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Dexamethasone as a chemoprotectant in cancer chemotherapy: hematoprotective effects and altered pharmacokinetics and tissue distribution of carboplatin and gemcitabine

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Abstract Purpose: Hematoprotective strategies may offer new approaches to prevent chemotherapy-induced hematotoxicity. The present study was undertaken to investigate the chemoprotective effects of dexamethasone and its optimal dose and the underlying mechanisms. **Methods:** Lethal toxicity and hematotoxicity of carboplatin were compared in CD-1 mice with or without dexamethasone pretreatment. Plasma and tissue pharmacokinetics of carboplatin were determined in CD-1 mice. Carboplatin was quantified by HPLC. Gemcitabine was analyzed by radioactivity counting. **Results:** Pretreatment with dexamethasone prevented lethal toxicity of carboplatin in a dose- and schedule-dependent manner. The best protective effects of dexamethasone pretreatment as measured by survival were observed at the dose level of 0.1 mg/mouse per day for 5 days (80% vs 10% in controls). In contrast, post-treatment with dexamethasone had no protective effects. Pretreatment with dexamethasone significantly prevented the decrease in granulocyte counts. To elucidate the mechanisms by which dexamethasone pretreatment reduces hematotoxicity, we examined the effects of dexamethasone pretreatment on the pharmacokinetics of carboplatin and gemcitabine in CD-1 mice. No

significant differences in plasma pharmacokinetics of carboplatin or gemcitabine were observed between control and mice pretreated with dexamethasone. However, dexamethasone pretreatment significantly decreased carboplatin and gemcitabine uptake in spleen and bone marrow with significant decreases in AUC, $T_{1/2}$, and C_{max} , and an increase in CL. **Conclusions:** To our knowledge, this is the first time that dexamethasone has been shown to significantly decrease host tissue uptake of chemotherapeutic agents, suggesting a mechanism responsible for the chemoprotective effects of dexamethasone. This study provides a basis for future study to evaluate dexamethasone as a chemoprotectant in cancer patients.

Keywords Dexamethasone · Chemoprotection · Carboplatin · Gemcitabine

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Introduction

There is considerable interest in the development of clinical strategies to prevent or reverse the side effects of cancer chemotherapy [1, 2]. Most clinically used cancer chemotherapeutics are DNA-interactive agents with various mechanisms of action. Amongst many side effects of these chemotherapeutic agents is bone marrow suppression. These agents cause DNA damage to the lymphohematopoietic precursors, decreasing blood cellular elements [2], which is seen as blood cytopenia (decrease in circulating platelets, and white and red blood cells) in the clinic [2, 3]. In clinical practice, hematopoietic growth factors such as granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are frequently used after chemotherapy with the aim of reducing hematotoxicity [4, 5]. These cytokines have been used in the treatment of primary bone marrow failure states and after myelosuppressive chemotherapy or radiotherapy. In most studies with G-CSF and GM-CSF, acceleration of granulocyte

recovery after chemotherapy and radiotherapy has been observed, resulting in a reduction in infectious risk, a shortening of drug- and radiation-induced myelosuppression, and a higher chemotherapy dose intensity; however, an improved remission rate and improved long-term survival rates have not yet been definitively documented [4, 5]. In addition, post-therapy administration of hematopoietic growth factors is expensive and fails to prevent genomic damage and hematopoietic progenitor depletion.

Alternatively, administration of hematopoietic growth factors prior to chemotherapy or radiotherapy may offer preventive benefits to cancer patients, although the pretreatment hematoprotective strategy has not been extensively evaluated. Several approaches have been tested in animal models, including the use of corticosteroids [6, 7, 8, 9, 10], cytokines [11, 12, 13, 14, 15, 16], scavengers of chemotherapeutic agents or their metabolites [17], and vectors to introduce chemotherapy resistance into stem cells [18]. It is believed that corticosteroids have the ability to suppress the production of growth factors and cytokines and are thus implicated in the negative regulation of hematopoiesis. In a study with mouse models, Kriegler et al. [7] demonstrated that the corticosteroids, prednisolone and dexamethasone (DEX), effectively protect progenitor cells against the chemotherapeutic agent 5-fluorouracil, with better protective effects being observed with DEX than with prednisolone. In murine models, administration of corticosteroids prior to chemotherapy reduces carboplatin-induced hematotoxicity [8, 9, 10]. These findings indicate that the current clinical schedules of corticosteroids during cancer therapy need to be reexamined to obtain the maximum benefits for cancer patients with chemotherapy or radiotherapy. Thus far, at least three agents, DEX [19], GM-CSF [20, 21, 22, 23], and amifostine [24], have been shown to have hematoprotective effects in cancer patients receiving chemotherapy.

