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Preclinical report

Inhibition of aldose reductase enhances HeLa cell sensitivity to chemotherapeutic drugs and involves activation of extracellular signal-regulated kinases

Eun Kyoung Lee,[1] William T Regenold[2] and Paul Shapiro[1]
[1]Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy and
[2]Department of Psychiatry, Division of Geropsychiatry, University of Maryland School of Medicine, Baltimore, MD 21201, USA.

Changes in glucose metabolism during diabetes are linked to an increased risk for the development of cancer. Increased activity of aldose reductase, the rate-limiting polyol pathway enzyme that converts glucose into sorbitol, mediates pathologies associated with diabetes and is thought to be involved in increased resistance to chemotherapeutic drugs. Thus, increased intracellular sorbitol levels may serve a protective function in cancer cells. In these studies we determined whether an inhibitor of aldose reductase could enhance the effectiveness of anticancer agents. Our findings indicate that treatment with the aldose reductase inhibitor, ethyl 1-benzyl-3-hydroxy-2(5H)-oxopyrrole-4-carboxylate (EBPC), enhances the cytotoxic effects of the anticancer agents doxorubicin and cisplatin in HeLa cervical carcinoma cells. To establish a mechanistic basis for the increased cytotoxicity by EBPC, we examined the activity of the extracellular signal-regulated kinase (ERK) pathway, which is an important regulator of cell growth. Interestingly, treatment with EBPC in combination with the chemotherapeutic drugs increased ERK activity as compared to treatment with the chemotherapeutic drugs, suggesting a possible role for the ERK pathway in mediating doxorubicin- or cisplatin-induced cell death. Consistent with this possibility, inhibition of ERK activation by the MEK inhibitor, U0126, reversed the EBPC-mediated enhancement of cell death. In summary, these data provide evidence that adjuvant therapy with aldose reductase inhibitors improves the effectiveness of chemotherapeutic drugs, possibly through an ERK pathway-mediated mechanism.

Key words: Aldose reductase, chemotherapy, cytotoxicity, MAP kinase, polyols.

Correspondence to P Shapiro, Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, 20 N Pine Street, Baltimore, MD 21201, USA.
Tel: (+1) 410 706-8522; Fax: (+1) 410 706-0346;
E-mail: pshapiro@rx.umaryland.edu

Introduction

Resistance to chemotherapeutic drugs and the debilitating side effects associated with increasing doses of chemotherapy remain a major barrier for the effective treatment of cancer. Several studies indicate that changes in glucose metabolism correlate with increased tumorgenicity, chemotherapeutic drug resistance and cytotoxic immunologic responses.[1,2] In addition, the correlation between diabetes mellitus and the development of endometrial and breast cancer suggests that changes in glucose metabolism contribute to enhanced cell proliferation.[3,4] Furthermore, chemotherapeutic toxicity and counter-therapeutic effects may be mediated by de-regulation of glucose-responsive proteins or through upregulation of proteins that promote glucose transport and metabolism.[5,6] Therefore, proteins that are involved in glucose metabolism may be targeted for developing effective chemotherapeutic protocols.

Increased flux of glucose through the polyol pathway (also called the sorbitol pathway) has been linked to a number of diabetes complications including neuropathy and retinopathy.[7] Aldose reductase is the initial rate-limiting polyol pathway enzyme that converts glucose to sorbitol.[8,9] Thus, aldose reductase inhibitors are currently being tested in human trials and may provide some level of benefit for treating diabetic neuropathy.[10–12] Moreover, aldose reductase activity has been linked to cell growth. For example, aldose reductase is reportedly involved in glucose-induced proliferation of smooth muscle cells.[13] Furthermore, increases in

aldose reductase expression have been observed in carcinoma cells.[2,15] Importantly, increased aldose reductase expression and intracellular sorbitol accumulation may be responsible for reduced efficacy of chemotherapeutic drugs used to treat breast cancer.[6] In such cases, aldose reductase activity appears to influence cell growth and the response of tumor cells to anticancer drugs. The mechanistic basis for aldose reductase activity, sorbitol production and tumor cell growth is unclear, but may involve activation of growth and survival signals, osmotic sensing signaling pathways, and the regulation of gene expression.

