

Dexamethasone as a chemosensitizer for breast cancer chemotherapy: Potentiation of the antitumor activity of adriamycin, modulation of cytokine expression, and pharmacokinetics

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Abstract. Dexamethasone (DEX) is mainly used as an antiemetic agent in cancer therapy. We have recently demonstrated that DEX pretreatment increases the antitumor activity of the cancer chemotherapeutic agents carboplatin and gemcitabine, and decreases host toxicity in nude mouse xenograft models of human cancer. However, the underlying mechanisms are not fully understood. The present study was designed to determine the effects of DEX pretreatment on the anticancer activity of adriamycin (ADR) in a syngeneic model of breast cancer (4T1), emphasizing the effects of DEX on cytokine expression and modulation of ADR pharmacokinetics. We have demonstrated five major new findings about DEX pretreatment: a) it enhances the therapeutic effect of ADR, inducing almost complete inhibition of tumor growth; b) it increases tumor ADR accumulation; c) it modulates the expression of cytokines produced by the tumor, increasing TNF α and decreasing IL-1 β and VEGF expression; d) it enhances the effects of ADR on induction of apoptosis and inhibition of cell proliferation; and e) it suppresses nuclear NF κ B activation and inhibits ADR-induced NF κ B activation, possibly via I κ B up-regulation. These findings suggest that

DEX can be used as a chemosensitizer and chemoprotectant. These results provide a rationale for the expanded clinical use of DEX for cancer therapy.

Introduction

Although early detection and improvements in surgery, chemotherapy, and radiation therapy have led to increased survival of patients with breast cancer, it remains the second cause of cancer death in women in North America (1). Additionally, although there have been improvements, patient quality of life is still an issue due to the side effects of many of the agents used for therapy. Therefore, increasing efforts need to be devoted to the development of more effective, less toxic therapeutic approaches.

Among therapeutic agents used for breast cancer, adriamycin (ADR) remains a primary chemotherapeutic agent, achieving response rates of 40-50% as a single agent and 60-80% in combination (2,3). For example, the combination of ADR with cyclophosphamide and 5-fluorouracil (5-FU) leads to response rates of 50-80% (3). Unfortunately, as is seen with other DNA-interactive chemotherapeutic agents, ADR causes various side effects, among which myelosuppression is one of the most serious. Several bone marrow protective and stimulatory agents, such as granulocyte colony stimulating factor (G-CSF), have been applied following chemotherapy to reduce the associated hematotoxicity.

Inflammatory responses are believed to play an important role in tumor initiation, progression, and metastasis (4,5). Pro-inflammatory cytokines, such as tumor necrosis factor (TNF) α , interleukin (IL)-1, and IL-6, are produced not only by inflammatory cells but also by tumor and stromal cells (4,5). Acting as essential communicating molecules between tumor and inflammatory cells, pro-inflammatory cytokines promote tumor cell proliferation and progression. Dexamethasone (DEX), a synthetic glucocorticoid, is widely used in autoimmune diseases as an anti-inflammatory agent and

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Abbreviations: DEX, dexamethasone; ADR, adriamycin; FBS, fetal bovine serum; PBS, phosphate-buffered saline; HPLC, high performance liquid chromatography; TNF α , tumor necrosis factor α ; IL-1 β , interleukin 1 β ; VEGF, vascular endothelial growth factor; NF κ B, nuclear factor kappa B; I κ B, NF κ B inhibitory protein; IFP, interstitial fluid pressure

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immunosuppressant, mainly due to its capacity to inhibit the production of pro-inflammatory cytokines (6,7). DEX is also clinically used in the treatment of human malignancies, especially leukemia and myelomas. It has also been given during and after chemotherapy for patients with solid tumors (including breast cancer) to prevent nausea and vomiting and reduce acute toxicity (8-11).

