragmentation and LDH release. (*A*) L929 cells (1×10^6 per plate) were incubated for 24 h with or without cisplatin (20 µM) and/or AE (20 µM), and then stained with PI as described in Materials and methods. The histogram of flow cytometric cell cycle analysis of PI-stained cells from a representative experiment is shown, while the percentage of apoptotic (sub-G) cells given in each histogram is the mean ± SD of values obtained in three separate experiments [*$p < 0.05$ in 'AE' and 'cisplatin' groups is relative to untreated cells ('medium'), while *$p < 0.05$ in 'cisplatin + AE' is relative to 'cisplatin'; ANOVA]. (*B*) C6 (left) or L929 (right) cells (3×10^4/well) were treated with cisplatin (20 µM), in the presence or absence of AE (20 µM) for 24 h, and LDH release was subsequently assessed. The data are presented as the mean ± SD from three experiments.

AE caused only a slight, but significant increase in the number of hypodiploid, apoptotic tumor cells (fig. 2A), which was not associated with increased cell membrane permeability and subsequent release of LDH (fig. 2B). Moreover, both cisplatin-induced DNA fragmentation and LDH release were efficiently downregulated by concomitant treatment with AE (fig. 2A, B). Results similar to those presented in figure 2A were also obtained with C6 cells (data not shown). Double staining with annexin-FITC and PI confirmed the ability of AE to interfere with cisplatin-induced apoptosis and necrosis, as a significantly lower number of both apoptotic (annexin$^+$/PI$^-$) and necrotic (annexin$^+$/PI$^+$) cells was found in cisplatin-treated L929 cell cultures in the presence of AE (fig. 3).

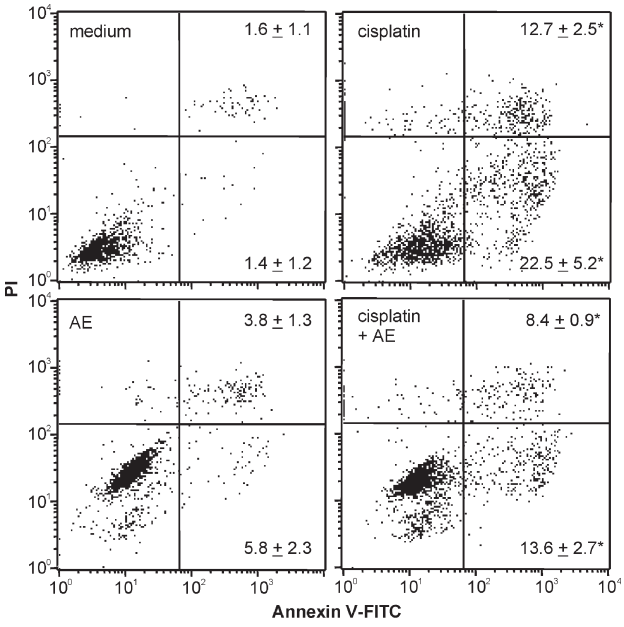

Figure 3. AE decreases cisplatin-induced apoptosis and necrosis of L929 cells. L929 cells (1×10^6 per plate) were incubated for 18 h with or without cisplatin (20 µM) and/or AE (20 µM), and then stained with annexin V-FITC and PI. The dot plots of flow cytometric analysis are from a representative experiment, while the percentages of apoptotic (annexin$^+$/PI$^-$) and necrotic (annexin$^+$/PI$^+$) cells given in each dot plot are mean ± SD values from three separate experiments [*$p < 0.05$ in the 'cisplatin' group is relative to untreated cells ('medium'), while *$p < 0.05$ in 'cisplatin + AE' is relative to 'cisplatin'; ANOVA].