The three major families of mitogen-activated protein (MAP) kinase pathways that regulate cell growth and stress responses include the extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs) and p38 proteins.[16] The ERK MAP kinases function primarily in regulating cell proliferation and differentiation, whereas the JNK and p38 MAP kinases mediate stress and apoptotic signals.[16] However, in some cases ERK pathway activation has been linked to the induction of cell death.[17] Thus, depending on the extracellular signals and cell type, MAP kinase pathways may function in promoting or inhibiting cell growth. Increased extracellular glucose concentrations activate the ERK and p38 MAP kinase pathways in several cell and animal models of diabetes.[18,19] In addition, animal models of long-term diabetes show higher basal levels of ERK activity compared to non-diabetic controls.[20] Whether MAP kinase activation functions in promoting or protecting against the effects of high glucose concentrations during diabetes is not yet known. Similarly, ERK activation in response to changes in glucose metabolism could function in promoting cell survival and proliferation of cells susceptible to transformation.

In these studies, we addressed whether aldose reductase activity functions in sensitizing cells to the genotoxic chemotherapeutic agents, doxorubicin and cisplatin. We find that inhibition of aldose reductase in combination with doxorubicin or cisplatin treatment enhances the cell death response of cultured cancer cells. Our data also indicate that inhibition of aldose reductase enhances ERK activity, and that this activity is required for the enhanced cell death observed in the presence of the aldose reductase inhibitor. Thus, in agreement with previous studies,[17] ERK activity can also mediate cell death. These data provide a rationale for further study of the use of aldose reductase inhibitors as adjuvant therapy for the treatment of cancer.

Materials and methods

Cell culture

Cervical adenocarcinoma (HeLa) cells were grown in complete media [Dulbecco's modified Eagle's medium (DMEM) + 10% fetal bovine serum (FBS)], supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml). For protein analysis following treatments, cells were trypsinized and seeded onto six-well (35-mm) plates and allowed to grow for 24 h. Cells were then cultured in the absence or presence of doxorubicin at doses ranging from 0.1 to 10 μg/ml or treated with 0–240 μM of cisplatin. In some experiments, cells were also treated in the absence or presence of aldose reductase inhibitors at concentrations of 10–60 μM. Treated and untreated cells were further incubated for 2–48 h prior to harvesting and analysis.

Reagents

The aldose reductase inhibitor, ethyl 1-benzyl-3-hydroxy-2(5H)-oxopyrrole-4-carboxylate (EBPC), was purchased from Tocris Cookson (Ballwin, MO) and stored as 10 mM stock solutions in ethanol. Stock solutions of doxorubicin hydrochloride (10 mg/ml), cisplatin (60 mM) and MTT (5 mg/ml) were purchased from Sigma (St Louis, MO), and stored in DMSO (doxorubicin and cisplatin) or phosphate-buffered saline (PBS). The MEK1/2 inhibitor, U0126, was purchased from Promega (Madison, WI) and stored at a 10 mM stock solution in DMSO. The monoclonal antibody recognizing phosphorylated ERK1/2 (pT183, pY185) was purchased from Sigma. Polyclonal antibodies recognizing poly(ADP-ribose)-polymerase (PARP; H-250) and ERK2 (C-14) were purchased from Santa Cruz Biotechnolgy (Santa Cruz, CA).

Cell proliferation assays

Cell proliferation assays were performed using direct cell counts following Trypan blue staining or by MTT assay. For cell counts using Trypan blue, cells were seeded on 12-well plates and incubated in the absence or presence of doxorubicin, cisplatin or aldose reductase inhibitors for 24–48 h. Floating and adherent cells were collected following trypsinization, stained with Trypan blue (0.2%) in PBS and counted using a hemocytometer. Dead cells were

scored by their failure to exclude Trypan blue and expressed as a percentage of the total number of cells counted under each condition. For the MTT assay, cells were seeded on 24-well plates containing 1 ml of media and treated as indicated above for the Trypan blue assay. Following treatments, 100 μl of MTT stock was added to each well and incubated for 3 h. The reaction mixture was stopped with DMSO, transferred to a 96-well plate, and absorbance was measured at 570 nm to quantify the formazan product and 690 nm for background levels using a multi-well plate reader (Multiskan Ascent; MTX Lab Systems, Vienna VA).