We recently demonstrated that pretreatment with DEX decreased host tissue uptake of carboplatin and gemcitabine, indicating a chemoprotective action of DEX (12). Furthermore, DEX pretreatment enhanced the anticancer effects of carboplatin and gemcitabine in several human cancer *in vivo* models, including those of colon, lung, breast, and brain cancers, indicating that DEX may act as a chemosensitizer for cancer chemotherapy (13). The mechanisms responsible for the chemoprotective effects of DEX, however, are not fully understood. One possibility is that DEX exerts its effects through the suppression of inflammatory responses that occur during tumor development in the host. Mechanistic studies have shown that DEX prevents NF κ B activation via increasing I κ B synthesis, and that this action depends on the early and transient activation of NF κ B (14,15). Our present work focused on investigation of the effects of DEX pretreatment on breast cancer chemotherapy, as well as mechanisms responsible for these effects. Our hypothesis was that pretreatment with DEX may affect expression of pro-inflammatory cytokines through regulation of the NF κ B pathway, thereby enhancing the effects of chemotherapeutic agents in the treatment of breast cancer. This could potentially lead to a change in the clinical use of DEX, possibly altering the administration schedule, and indicating that use of DEX may be preferential to other anti-nausea and hematoprotective agents because of its numerous other protective effects.

Materials and methods

Chemicals and reagents. All chemicals and solvents were high-performance liquid chromatography (HPLC) grade or certified grade. Methanol, acetonitrile and ammonium formate were purchased from Fisher Chemicals (Atlanta, GA). DEX (clinical grade) was purchased from American Regent Laboratories (Shirley, NJ), and ADR (doxorubicin hydrochloride injection) was purchased from NovaPlus. Centrifree micropartition systems (catalog no. 4104) were purchased from Millipore Corp. (Bedford, MA). Cell culture media, fetal bovine serum, sodium pyruvate, nonessential amino acids, penicillin-streptomycin, and other cell culture supplies were provided by the Comprehensive Cancer Center Media Preparation Shared Facility, University of Alabama at Birmingham. Tissue solubilizer (TS-2) was purchased from Research Products Inc. (Mount Prospect, IL).

Animals. The protocol for animal care and use was approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham. Female BALB/c mice (4-6 weeks of age) were obtained from Frederick Cancer Research Facility (Frederick, MD). For 1 week before the study, all animals were fed with commercial diet and provided water *ad libitum*.

Cell culture. The mouse mammary cancer cell line 4T1 was obtained from the ATCC and cultured according to their instructions. The culture medium contained 90% DMEM, 10% fetal bovine serum, 1% penicillin-streptomycin, 1 mM sodium pyruvate, and 1 mM non-essential amino acids. Cells were incubated at 37°C in 5% CO₂.

Animal syngeneic tumor model. The mouse mammary cancer model was established in female BALB/c mice. When cultured 4T1 cells in monolayers reached a confluence of 80%, cells were trypsinized, harvested by centrifugation, washed with the serum-free DMEM medium, and resuspended in the same medium. The cell suspension was then injected subcutaneously (s.c.) (10⁵ cells; total volume, 0.2 ml) into the left inguinal area of 5 to 7-week-old female BALB/c mice. The animals were monitored for activity, physical condition, body weight, and tumor growth. Tumor growth was determined by caliper measurement in two perpendicular diameters of the implant. As reported previously (13,16-18), tumor mass (in g) was calculated by the formula $1/2a \times b^2$, where a is the long diameter, and b is the short diameter (in cm).

In vivo chemotherapy. The mice bearing 4T1 tumors were randomly divided into various treatment and control groups (5 mice/group). Animals were treated with DEX by s.c. injection at a dose of 0.1 mg/day for 5 days or with saline (as controls) before chemotherapy (day -4 to 0). On day 0, ADR was administered intravenously (i.v.) at a single, clinically relevant dose of 10 mg/kg (30 mg/m²).

Pharmacokinetics of adriamycin. The 4T1 tumor-bearing mice were pretreated with DEX (s.c., 0.1 mg/day/mouse for 5 days) or saline (as controls) and, 1 h after the fifth dose of DEX, were given a single i.v. bolus administration of ADR (10 mg/kg) via a tail vein. At various times, blood samples were collected in heparinized tubes, and plasma was separated by centrifugation at 20,000 x g for 5 min. Tissues were collected at various times and immediately blotted on Whatman No. 1 filter paper, trimmed of extraneous fat or connective tissue, weighed, and homogenized in physiological saline (0.9% NaCl, 5 ml/g wet weight). The resulting homogenates were stored at -70°C until further analysis.