Carboplatin, representing a new generation of platinum anticancer compounds, shares some of the therapeutic advantages of cisplatin, but without a significant incidence of the dose-limiting neurotoxicity and nephrotoxicity which is experienced with cisplatin [25]. However, its use is associated with dose-limiting bone marrow suppression. In preclinical models, pretreatment with corticosteroids markedly reduced carboplatin-induced hematotoxicity [8, 9, 10]. In a phase I clinical trial, the hematoprotective effect of DEX was demonstrated in patients with metastatic cancer who were treated with carboplatin and ifosfamide [19]. Gemcitabine has been established as a new standard for the treatment of pancreatic cancer [26]. It has been shown to improve clinical benefits including response, time to progression, and survival, compared with other chemotherapeutic agents such as 5-fluorouracil. In clinical trials, the combination of cisplatin and gemcitabine significantly improved tumor response and time to progression as compared with gemcitabine alone [26]. The present study was designed to test the hypothesis that DEX pretreatment would reduce

hematotoxicity and increase therapeutic efficiency of carboplatin- gemcitabine chemotherapy. Moreover, we hypothesized that DEX modulation of carboplatin pharmacokinetics may play a role in altering carboplatin-associated host hematotoxicity. Therefore, we examined the effects of DEX on the plasma and tissue pharmacokinetics of carboplatin in experimental animals.

Materials and methods

Chemicals, reagents, and animals

All chemicals and solvents were high-performance liquid chromatography (HPLC) grade or of the highest analytical grade available. Methanol, acetonitrile, and acetic acid were purchased from Fisher Chemicals (Atlanta, Ga.). DEX (analytical grade), carboplatin (analytical grade), and triethylamine were purchased from Sigma (St. Louis, Mo.). Perchloric acid was purchased from J.T. Baker (Phillipsburg, N.J.). Centrifree micropartition system (cat. no. 4104) was purchased from Millipore Corporation (Bedford, Mass.). Carboplatin (clinical grade) was purchased from Bristol-Myers Squibb Company (Princeton, N.J.) and gemcitabine (clinical grade) was purchased from Eli Lilly and Company (Indianapolis, Ind.). DEX (clinical grade) was purchased from American Regent Laboratories (Shirley, N.J.). [³H]-Gemcitabine was obtained from Moravék Biochemicals (Brea, Calif.). Tissue solubilizer (TS-2) was purchased from Research Products (Mt. Prospect, Ill.). The animal use and care protocol was approved by the Institutional Animal Use and Care Committee of the University of Alabama at Birmingham. Male CD-1 mice (4–6 weeks old) were obtained from Charles River Laboratories (Cambridge, Mass.). All animals were fed with commercial diet and water ad libitum for 1 week prior to the study.

Animal survival study

Male CD-1 mice (25–27 g) randomly divided into multiple treatment and control groups (ten mice per group) were given DEX by subcutaneous (s.c.) injection at doses of 0.01, 0.05, 0.1 and 0.3 mg/mouse per day or saline (as controls) for 5 days prior to (day –4 to 0) or after (day 0 to 4) a single intraperitoneal (i.p.) injection of carboplatin (600 mg/m² or 200 mg/kg) on day 0. Animals were monitored daily for activity, physical condition, body weight and 14-day survival rates.

Peripheral blood cell counts

Using a protocol similar to that used in the above survival study, the effects of DEX on chemotherapy-induced bone marrow toxicity were studied in male CD-1 mice. DEX (s.c., 0.1 mg/mouse per day for 5 days) was given prior to (day –4 to 0) or after (day 0 to 4) a single i.p. dose of carboplatin (360 mg/m² or 600 mg/m²). On various days peripheral blood samples (60 µl) were obtained from the postorbital venous plexus using a microcapillary tube coated with 5% EDTA, and cell counts were obtained using a Coulter counter. Wright's stained peripheral blood smears were examined by light microscopy and the percentage of lymphocytes, granulocytes and monocytes were recorded.