Immunoblotting

Untreated and treated cells were washed twice with cold PBS, lysed with 300 μl of tissue lysis buffer (20 mM Tris, pH 7.4, 137 mM NaCl, 2 mM EDTA, 1% Triton X-100, 25 mM β-glycerophosphate, 2 mM sodium pyrophosphate, 10% glycerol, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride and 1 mM benzamidine) and centrifuged at 15 000 r.p.m. to clarify lysates. Lysates (around 20 μg of total protein) were diluted with an equal volume of 2 $\times$ SDS sample buffer and resolved by SDS–PAGE. Proteins were transferred to PVDF membrane, blocked for 1–2 h with 5% non-fat dry milk in Tris-buffered saline (TBS, 50 mM Tris, pH 7.5, 0.15 M NaCl and 0.1% Tween 20) and incubated with primary antibodies diluted in TBS + 1% BSA for 2 h to overnight. Membranes were washed several times in TBS and incubated with horseradish peroxidase-conjugated anti-mouse or anti-rabbit antibodies (Jackson ImmunoResearch; diluted 1:10,000). Protein immunoreactivity was detected by enhanced chemiluminescence (NEN/DuPont, Boston, MA).

Statistics

Statistical significance between treatment groups was determined using analysis of variance.

Results

Induction of cell death in response to varying doses of doxorubicin and cisplatin

Initial experiments characterized the cytotoxic effects of doxorubicin or cisplatin on HeLa cells. Cells were plated and allowed to grow for 24 h before being exposed to varying concentrations of doxorubicin or cisplatin for an additional 24–48 h. Dead cells that failed to exclude Trypan blue dye were counted and expressed as a percentage of the total cells, and cell proliferation was determined using MTT assays under each condition. Cells showed a dose- and time-dependent cytotoxic response to both doxorubicin and cisplatin (Figure 1). After a 2-day exposure to drugs, cells treated with cisplatin showed increased cell death with increasing drug doses, whereas the maximum cell death as measured by Trypan blue dye exclusion occurred with approximately 0.25 μg/ml for doxorubicin (Figure 1A and B). Cell proliferation was also inhibited in a dose-dependent manner following a 2-day exposure to doxorubicin or cisplatin as measured using the MTT assay (Figure 1C and D). Similar to the dead cell counts in Figure 1(A and B), a 1-day exposure to drugs showed only a smaller degree of cell growth inhibition at the highest concentrations as measured by the MTT assay (Figure 1C and D).

Induction of PARP cleavage with varying doses of cisplatin and doxorubicin

The cleavage of PARP, as an indicator of apoptotic cell death, was examined in cells treated for up to 28 h with varying doses of doxorubicin or cisplatin. Doxorubicin or cisplatin caused a dose- and time-dependent cleavage of the 112-kDa full-length PARP protein and the subsequent generation of the 85-kDa fragment (Figure 2). The concentrations of doxorubicin or cisplatin that stimulated PARP cleavage correlated well with the drug concentrations that stimulated cell death and inhibited cell proliferation as shown in Figure 1. Thus, the experiments in Figures 1 and 2 established the conditions for examining the effects of aldose reductase inhibitors on cisplatin or doxorubicin-mediated cytotoxicity.

Inhibition of aldose reductase enhances cisplatin- and doxorubicin-mediated cell death

Since HeLa cells express high levels of aldose reductase,[21] we chose these cells to test whether the presence of an aldose reductase inhibitor could enhance the cytotoxic effects of anticancer agents. For these experiments, cells were exposed to suboptimum doses of doxorubicin or cisplatin that typically cause minimal cell death (Figure 1) in the presence or absence of the aldose reductase