HPLC analysis of adriamycin in plasma and tissues. Plasma or tissue samples (200 μ l) were added to 400 μ l of acetonitrile and mixed thoroughly by vortexing for 1 min. After centrifugation at 14,000 x g for 10 min, the organic layer was removed to a glass test tube and evaporated by air at room temperature. The residue was reconstituted in 300 μ l of mobile phase that consisted of 0.1 M ammonium formate (pH 4.0) and acetonitrile (85:15), vortexed thoroughly, and filtered through a 0.2- μ m polypropylene syringe filter for HPLC injection. ADR levels in plasma and tissue samples were determined by a method previously reported (19) with some modifications. Briefly, the mobile phase, which consisted of 0.1 M formate buffer and acetonitrile, was used in a linear gradient from 85% A (ammonium formate) and 15% B (acetonitrile) to 70% A and 30% B up to 6 min, then changed to 50% A/50% B within 4 min. This gradient continued to 100% B during 2 min, then returned to 85% A

and 15% B within an additional 2 min. The flow rate was maintained at 1 ml/min. ADR, eluted from a RP-C₁₈ column (ZORBAX SB-C₁₈, 5 μ M, 4.6x150 mm, Agilent), was monitored using a fluorescence detector (Agilent 1050 series) at an excitation wavelength of 480 nm and an emission wavelength of 550 nm. Quantitation of plasma or tissue ADR was accomplished by use of an external standard curve (0-5,000 ng/ml) that was prepared on a daily basis. Linear regression and correlation analysis were completed to establish the standard peak-area/concentration curves for ADR.

Real-time quantitative PCR (RT-Q-PCR) for analyses of cytokine mRNA expression. The mRNA levels of three cytokines in tumor tissue, TNF α , IL-1 β , and VEGF, were measured by quantitative real-time RT-PCR. A small piece of tumor tissue (9 mm³) was cut and stored in RNAlater (Ambion, Austin, TX) at the time of animal dissection. Total RNA was isolated and purified with the RNeasy Minikit (Qiagen, Valencia, CA). With a TaqMan Reverse Transcription kit (ABI, Foster City, CA), 2 μ g of total RNA were used to form cDNA in a 25- μ l solution, following the manufacturer's suggested protocol. Real-time quantitative PCR was accomplished with an ABI PRISM 7900 machine. Assays-on-demand probes and primer master mixes from ABI were used for all reverse transcription (RT)-PCR reactions. The comparative C_T (threshold cycle) method described in the manufacturer's protocol was used for relative quantitation of expression, comparing treated samples to concordant untreated controls and to the internal control, 18s rRNA. Each sample was run in duplicate.

Cell viability assay. The viability of cultured 4T1 cells was assayed by using the MTT [(tetrazolium salt 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)] assay as reported previously (16,17). In brief, cells were seeded in 96-well microtiter plates (500-1,000 cells/well) and exposed to DEX for 24 h. ADR was then added to the cells that were incubated for additional 24 h. Fresh media, as well as MTT stock solution, were added to each well, reaching a final MTT concentration of 0.5 μ g/ml. Cells were cultured for 2-4 h to allow color development and, at that time, DMSO was added to the wells to solubilize the formazan products. Absorbances were measured at 570 nm.

Cell proliferation assay. Cell proliferation was detected by labeling cells with BrdU using a cell proliferation assay kit (Oncogene Science, San Diego, CA) as reported previously (16-18). After the same treatment protocol described in the MTT assay, cells were incubated with BrdU (1:2,000) for 6-8 h according to the manufacturer's protocol. The incorporated BrdU was detected by monoclonal anti-BrdU antibody via measuring absorbance at dual wavelengths of 450/540 nm with an OPTImax microplate reader (Molecular Devices, Sunnyvale, CA).

Detection of apoptosis. After the same treatment protocol as above, apoptotic cells were analyzed using an annexin V-FITC apoptosis detection kit (BioVision, Mountain View, CA), following the previously reported protocol (16-18). Briefly, total cells collected by centrifugation were resuspended

in 500 μ l of 1x binding buffer, followed by addition of 5 μ l of Annexin V-FITC and 5 μ l of propidium iodide (PI). Samples were incubated in the dark for 5 min, then analyzed by using a Becton Dickinson FACSCalibur (Ex = 488 nm; Em = 530 nm). Each sample was evaluated in duplicate and data were expressed as the percentage of the control.

Western blot analysis. The protein levels of cytoplasmic and nuclear NF κ B (p65) and I κ B were measured by Western blot analysis as described previously (13). Cells were exposed to DEX either alone or in combination with ADR, followed by fractionation of cytoplasmic and nuclear proteins using nuclear and cytoplasmic extraction reagents from Pierce (Rockford, IL). The polyclonal antibodies against NF κ B (p65) and I κ B were purchased from Oncogene Research Products (Boston, MA).