Pharmacokinetics and tissue distribution of carboplatin

Pharmacokinetic studies were carried out using a protocol similar to that previously described [27] using metabolism cages. Male CD-1 mice were pretreated with DEX (s.c., 0.1 mg/day per mouse for 5 days) or saline (as controls), and, at 1 h after the fifth dose of DEX, were given a single intravenous (i.v.) bolus administration of carboplatin (60 mg/kg) via a tail vein. At various times (5, 15 and

30 min, 1, 2, 4, 8 and 24 h after drug dosing; three animals for each time point) blood samples were collected into heparinized tubes and tissue samples removed. Plasma was separated by centrifugation at 20,000 *g* for 5 min. Tissues including liver, kidneys and spleen were immediately blotted on Whatman no. 1 filter paper, trimmed of extraneous fat or connective tissue, weighed, and homogenized in physiological saline (5 ml per g of wet tissue weight). The resultant homogenates were stored at -70°C until further analysis. Bone marrow cells were harvested by flushing the femurs with sterile physiological saline as reported previously [28]. The resultant bone marrow cell suspension was weighed and lysed by sonicating five times for periods of 10 s. Following centrifugation at 20,000 *g* for 30 min, the supernatant was removed and stored at -70°C until further analysis.

HPLC analysis of carboplatin in plasma and tissues

Carboplatin in biological samples was analyzed by a procedure involving microfiltration and reversed-phase HPLC [29]. Plasma or tissue homogenate (200 μl) or bone marrow suspension was added to the reservoir of a Centrifree micropartition system. The latter was capped and centrifuged at 2000 *g* for 5 min. All filtrates were transferred into a new microcentrifuge tube and 6 μl of the filtrates was injected onto the HPLC column. The HPLC system consisted of a Hewlett Packard 1050 ChemStation with a UV detector (Agilent 1050 series). Determination of carboplatin was achieved using a LiChrosorb diol (10 μm , 250 \times 4.6 mm) analytical column with a LiChroCART 100 RP-18 guard column. The mobile phase for plasma was composed of 98:2 acetonitrile/ H_2O (vol/vol) and 89:11 acetonitrile/0.015% H_3PO_4 (vol/vol) for urine and tissue samples. The flow rates were 2 ml/min (for plasma) and 1.1 ml/min (for urine and tissue samples). The column eluate was monitored by UV at 229 nm. Quantitation of plasma or tissue carboplatin was carried out using an external standard curve (0–4000.0 $\mu\text{g}/\text{ml}$) that was freshly prepared daily. Linear regression and correlation analysis were carried out to establish the standard peak-area/concentration curves for carboplatin. The intra- and interday variations (CV) were less than 5% for plasma and tissue samples. The lower limit of quantitation was 1.0 and 2.0 $\mu\text{g}/\text{ml}$ for plasma and tissue samples, respectively. The recovery rates from plasma and tissues extracts were $97 \pm 3\%$ and $83 \pm 10\%$, respectively.

Pharmacokinetics and tissue distribution of gemcitabine

Pharmacokinetic studies of gemcitabine were carried out using a protocol similar to that used for carboplatin pharmacokinetics as described above. Male CD-1 mice (three animals for each time

point) were pretreated with DEX as described above, and then given a single i.v. bolus administration of [^3H]-gemcitabine (160 mg/kg) via a tail vein. At various times (5, 15 and 30 min, 1, 2, 4, 8 and 24 h after drug dosing) plasma, bone marrow, and tissues including liver, kidneys, heart, lungs, spleen, brain and tumor were collected and treated as described above.

Quantitation of gemcitabine by radioactivity measurements

The total gemcitabine-derived radioactivity in tissues and body fluids were determined by liquid scintillation spectrometry (LS 6000T A; Beckman, Irvine, Calif.), using a method described previously [27, 30]. In brief, plasma samples (50 μl) were mixed with 5 ml scintillation solvent (Beckman) to determine total radioactivity. Tissue homogenates (50–200 μl) were mixed with 200 μl solubilizer (TS-2) overnight, neutralized with 400 μl 0.3% acetic acid, and then mixed with scintillation solvent (5 ml) to quantitate the total radioactivity.