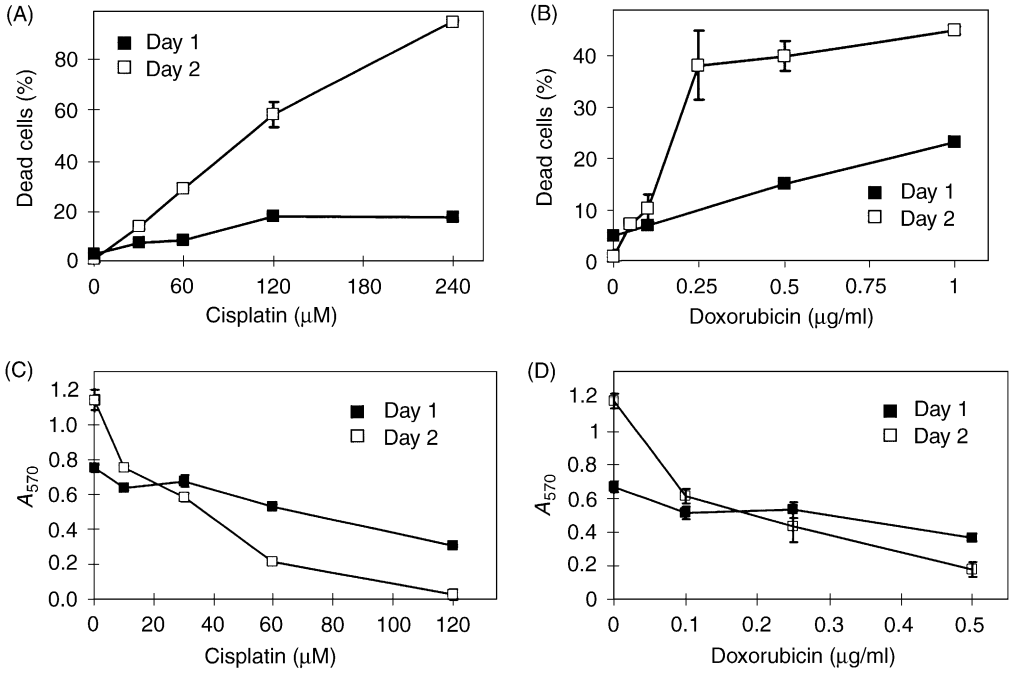

Figure 1. Effect of doxorubicin or cisplatin on cytotoxicity of HeLa cells. The percentage of dead cells was determined following incubation with varying concentrations of cisplatin (A) or doxorubicin (B). Detached cells and adherent cells were collected, stained with Trypan blue, and counted using a hemocytometer after 1 (closed squares) or 2 (open squares) day incubation with the drugs. The data represents averages ± SD from four separate culture dishes. Cell proliferation was also determined using the MTT assay in cells treated with varying concentrations of cisplatin (C) or doxorubicin (D) for 1 (closed squares) or 2 (open squares) days. Day 1 samples contained fewer cells, hence the lower absorbance readings in the untreated condition as compared to day 2 cells. Data represents average ± SD from three separate culture dishes.

inhibitor, EBPC. Cells treated for 24 h with 0.25 μg/ml doxorubicin or 30 μM cisplatin in the presence of 30 or 50 μM of EBPC showed a significant increase in the percentage of dead cells as compared to untreated controls (Figure 3A and B). EBPC also enhanced the cytotoxic effects of doxorubicin and cisplatin on cell proliferation as evaluated by MTT assays (Figure 3C and D). Treatment with EBPC alone at these concentrations had no effect on cell death or proliferation (data not shown). Furthermore, the presence of EBPC also enhanced the cleavage of the PARP protein in cells treated with doxorubicin or cisplatin for 16 h (Figure 4A and B). These data suggest that inhibition of aldose reductase activity increases the effectiveness of doxorubicin and cisplatin in promoting cytotoxic effects in HeLa cells.

ERK activation following treatment with doxorubicin or cisplatin is enhanced by EBPC

The ERK MAP kinase pathway has been implicated in mediating cisplatin-induced cell death.[17] To determine the effects of chemotherapeutic drugs on ERK activation, HeLa cells were treated with varying concentrations of doxorubicin or cisplatin for up to 28 h, and protein lysates were immunoblotted for active ERK1 and ERK2 using a phosphorylation-specific ERK1/2 antibody. ERK proteins showed a dose-dependent increase in activity in response to doxorubicin or cisplatin (Figure 5A and B). Doxorubicin and cisplatin treatment caused ERK activation within 4 h of treatment, suggesting that ERK activity is involved in early events that regulate the cellular response to these anticancer drugs (Figure 5C and D).

We next examined whether the ERK pathway was involved in the enhanced chemotherapeutic drug-induced cell death observed in the presence of EBPC (Figure 3). Cells were treated for 4 h with cisplatin (30 μM) or doxorubicin (0.25 μg/ml) in the absence or presence of EBPC (30 μM) only or EBPC plus the MEK1/2 inhibitor U0126 (10 μM), which prevents ERK activation. The 4-h time point was chosen because it was after the ERK proteins were activated in response to doxorubicin or cisplatin but before the cytotoxic effects of these drugs are observed. The presence of EBPC significantly enhanced the activity