AE downregulates cisplatin-triggered activation of ERK in tumor cells

Activation of various members of the MAP kinase signaling pathway, including ERK and JNK, is one of the major intracellular events induced in tumor cells by cisplatin treatment and responsible for its toxicity [4]. Accordingly, the cell-based ELISA revealed that cisplatin administration led to a time-dependent increase in the amount of activated, phosphorylated forms of ERK in both L929 and C6 cells (fig. 4A). In contrast, AE significantly reduced the level of activated ERK in untreated tumor cells and almost completely prevented cisplatin-induced ERK activation in both cell lines (fig. 4A). The ability of AE to markedly reduce cisplatin-triggered activation of both ERK1 and ERK2 in L929 cells was confirmed by Western blot analysis of the phosphorylation status of ERK (fig. 4B). Similar to the results previously obtained with cisplatin-treated ovarian carcinoma and breast cancer cell lines [24, 25], cisplatin-induced activation of another MAP kinase family member, JNK, reached its peak after 4 h and then declined in both C6 and L929 cells (fig. 4C). The addition of AE, however, failed to significantly affect either basal or cisplatin-triggered activation of JNK in L929 and C6 cells (fig. 4C), indicating that the observed inhibitory effect of the drug was fairly selective for ERK.

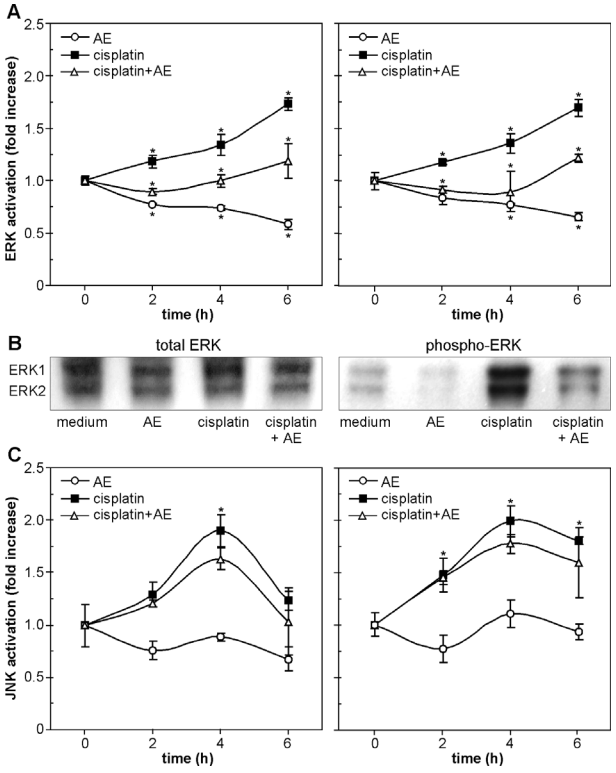

Figure 4. AE blocks cisplatin-triggered activation of ERK, but not JNK. (*A*, *C*) C6 cells (left) or L929 cells (right) (both 3×10^4/well) were incubated with or without cisplatin (20 µM) in the presence or absence of AE (20 µM). The activation of ERK or JNK was assessed at indicated time points by cell-based ELISA for phospho-ERK or phospho-JNK. The data from a representative of three separate experiments are presented as the mean ± SD of triplicate observations [*$p < 0.05$ relative to 0 h time point (untreated cells) for 'cisplatin' and 'AE,' or to corresponding 'cisplatin' values for 'cisplatin + AE'; ANOVA]. (*B*) L929 cells (2×10^6) were incubated for 6 h with or without cisplatin (20 µM) in the presence or absence of AE (20 µM). The levels of total and phosphorylated ERK1 and ERK2 were determined by Western blotting followed by chemiluminescent detection. Similar results were obtained in another experiment.