NF κ B-luciferase promoter assay. NF κ B-luciferase promoter activity was detected by the Dual-Luciferase[®] reporter assay system (Promega, Madison, WI) following the manufacturer's protocol. Briefly, 4T1 cells were seeded in a 6-well microtiter plate (10⁴ cells/well), then transfected with NF κ B-luciferase promoter vector that was kindly provided by Dr Chuanshu Huang, New York University School of Medicine. After 8 h, cells were pre-treated with or without DEX for 24 h, followed by exposure to ADR for an additional 24 h. Cells were then harvested and activity was detected using the Dual-Luciferase[®] reporter assay system. The results were expressed as NF κ B luciferase activity relative to that of the internal control.

Results

Pretreatment with DEX enhances the therapeutic effects of ADR in vivo. The effect of DEX pretreatment on the antitumor activity of ADR was studied in a 4T1 syngeneic mouse model of breast cancer. The animals were treated with s.c. DEX at a dose of 0.1 mg/day for 5 days (days -4 to 0), followed by i.v. ADR (on day 0). As illustrated in Fig. 1A, DEX alone showed a modest inhibitory effect on tumor growth. Pretreatment with DEX, however, enhanced the therapeutic effect of ADR, inducing almost complete inhibition of tumor growth. DEX alone produced no observable toxicity, as determined by clinical inspection and measurement of animal weights as indices of toxicity or side effects. ADR was also well tolerated. Further, DEX pretreatment had no effect on the toxicity profiles of ADR (Fig. 1B).

Pretreatment with DEX increases the accumulation of ADR in tumor tissues in tumor-bearing mice. The time-concentration curves are illustrated in Fig. 2. Although there were no significant differences in plasma pharmacokinetics of ADR between control mice and mice pre-treated with DEX (Fig. 2A), DEX pretreatment significantly increased the ADR concentration in tumor tissue, especially for later time points (4 and 24 h after dosing) (Fig. 2B). No appreciable differences in the concentrations of ADR were observed in spleen (Fig. 2C), kidney, liver or heart (data not shown).

Pretreatment with DEX influences cytokine expression at the mRNA level in vivo. In the 4T1 tumors, expression levels of

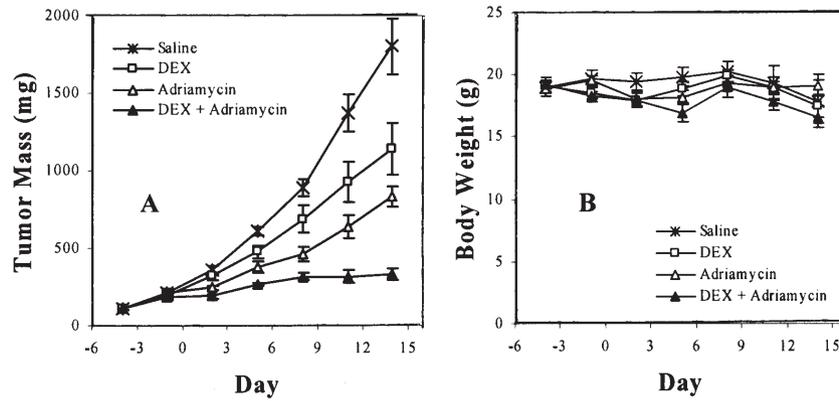


Figure 1. Effects of DEX pretreatment on antitumor activity (A) and body weight (B) following ADR chemotherapy in BALB/c mice bearing 4T1 syngeneic tumors. Animals, randomly divided into various treatment and control groups (5 mice/group), were pretreated with DEX (s.c., 0.1 mg/day for 5 days, days -4 to 0) or saline (as control). ADR was administered i.v. at a single dose of 10 mg/kg (30 mg/m²) on day 0. Tumor mass was expressed as mean ± SD.