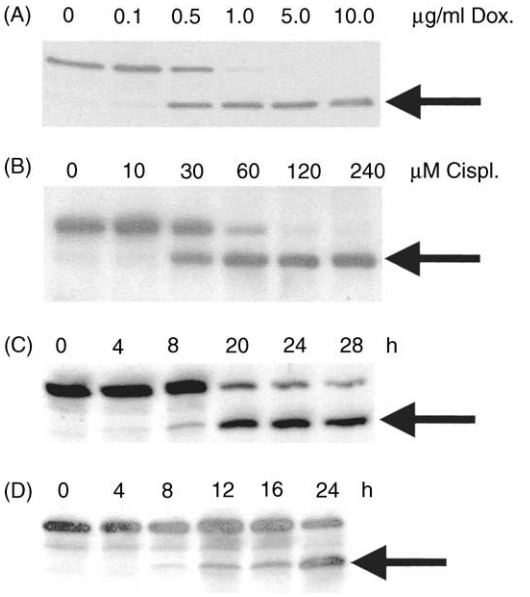

Figure 2. Effect of doxorubicin or cisplatin on PARP cleavage. Cells were incubated with the indicated concentrations of doxorubicin (Dox., A) or cisplatin (Cispl., B) for 24 h. Time course of PARP cleavage following exposure to 1 μg/ml doxorubicin (C) or 60 μM cisplatin (D). Cell lysates were collected and the proteins were separated by SDS–PAGE and immunoblotted for PARP. Arrows indicate the 85-kDa cleaved form of PARP that is generated following apoptotic stimuli. The data are representative of at least three separate experiments.

of the ERK proteins in response to doxorubicin and cisplatin (Figure 6A and B). EBPC treatment alone had no effect on ERK activity (data not shown). As expected, the presence of U0126 blocked ERK activation under all conditions in Figure 6. These data indicate that aldose reductase activity during exposure to doxorubicin or cisplatin suppresses ERK activation and may function to protect cell survival.

Inhibition of ERK activity reverses EBPC-induced enhancement of doxorubicin- or cisplatin- mediated cytotoxicity

Lastly, to demonstrate that ERK activity mediates the enhanced cell death observed during aldose reductase inhibition, cytotoxicity assays were performed in the presence of U0126. Cells were treated with low concentrations of doxorubicin or cisplatin in the absence or presence of 30 μM EBPC and U0126 (10 μM) for 2 days, and the percentage of dead cells was determined following staining with Trypan blue. Similar to the data presented in Figure 3, treatment with EBPC significantly enhanced the cytotoxic response to both doxorubicin and cisplatin (Figure 7). The presence of U0126 effectively reversed the EBPC-mediated cytotoxic response to doxorubicin

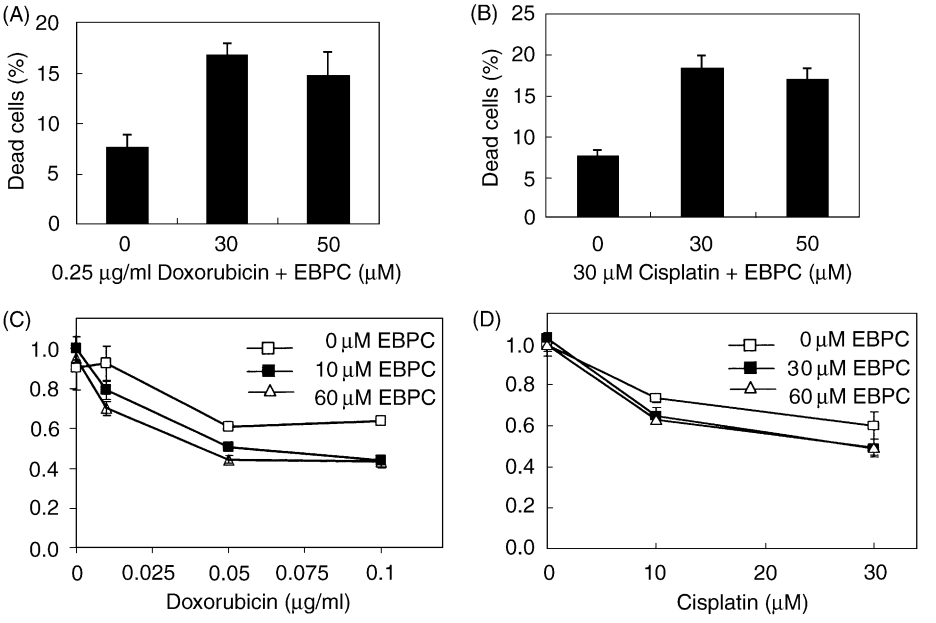

Figure 3. Aldose reductase inhibitors enhance the cytotoxic response to doxorubicin or cisplatin. Cells were incubated with 0.25 μg/ml doxorubicin (A) or 30 μM cisplatin (B) for 24 h in the absence or presence of the aldose reductase inhibitor EBPC (30 or 50 μM). Detached and adherent cells were collected and the percentage of dead cells was determined following staining with Trypan blue. Data are representative of two separate experiments each done in quadruplicate. Cell proliferation was determined using MTT assay following treatment for 2 days with varying doses of doxorubicin (C) or cisplatin (D) in the absence or presence of 0–60 μM EBPC. The difference between non-treated and EBPC-treated cells was statistically significant by analysis of variance ($p < 0.05$).

and cisplatin (Figure 7). The ability of U0126 to inhibit cisplatin-mediated cell death is in support of previous studies.[17] These data support a role for ERK activation in mediating genotoxic drug-induced apoptosis in HeLa cells.