Data and statistical analysis

The peripheral blood counts were expressed as mean and standard deviations and the significance of differences were analyzed by ANOVA; survival rates in the toxicity study were analyzed by χ^2 analysis. The following pharmacokinetic parameters of carboplatin were estimated using WinNonlin programs (version 2.1; Pharsight, Mountain View, Calif.): the area under the drug concentration-time curve (AUC), the maximal concentration (C_{max}), the elimination half-life ($T_{1/2}$), clearance (CL), and the volume of distribution at steady-state (V_{ss}). The significance of the differences among treated groups and controls were analyzed by ANOVA.

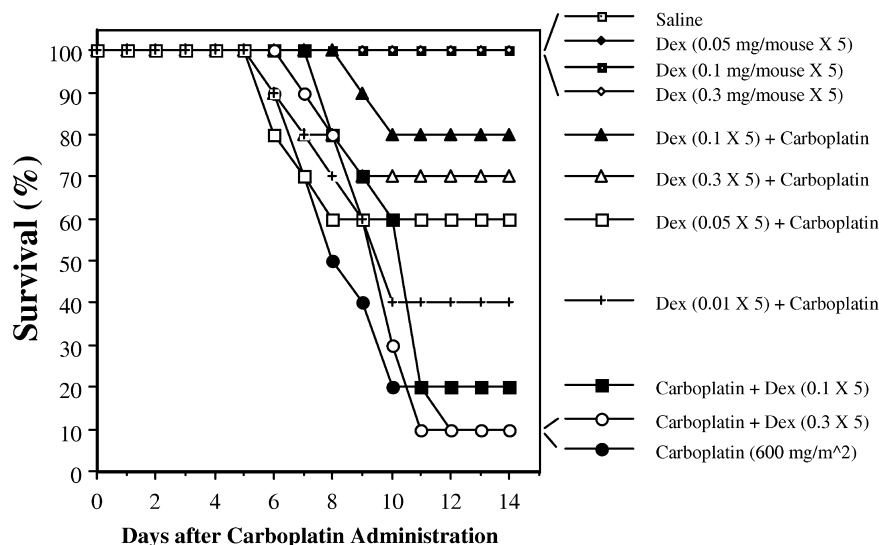
Results

Pretreatment with DEX alters mortality and hematotoxicity in CD-1 mice treated with carboplatin

The effect of pretreatment with DEX on reduction in lethal carboplatin hematotoxicity is schedule- and dose-dependent

DEX pretreatment significantly reduced mortality of carboplatin in CD-1 mice (Fig. 1, ten animals per

Fig. 1 DEX decreases host toxicity of carboplatin chemotherapy in CD-1 mice. Animals were randomly divided into multiple treatment and control groups (ten mice per group). DEX (s.c., 0.1 mg/mouse per day for 5 days) or saline (in controls) was given prior to or after a single i.p. injection of carboplatin (600 mg/ m^2 or 200 mg/kg)



group). No death occurred in groups treated with DEX (s.c., 0.01–0.3 mg/mouse per day for 5 days) alone or saline. The single i.p. dose of carboplatin alone (600 mg/m² or 200 mg/kg) resulted in a 90% mortality rate, which is similar to the previously reported lethal toxicity of carboplatin [8, 9]. Although the exact causes of death were not determined, based on the time of death (between 6 and 10 days after chemotherapy) and clinical observations, hematotoxicity may have been the major cause of death. The major toxicities included weakness, decreased activity, decreases in food and water uptake, body weight loss, and death. Pretreatment with DEX prevented lethal toxicity of carboplatin in a dose-dependent manner. At a lower dose (0.01 mg/mouse per day for 5 days), DEX pretreatment slightly increased the survival rate (40% vs 10% in carboplatin controls). The best protective effect as measured by survival were observed at the dose of 0.1 mg/mouse per day (80% vs 10% in carboplatin controls). In contrast, posttreatment with DEX did not have a protective effect at either of the dose levels tested (0.1 and 0.3 mg/mouse per day for 5 days), further confirming the schedule-dependent protective effects of DEX. DEX alone at various dose levels had no host toxicity as measured by general clinical observation and body weights. In addition, no significant changes in body weights were observed in DEX-pretreated mice compared with the untreated controls.