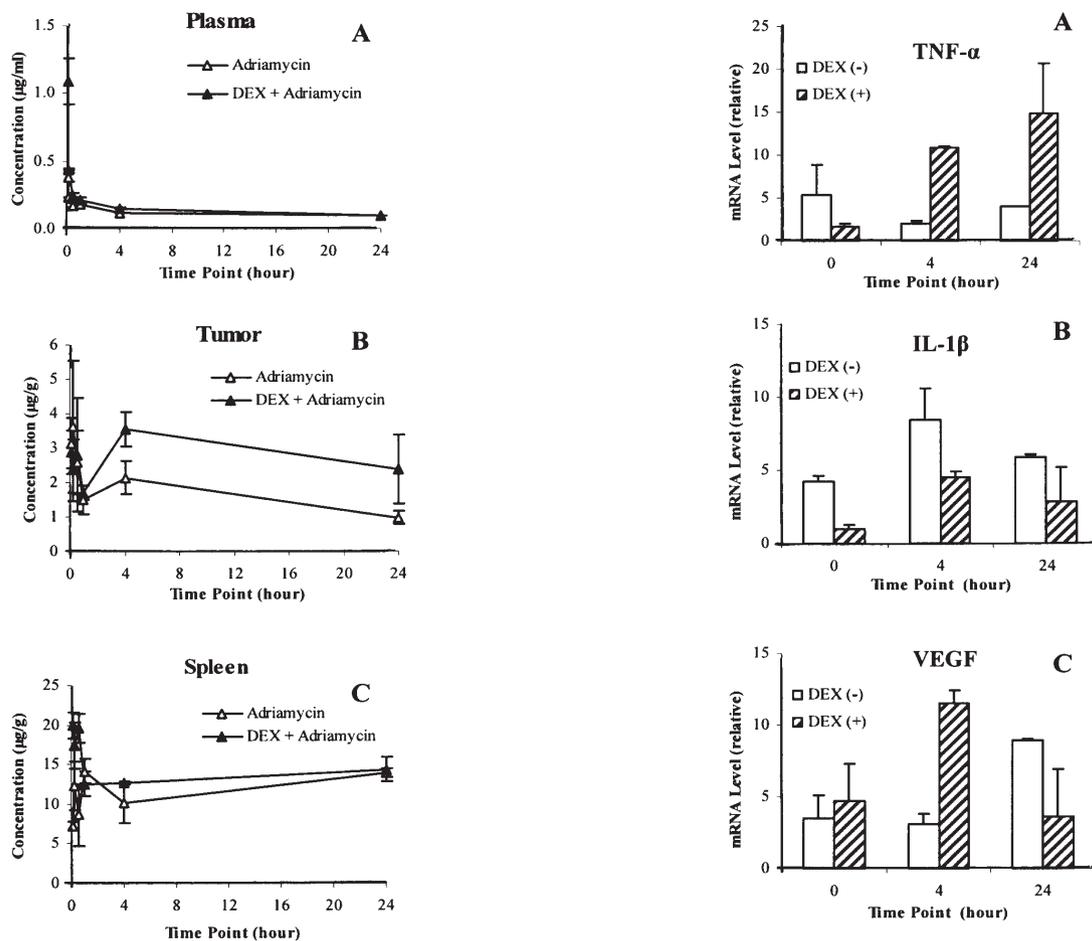


Figure 2. Pharmacokinetics of ADR in BALB/c mice bearing 4T1 tumors. Animals were pretreated with DEX (s.c., 0.1 mg/day/mouse for 5 days) or saline (as controls) and given ADR at a single i.v. dose of 10 mg/kg (30mg/ m²). Plasma (A), tumor (B) and spleen (C) samples were collected at various times up to 24 h. The ADR levels were analysed by high-performance liquid chromatography.

Figure 3. Cytokine mRNA levels in tumor samples of BALB/c mice bearing 4T1 syngeneic mammary cancer. Animals were pretreated with DEX (s.c., 0.1 mg/day/mouse for 5 days) or saline (as controls) and given ADR at a single i.v. dose of 10 mg/kg (30 mg/m²). Tumor samples were collected at various times up to 24 h. mRNA levels of three cytokines, TNF α (A), IL-1 β (B) and VEGF (C), were determined by quantitative real-time RT-PCR. Time points relate to time after ADR administration.