Discussion

This study presents evidence that inhibition of aldose reductase, the polyol pathway enzyme that converts glucose to sorbitol, sensitizes cancer cells to chemotherapeutic agents. These findings provide the basis for further studies of aldose reductase inhibitors as adjuvant therapy in the treatment of cancer. Importantly, aldose reductase inhibitors may prove beneficial to cancer patients by affording the use of lower doses of doxorubicin or cisplatin that would be similarly effective in promoting cancer cell death, while reducing the toxic effects of these chemotherapeutic compounds on normal cells. Other studies also provide evidence that targeting the aldose reductase enzyme may be a beneficial approach for treating cancer. For example, liver cancer cells overexpressing aldose reductase are resistant to treatment with doxorubicin and this resistance is reversible by treatment with aldose reductase inhibitors.[22] Since aldose reductase expression is high in HeLa cells used in this study and in other cancer cell lines isolated from renal, ovarian, brain, breast, non-small cell lung and small cell lung tissue,[2] inhibition of this protein may affect tumor cell responsiveness to chemotherapeutic compounds. Importantly, aldose reductase inhibitor compounds are being tested in clinical trials in the US for treating diabetic neuropathy[23] and could be rapidly transferred for adjuvant therapy in the treatment of cancer.

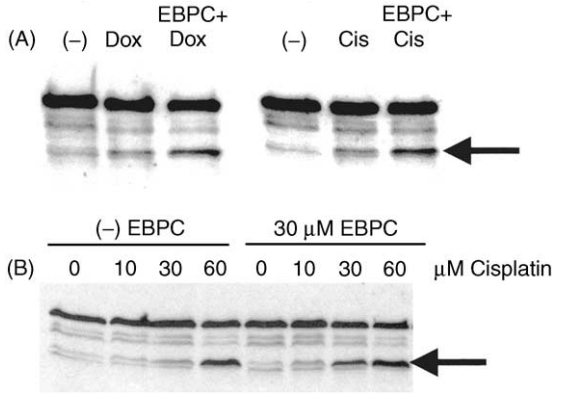

Figure 4. Inhibition of aldose reductase enhances PARP cleavage. (A) PARP cleavage immunoblots in cells were left untreated or exposed to 0.25 μg/ml doxorubicin (Dox, left panel) or 30 μM cisplatin (Cis, right panel) in the absence or presence of EBPC (60 μM) for 16 h. (B) Cells were treated with varying concentrations of cisplatin in the absence or presence of 30 μM EBPC. Arrows indicate the 85-kDa cleaved form of PARP that is generated following apoptotic stimuli.

Potential mechanisms by which aldose reductase facilitates cancer cell resistance to chemotherapeutic compounds could be by increasing drug metabolism or decreasing drug transport into the cell. For example, increased expression of aldose reductase may account for carbonyl reduction and increased detoxification of doxorubicin in doxorubicin-resistant human stomach carcinoma cells.[24] Furthermore,