In a separate study, the protective effects of DEX were also demonstrated at various doses of carboplatin (Fig. 2A). In the combination treatment with carboplatin and gemcitabine, pretreatment with DEX also reduced the mortality of treated animals (Fig. 2B).

Pretreatment with DEX reduces chemotherapy-induced cytopenias

To examine possible mechanisms responsible for reduced mortality in mice pretreated with DEX, male CD-1 mice (5 weeks old) were divided into multiple treatment groups in addition to a control group (ten mice per group). DEX (s.c., 0.1 mg/mouse per day for 5 days) was given prior to a single i.p. dose of carboplatin (600 mg/m² or 200 mg/kg) alone or in combination with gemcitabine (510 mg/m² or 170 mg/kg). On day 1 (24 h after chemotherapy) and day 8, peripheral blood samples (60 µl) were obtained from the postorbital venous plexus using a microcapillary tube coated with 5% EDTA, and cell counts were obtained. As illustrated in Fig. 3, treatment of DEX induced granulocytosis and lymphopenia. Pretreatment with DEX significantly prevented the decrease in granulocyte counts and reduced recovery time after carboplatin-gemcitabine therapy (Fig. 3A). DEX-treated mice showed lymphopenia on day 1 (6 days after DEX treatment) but recovered within a week (on day 8). In addition, there were smaller decreases in platelet counts in animals treated with DEX compared

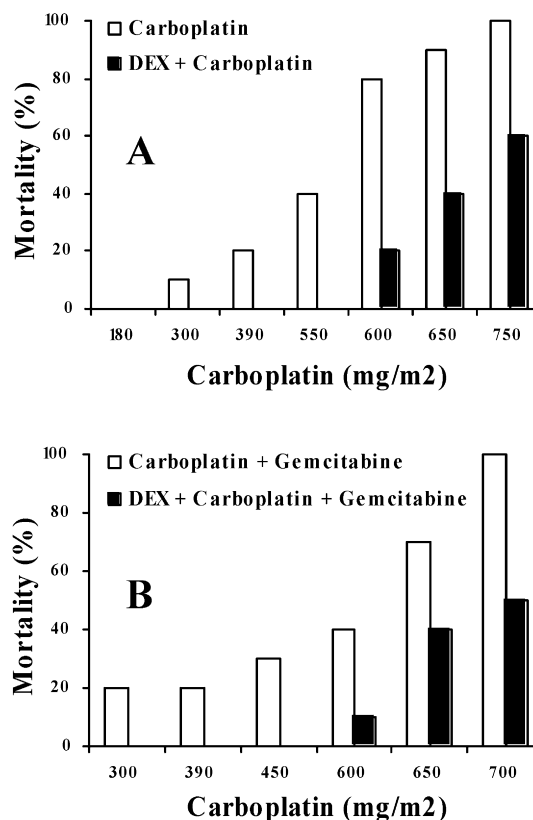


Fig. 2A, B DEX decreases host toxicity of carboplatin alone or in combination with gemcitabine in CD-1 mice. Animals were randomly divided into multiple treatment and control groups (ten mice per group). DEX (s.c., 0.1 mg/mouse per day for 5 days) or saline (in controls) was given prior to a single i.p. injection of carboplatin alone at various doses (180–750 mg/m²) (A) or in combination with gemcitabine (500 mg/m²) (B)

with those without DEX treatment following carboplatin-gemcitabine treatment (Fig. 3C).