TNF α , IL-1 β and VEGF mRNA were determined by quantitative real-time RT-PCR; the results are shown in Fig. 3. In mice treated with ADR alone, the expression of TNF α mRNA did not show any appreciable change. Pretreatment with DEX, however, enhanced the expression at 4 and 24 h

after ADR administration (Fig. 3A). ADR alone increased the expression of IL-1 β mRNA at 4 h after ADR administration, but the value returned to normal at 24 h. Pretreatment with DEX reduced IL-1 mRNA expression at all three time points (Fig. 3B). In the absence of DEX, VEGF expression

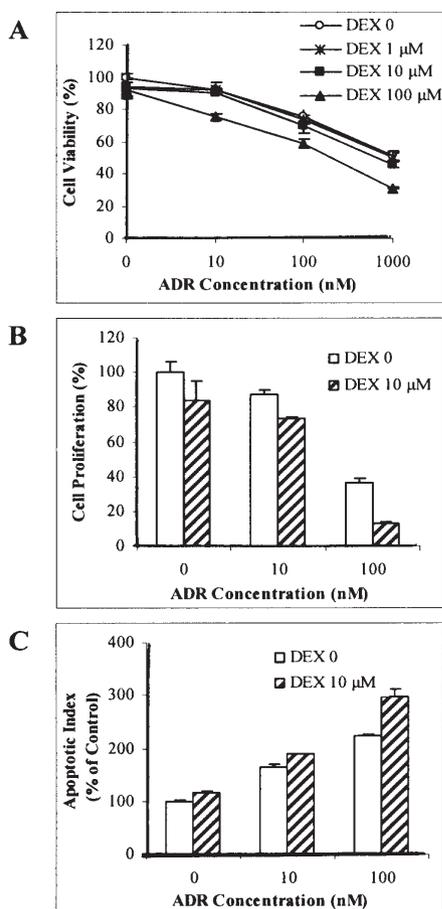


Figure 4. Effects of DEX pretreatment on ADR-induced cell viability, proliferation, and apoptosis for the 4T1 cell line. (A) Cell viability: 4T1 cells were seeded on 96-well microtiter plates (500-1,000 cells/well) and pretreated with gradient concentrations of DEX for 24 h. Cells were then exposed to ADR at gradient concentrations for 24 h. Cell viability percentages were determined by the MTT assay for cell viability. Data were obtained from triplicates of each experiment. (B) Cell proliferation rate: following a similar treatment protocol, the cell proliferation rate was determined by BrdU. Data were obtained from duplicates of each experiment. (C) Apoptosis: following a similar treatment protocol, cells in early and late stages of apoptosis were detected with an annexin V-FITC apoptosis detection kit.

was enhanced at 24 h after ADR administration. With DEX pretreatment, the expression was enhanced at 4 h, but the level returned to normal at 24 h after ADR treatment, reversing the ADR-induced effects on VEGF expression (Fig. 3C).

Pretreatment with DEX enhances ADR-induced cell death and apoptosis, and inhibits proliferation in cultured cells. In a concentration-dependent manner, ADR alone decreased cell viability; the IC_{50} value was approximately 1 μ M. Pretreatment with DEX contributed to the effects of ADR. At a DEX concentration of 100 μ M, the IC_{50} for ADR decreased to about 250 nM (Fig. 4A). Prior exposure to DEX at 10 μ M increased the inhibitory effect of ADR on cell proliferation (Fig. 4B), and increased ADR-induced cell apoptosis (Fig. 4C).

DEX inhibits activation of nuclear NF κ B, likely by increasing the production of I κ B. Analysis of Western blots showed that DEX decreased nuclear NF κ B in a concentration-dependent manner, whereas cytoplasmic NF κ B accumulated. DEX also increased the expression of the NF κ B inhibitory protein, I κ B, which is thought to be involved in the biological effects of DEX (Fig. 5A). ADR alone (500 nM) induced nuclear NF κ B expression, which was suppressed by prior exposure to DEX (Fig. 5B). Similar results were observed with the NF κ B-luciferase promoter assay (Fig. 5C). DEX alone inhibited NF κ B activation, and prior exposure to DEX (1-100 μ M) suppressed the ADR (500 nM)-induced NF κ B activation.