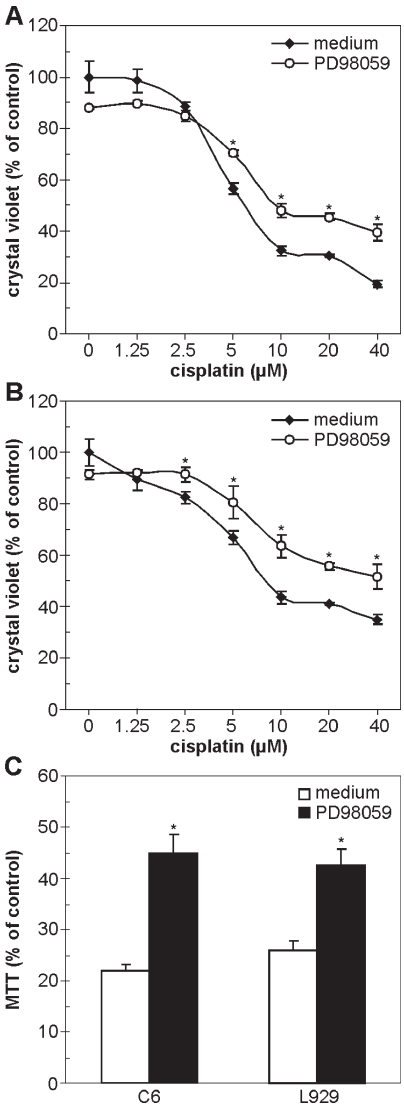

Figure 5. ERK antagonism mimics the protective action of AE. C6 (*A*) or L929 (*B*) cells (3×10^4/well) were incubated with different doses of cisplatin in the presence or absence of the ERK antagonist PD98059 (20 µM), and cell viability was determined after 24 h by crystal violet assay. (*C*) Tumor cell lines were incubated with cisplatin (20 µM) in the presence or absence of PD98059 (20 µM), and an MTT test was performed after 24 h. The data from a representative of three independent experiments are presented as the mean ± SD of triplicate observations (*$p < 0.05$ represents significantly higher viability of PD98059-treated vs cisplatin alone-treated cells; t test).

ERK inhibitor PD98059 mimics AE-mediated protection against cisplatin toxicity

To investigate whether the interference with ERK activation might be involved in AE-mediated impairment of the antitumor action of cisplatin, we tested the ability of PD98059, a selective inhibitor of ERK activation, to mimic the protective effect of AE. Preliminary experiments confirmed by cell-based ELISA that PD98059 can markedly downregulate both basal and cisplatin-induced activation of ERK in tumor cell lines (data not shown). This inhibition of ERK was associated with only a slight decrease in tumor cell number, as assessed by crystal violet staining of C6 and L929 cells (fig. 5A, B). However, similar to the results previously obtained with AE, the addition of PD98059 significantly improved the survival of tumor cell lines in the presence of cisplatin, as demonstrated by both the crystal violet (fig. 5A, B) and MTT (fig. 5C) assay. Blockade of ERK activation appears, therefore, to contribute to AE-mediated protection of tumor cells against cisplatin toxicity.

AE does not prevent the ERK-independent anticancer action of [Pt(dbeddp)Cl_4]

To further corroborate our assumption about ERK involvement in the tumor-protective action of AE, we took advantage of our recent finding that the cytotoxic action of a