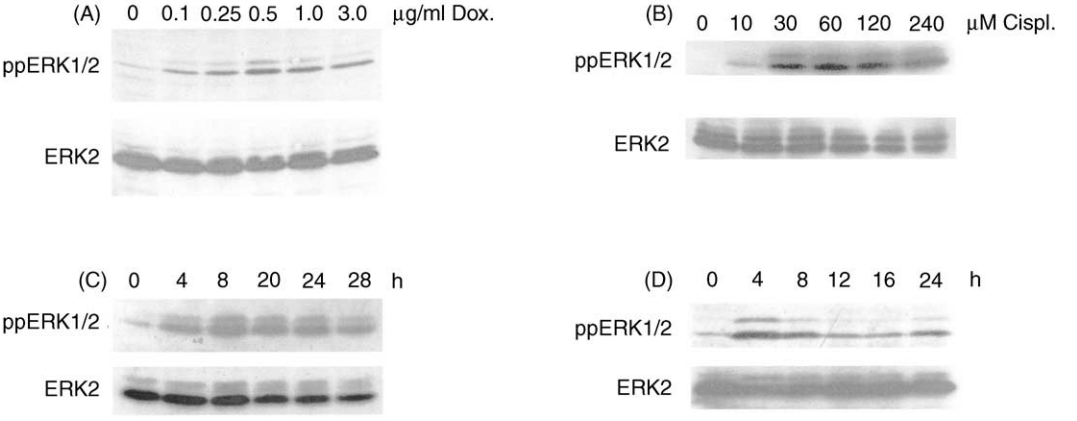

Figure 5. Activation of ERK1 and ERK2 in cells treated with doxorubicin or cisplatin. Cells were incubated with varying doses of doxorubicin (A) or cisplatin (B) for 20 h, and protein lysates were collected and immunoblotted for active ERK1 and ERK2 (ppERK1/2, top panels) and total ERK2 (bottom panels) for a protein loading control. The time course for ERK activation (ppERK1/2, top panels) in response to 0.5 μg/ml doxorubicin (C) or 60 μM cisplatin (D) is shown. Total ERK2 is shown for a protein loading control in the bottom panels.

treatment of cells with sorbitol decreased the sensitivity of non-small cell lung cancer cell lines to cisplatin through a mechanism involving inhibition of Na^+/K^+ ATPase activity, which appears to be important for cellular uptake of cisplatin.[25]

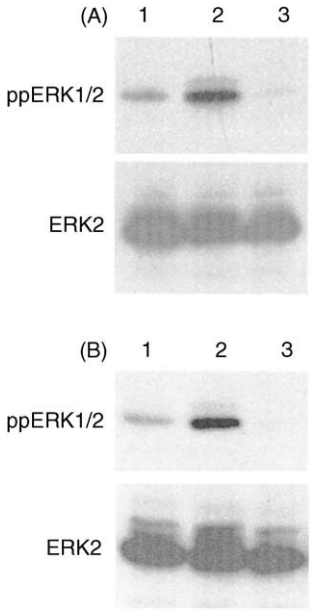

Figure 6. Inhibition of aldose reductase enhances ERK activity. Cells were incubated with 0.25 μg/ml doxorubicin (A) or 30 μM cisplatin (B) and EBPC (30 μM) for 4 h in the absence or presence of the MKK inhibitor, U0126 (10 μM). Cell lysates were collected and immunoblotted for active ERK proteins (ppERK1/2, top panels) and total ERK2 for a protein loading control (bottom panels). Lane 1 indicates doxorubicin or cisplatin only treated cells. Lane 2 indicates doxorubicin or cisplatin plus EBPC-treated cells. Lane 3 indicates doxorubicin or cisplatin plus EBPC and U0126-treated cells.

Aldose reductase serves an important function in preserving cell integrity in stressful environments. For example, increased aldose reductase activity and concomitant increases in intracellular sorbitol concentrations are used to maintain an osmotic balance with the extracellular environment. This is best described in the kidney where renal medullary cells that are exposed to a high salt environment generate sorbitol as an organic osmolyte to maintain osmotic balance.[26,27] Aldose reductase-mediated production of sorbitol also serves as a cryopreservative in order to promote cell survival. This is evident in overwintering insects and hibernating animals that use increased intracellular sorbitol levels to lower metabolic requirements and protect cellular functions.[28,29] Similarly, we propose that aldose reductase activity in cancer cells acts as a survival factor in permitting other growth regulatory enzymes to function more efficiently. Increased aldose reductase activity in cancer cells may also provide additional substrates to meet the high-energy requirements of rapidly proliferating cells. This can be demonstrated in proliferating sperm germ cells, which use fructose derived from sorbitol as an energy source during normal metabolic processes.[30]

To determine the mechanisms involved in increased cell death following aldose reductase inhibition, the activity of the ERK signaling pathway following treatment with low doses of doxorubicin or cisplatin was examined. Previous studies demonstrated that both cisplatin and doxorubicin cause