Discussion

There have been intensive investigations into the concept that inflammation is an essential component in cancer development and progression (4,14). The potential for using anti-inflammatory agents as chemosensitizers and chemoprotectants is promising (5,15). The anti-inflammatory agent, DEX, has been used in clinical cancer therapy, especially for treatment of leukemia and lymphoma during and after chemotherapy (8-11). Our previous data indicate that pre-treatment with DEX results in two distinct anticancer

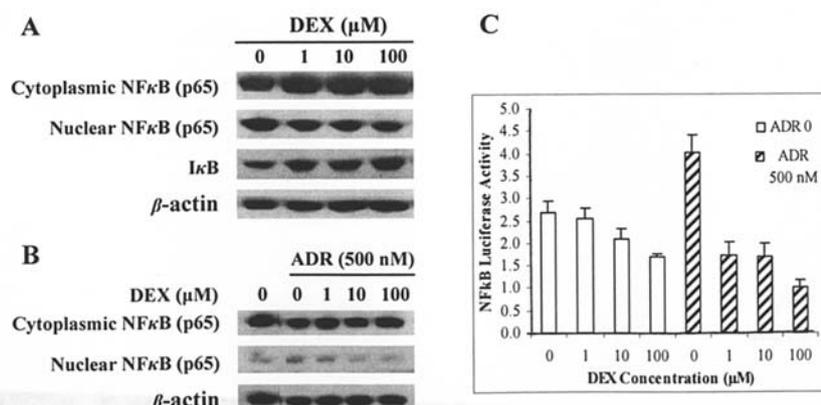


Figure 5. Protein levels of various genes and NF κ B-luciferase promoter activity in 4T1 cells treated with DEX alone or in combination with ADR. (A) 4T1 cells were exposed to DEX for 24 h, and cytoplasmic and nuclear proteins were fractionated by nuclear and cytoplasmic extraction reagents. Proteins were analyzed by Western blotting. (B) 4T1 cells were pretreated with DEX for 24 h, followed by the incubation with ADR for 24 h and cytoplasmic and nuclear proteins were fractionated. (C) 4T1 cells were transfected with an NF κ B-luciferase vector for 8 h, then exposed to DEX for 24 h, followed by incubation with ADR or saline (as a control) for an additional 24 h. Cell lysates were collected and tested for NF κ B-luciferase activity using the Dual-Luciferase Reporter Assay System.

effects: reduction of myelosuppression and enhancement of the antitumor activity of chemotherapeutic agents (12,13). These effects are likely to be associated with the fact that DEX modulates the tissue distribution of various drugs by decreasing host tissue uptake (12) and increasing tumor uptake (13) of chemotherapeutic agents such as carboplatin and gemcitabine. DEX also increases the cytotoxicity of these agents *in vivo* and *in vitro* (12,13). Since the efficacy data in our previous study were generated in immunocompromised animals (13), it was difficult to examine the involvement of cytokine expression in the DEX-mediated chemosensitization effects. In the present study, we have shown that pretreatment with DEX enhances the antitumor activity of another chemo-therapeutic agent (ADR) in a syngeneic mouse model of breast cancer, demonstrating that the antitumor activity is caused by the modulation of ADR distribution and cytokine expression.

Pro-inflammatory cytokines and chemokines, produced by tumor cells and/or tumor-associated leukocytes and platelets, may contribute directly to tumor development via various mechanisms, including by acting as growth and survival factors, bypassing the p53 tumor suppressor gene, and facilitating angiogenesis and tumor invasion (4,5,15). Our current data demonstrate that DEX pretreatment modulates the ADR-associated pro-inflammatory cytokine profiles as measured by mRNA expression. When evaluated 24 h after ADR administration, TNF α expression is enhanced and IL-1 β expression is reduced. Expression of VEGF, a marker for angiogenesis, is elevated in most primary and metastatic tumors (20,21). The present results demonstrate that pretreatment of mice with DEX leads to a reduction in the level of VEGF expression at 24 h after ADR administration (compared to animals treated with ADR alone), indicating that DEX has an anti-angiogenic effect in animals treated with ADR. These results suggest that modulation of cytokine expression might be a key mechanism by which DEX exerts its effects.

Expression of the aforementioned cytokines is believed to be associated with the NF κ B signaling pathway (15,22-25). NF κ B is a transcription factor that regulates inflammatory responses by inducing numerous genes, including those coding for cytokines and chemokines (26). Constitutive activation of NF κ B, observed in solid tumors (15), appears to support cancer cell survival and to reduce the sensitivity of cells to chemotherapeutic drugs (27). NF κ B is also a tumor promoter in inflammation-associated carcinoma, and activation of NF κ B constitutes a link between inflammation and cancer (28).