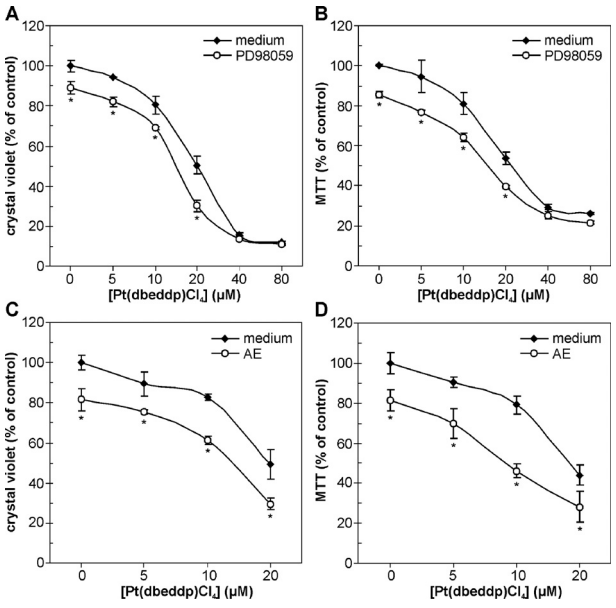

Figure 6. AE does not reduce ERK-independent toxicity of [Pt-(dbeddp)Cl4]. L929 cells (3×10^4/well) were incubated with different doses of [Pt(dbeddp)Cl_4] in the presence or absence of 20 μM PD98059 (*A*, *B*) or 20 μM AE (*C*, *D*), and cell viability was determined after 24 h by crystal violet (*A*, *C*) or MTT (*B*, *D*) assay. The data from one of two independent experiments are presented as the mean ± SD of triplicate observations (*$p < 0.05$ refers to corresponding cultures without PD or AE; t test).

novel Pt(IV) complex [Pt(dbeddp)Cl_4] toward human U251 glioma cells was ERK independent [26]. Treatment with [Pt(dbeddp)Cl_4] in a dose-dependent manner reduced the number and mitochondrial respiration of L929 cells, as determined by crystal violet and MTT assay, respectively (fig. 6A, B). In contrast to the results obtained with cisplatin, the ERK antagonist PD98059 did not protect L929 cells from [Pt(dbeddp)Cl_4]-mediated toxicity, which confirmed our previous observation of ERK-independent action of this Pt(IV) complex (fig. 6A, B). Similarly, AE completely failed to rescue tumor cells from the ERK-independent toxicity of [Pt(dbeddp)Cl_4] and even collaborated with the Pt(IV)-based agent to further reduce the viability of tumor cell lines in an additive fashion (fig. 6C, D). Results similar to those presented in figure 5 were also obtained with C6 glioma cells (data not shown), thus further suggesting that AE-mediated protection against cisplatin toxicity might depend on the ability of the anthraquninone to interfere with ERK activation in tumor cells.

Discussion

The results of the present study clearly demonstrate the ability of the hydroxyanthraquinone derivative AE to protect L929 fibrosarcoma and C6 glioma cell lines against the toxicity of the established anticancer therapeutic cisplatin. The observed effect of AE was associated with improved DNA and cell membrane integrity, indicating that the drug efficiently prevented both apoptotic (DNA fragmentation) and necrotic-like (cell membrane destruction) cell damage induced by cisplatin. The mechanisms underlying the protective action of AE apparently involved the suppression of cisplatin-induced activation of the MAP kinase family member ERK in tumor cells, while activation of JNK remained unaltered by AE treatment. Although AE-mediated protection against cisplatin toxicity has recently been demonstrated in normal lung fibroblasts [17], this is the first study to describe the ability of AE to prevent cisplatin-induced death of tumor cells.

The assumption that the effect of AE in the present report was due to inhibition of ERK is consistent with the finding that PD98059, a fairly selective inhibitor of an upstream ERK activator MEK [27], effectively mimicked the protective action of AE in our experiments. This is also consistent with a large body of evidence demonstrating the involvement of ERK in cisplatin toxicity against a variety of cancer cell lines [28–32]. Cisplatin-activated ERK is required for initiation of cytochrome c release, reduction in mitochondrial membrane potential and subsequent activation of caspase-3 and apoptosis in human cervical carcinoma HeLa cells, malignant human testicular germ cells, and the A172 human glioma cell line [28, 31, 32]. AE-mediated blockade of ERK activation might therefore prevent ERK-dependent signals that act upstream of mitochondrial dysfunction and caspase activation from initiating the apoptotic program in cisplatin-exposed tumor cells. Such an assumption is apparently strengthened by our observation that AE was unable to prevent ERK-independent tumor cell death induced by the novel Pt(IV)-based complex [Pt(dbeddp)Cl_4]. It should be noted, however, that AE was almost as potent as a selective ERK inhibitor in preventing cisplatin anticancer activity in our experiments, suggesting that some ERK-independent actions of AE might also be involved in the observed protection of tumor cells.