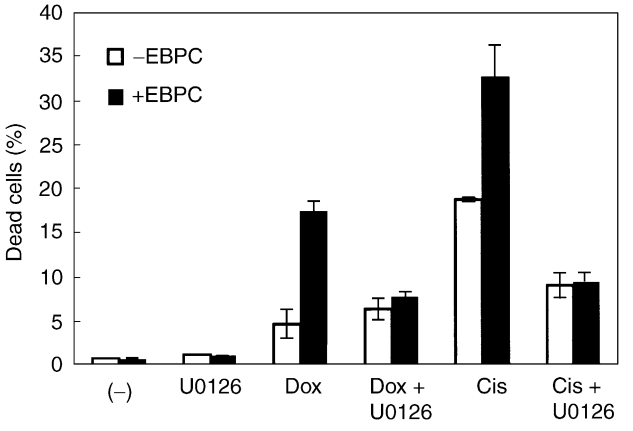

Figure 7. ERK activation mediates EBPC-induced cell death in response to low doses of doxorubicin or cisplatin. The percentage of dead cells was determined following a 2-day incubation with 0.1 μg/ml doxorubicin or 30 μM cisplatin in the absence or presence of EBPC (30 μM) and U0126 (10 μM). Both floating cells and adherent cells were collected, and the percentage of dead cells was counted following staining with Trypan blue. The percentage of dead cells treated only in the presence of EBPC or U0126 was similar to the untreated controls. Values represent the averages $\pm$ SD from three separate experiments. The difference between non-treated and EBPC treated cells was statistically significant as determined by analysis of variance ($p < 0.05$).

activation of the ERK and other MAP kinase pathways.[17,31,32] Furthermore, ERK and p38 MAP kinase activation occurs in response to increased extracellular osmolarity following sorbitol treatment.[33] Contrary to our hypothesis, aldose reductase inhibition enhanced ERK activity following treatment with doxorubicin or cisplatin in HeLa cells (Figure 6A and B). Moreover, the addition of a MEK inhibitor to block ERK activity reversed the increase in doxorubicin- or cisplatin-induced cell death observed in the presence of the aldose reductase inhibitor (Figure 7). Thus, under these conditions, ERK activation appears to mediate cell death induced by aldose reductase inhibition. The mechanism by which aldose reductase inhibition activates ERK is not clear, but may be related to changes in metabolism or transport of doxorubicin and cisplatin. These possibilities are currently being investigated.

ERK activation following treatment with chemotherapeutic agents may function in inhibiting or promoting a cell death response depending on the cell type and stimulus. Our findings suggest that ERK activation promotes cell death and is in agreement with other studies examining cisplatin-induced cell death in HeLa cells.[17] Further support of ERK activation as a promoter of apoptosis is demonstrated in neuronal cells, where ERK activation by taxol causes phosphorylation of the microtubule associated protein Tau and promotes apoptosis.[34] In addition, a novel vitamin K analog inhibits MCF7 growth through a mechanism that may involve enhanced ERK protein phosphorylation.[35] The mechanisms by which ERK proteins promote an apoptotic response are not fully defined. However, one possibility suggests that cisplatin-induced ERK activation phosphorylates and stabilizes the p53 tumor suppressor protein and its function in promoting apoptosis.[36]

In contrast, other reports suggest that the ERK pathway is not involved or protects against genotoxic-induced cell death. For example, a lack of involvement is suggested in some carcinoma cell lines where doxorubicin treatment does not activate the ERK pathway.[31] However, inhibition of the ERK pathway sensitizes ovarian carcinoma cells to cisplatin-mediated cytotoxicity, indicating that the ERK pathway may also serve a protective function against genotoxic stress.[37–39] Similarly, forced expression of oncogenic Raf-1, an indirect upstream activator of ERK proteins, protects cells against apoptosis.[40] However, the mechanism by which Raf-1 inhibits apoptosis appears to be through targeting and inactivating of the anti-apoptotic Bcl-2 protein at the mitochondrion and not by activating ERK proteins.[41] Thus, ERK's role in regulating cellular responses may be distinct from Raf-1 and may function in promoting or inhibiting an apoptotic event in a cell- and stimuli-dependent manner.

Conclusions

In summary, our studies provide additional support for the use of aldose reductase inhibitors as adjuvant therapy for treating cancer. Many cancer cells contain elevated levels of aldose reductase, indicating that this protein may be involved in cancer cell growth and survival. Our findings support this concept by demonstrating that inhibition of aldose reductase increases the toxicity of chemotherapeutic drugs. This may have significant implications with regard to reducing chemotherapy-related drug toxicity to normal tissue without a loss in the effectiveness of these anticancer agents in eliminating cancer cells. Future studies in animal models will provide additional support for using aldose reductase inhibitors as adjuvants in cancer chemotherapy. Importantly, aldose reductase inhibitors have been shown to be effective in clinical trials for treating complications associated with diabetes and thus could be readily transferred for testing in cancer patients.

Acknowledgments

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