Glucocorticoids, which are potent inhibitors of NF κ B, exert their action mainly through glucocorticoid receptor-dependent induction of the NF κ B inhibitory protein, I κ B (29). Our current data demonstrate that, in 4T1 cells, DEX up-regulates I κ B expression, leading to cytoplasmic NF κ B accumulation and reduction of nuclear NF κ B activation. Further, expression of IL-1 β and VEGF, two downstream cytokines in this pathway, are indirectly inhibited by DEX. Although the I κ B-NF κ B interaction has been known for several years, this is the first indication that DEX enhances antitumor activity by inhibiting nuclear NF κ B activation.

Our data also show that DEX pretreatment decreases TNF α production in tumors prior to ADR administration. At

4 h after ADR administration, however, DEX increases TNF α production. TNF α is suggested to play a dual role in cancer: endogenous TNF α produced in the tumor micro-environment enhances tumor development and metastasis, but administration of a high dose of TNF α can induce apoptosis and necrosis of tumor cells (30). Our results suggest that, by inducing TNF α production upon ADR treatment, DEX pretreatment may make tumor cells more sensitive to ADR-induced apoptosis.

The results from the present study should have an impact on both the use of chemotherapeutic agents, including ADR, and also on the application of DEX as a chemosensitizer and chemoprotectant. In this study, we confirmed the effects of DEX on the pharmacokinetics of chemotherapeutic agents (12,13) using a different class of anticancer agent (ADR). The data generated from the present study suggest that another mechanism may be responsible for the enhancement of the antitumor activity and tumor uptake of chemotherapeutic agents by DEX. Our results showed that DEX increased ADR distribution to tumor tissue. There is also evidence indicating that DEX may exert its effects by reducing the interstitial fluid pressure (IFP) in tumors (31).

Tumor blood vessel walls typically have high permeability to fluids and plasma proteins. In addition, functional lymphatic vessels are absent in tumor tissue (32). When fluid filters from tumor blood vessels into the interstitial space, it can be reabsorbed only slowly into the microvascular network and cannot be taken up by lymphatic vessels. Further, uncontrolled cell proliferation causes tumor cells to become densely packed in the confined environment. All these abnormalities cause fluid accumulation in the interstitial space, directly leading to the elevated IFP throughout the tumor (33,34). The penetration of anti-tumor agents through the interstitial space is a passive process, occurring predominantly by diffusion; interstitial hypertension is believed to be a major physiological barrier to antitumor agent transport. DEX may decrease the IFP by lowering the high vascular permeability of tumor blood vessels (31). From our previous and current work, we postulate that DEX may decrease the elevated volume and pressure in the interstitial space in tumors, allowing a greater concentration of chemotherapeutic agent to accumulate in tumors, thereby improving the antitumor activity of the chemotherapeutic agents. In addition, high IFP in tumor tissues is associated with overexpression of pro-inflammatory cytokines in tumor cells; the effects of DEX on cytokine expression seen in the present study are likely to contribute to increased ADR uptake in tumors by lowering the tumor IFP. Further studies to determine the relationship among tumor IFP, cytokine expression, NF κ B activation, and drug pharmacokinetics are needed in order to verify this hypothesis.

The ADR-mediated increase in pro-inflammatory cytokine expression may also be associated with its side effects in host tissue and drug resistance in tumor cells. Therefore, DEX pretreatment not only enhances the antitumor activity of ADR by affecting several important inflammatory cytokines in the NF κ B pathway, but also that it leads to the protection of normal tissues, especially bone marrow, against the toxicity frequently observed during breast cancer treatment. Although additional mechanistic studies, such as determination of

the effects of DEX on other inflammatory cytokines and cytokine-producing cells, are needed in order to provide a more complete understanding of the effects of DEX on cancer chemotherapy, the present study provides additional evidence for the clinical use of DEX as a chemosensitizer and chemoprotectant for a variety of cancer chemotherapeutic agents.

In summary, although DEX has been in use for many years, these data indicate that it may be of much greater clinical interest for cancer than was previously thought. We demonstrated in an earlier study that DEX could improve the pharmacokinetics of two chemotherapeutic agents and indicated that it might protect patients from hematotoxicity (12,13). In this study, we have demonstrated: a) that these effects are applicable to another class of chemotherapeutic agents; b) that the increased anti-cancer effects occur through inhibition of inflammatory and angiogenic pathways; and c) that the alteration of the tumor microenvironment may play a major role in the chemosensitizing effects of the drug. Therefore, to provide patients with the greatest benefit, new evaluations of the administration of DEX for cancer therapy should be undertaken.

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