There is a question of the mechanism(s) underlying the AE-mediated blockade of ERK activation in tumor cells. DNA damage is apparently required for the induction of ERK by cisplatin, as its trans-diaminodichloroplatinum isomer that does not readily cause DNA injury fails to activate ERK [24, 33]. As emodin has been reported to enhance the nucleotide excision repair of cisplatin-induced DNA damage in human fibroblasts [17], one could postulate that a similar protective effect on DNA might be responsible for AE-mediated inhibition of cisplatin-triggered ERK activation in our experiments. On the other hand, many tumor cells, unlike their normal counterparts, display constitutively up-regulated ERK activity [34, 35], and we have previously reported [15] and confirmed here the ability of AE to downregulate this basal ERK activation in tumor cells. Moreover, emodin was found to prevent ERK

activation in human cancer cell lines treated with 12-O-tetradecanoylphorbol-13-acetate [36], a phorbol ester that acts through induction of PKC activity [37]. Thus, AE could block cisplatin-induced ERK activation at least in part independently of its DNA-repairing action, probably by interfering with some ERK-activating signals that lie downstream of cisplatin-triggered DNA damage. As these signals have been shown to involve generation of reactive oxygen species [38] and activation of Ras [30], the antioxidant and Ras-antagonizing actions previously ascribed to emodin and/or its derivatives [39, 40] seem worthy of consideration as possible mechanisms involved in the AE-mediated block of cisplatin-triggered ERK activation.

Both emodin and AE have been generally considered as potential anticancer therapeutics due to their ability to induce apoptotic death and/or growth arrest in various tumor cell lines [5–16]. This antitumor activity of AE was also observed in C6 glioma and L929 fibrosarcoma cells, as described in our previous reports [15, 41] and confirmed in the present study. Moreover, emodin was found to sensitize HER-2/neu-overexpressing lung cancer cells and Merkel cell carcinoma to cisplatin treatment [11, 42]. Somewhat paradoxically, the present study demonstrated the protective effect of AE on cisplatin-treated L929 fibrosarcoma and C6 glioma cells. This apparent discrepancy might be due to a difference between the cancer cell lines used in the previous and present study. In other words, the selective ERK antagonism can either potentiate [24, 33, 43–46] or prevent [28–32, 45] cisplatin-induced toxicity depending on the tumor cell line used, indicating that cisplatin-triggered ERK could convey both cell death and survival signals in a cell type-dependent manner. Therefore, the outcome of combined cisplatin/AE treatment might at least partly depend on whether the AE-mediated ERK blockade is protective or damaging for the particular tumor cell line. Moreover, ERK inhibition might exert opposite effects even in the same tumor cell line, depending on the context. While AE-mediated inhibition of ERK was protective for cisplatin-exposed C6 glioma cells in the present study, the interference with ERK in untreated C6 cells was apparently responsible for their differentiation toward an astrocytic lineage in our previous report [15]. ERK activity, at least in C6 glioma cell line, apparently needs to be tightly controlled for optimal cell survival, with either an increase or decrease in ERK activation having detrimental effects on tumor cells, such as induction of apoptosis or differentiation, respectively. This complexity of ERK involvement in tumor cell survival and differentiation warrants further investigation and might eventually provide some clues for the fine-tuning of ERK activity in general, and AE-mediated ERK inhibition in particular, as potential anticancer strategies.

In conclusion, our results indicate that combining AE with cisplatin might undermine the anticancer effect of the latter drug through downregulation of ERK-dependent tumor cell death. Instead, the combination of AE with platinum-based drugs that do not require ERK for their cytotoxic action, such as the recently described Pt(IV) complexes with O,O-dibuthyl-ethylenediamine-N,N′-di-3-propanoato ester [26], seems worthy of consideration as an alternative therapeutic approach. On the other hand, the administration of AE as an adjunct to cisplatin therapy, separated in space or time to minimize the reduction of the anticancer effect, might be a feasible way for protecting normal tissues from the toxic effects of cisplatin. Further studies, however, are required to explore in more detail the interaction between AE and cisplatin or other platinum-based agents, particularly in light of the possible consequences for cancer therapy.

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