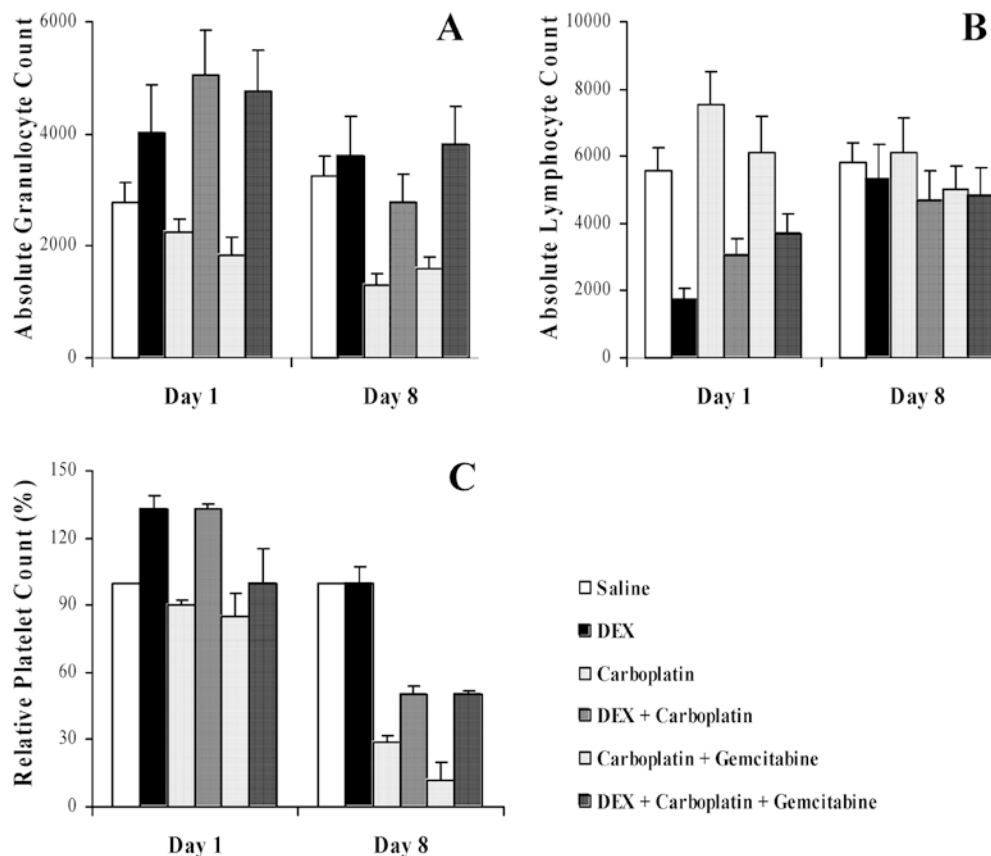
In a separate experiment, we used the same experimental design to examine the dose-schedule effects of DEX on prevention of neutropenia. DEX prevention of neutropenia was dose- and schedule-dependent, the optimal dose was 0.1 mg/kg and postcarboplatin treatment had no effect. As illustrated in Fig. 4, there were nadirs of granulocyte counts following different treatments with DEX following carboplatin (single dose, 360 mg/m²) on day 7.

Pretreatment with DEX modulates pharmacokinetics of carboplatin and gemcitabine in CD-1 mice

Carboplatin pharmacokinetics

The carboplatin pharmacokinetic study was performed in CD-1 mice with or without DEX pretreatment. The time-concentration curves are shown in Fig. 5. No significant differences in plasma pharmacokinetics of carboplatin were observed between control and mice

Fig. 3A–C Pretreatment with DEX prevents hematopoietic toxicity of carboplatin chemotherapy in CD-1 mice. Animals were randomly divided into multiple treatment and control groups (ten mice per group). Doses of DEX (s.c., 0.1 mg/mouse per day for 5 days) were given prior to a single i.p. dose of carboplatin (600 mg/m²) alone or in combination with gemcitabine (500 mg/m²). The data presented are granulocyte (A), lymphocyte (B), and platelet (C) counts, following chemotherapy



pretreated with DEX (Fig. 5A). However, DEX markedly decreased carboplatin concentrations in spleen ($P < 0.01$; Fig. 5C). There were significant decreases in AUC and C_{max} and increases in CL in mice pretreated with DEX ($P < 0.01$; Fig. 5E; Table 1). As shown in Fig. 5E, the AUC of carboplatin in spleen from animals pretreated with DEX was approximately 16.5% that of control mice ($P < 0.01$). We also found a 57.4% decrease in carboplatin AUC in bone marrow of animals pretreated with DEX compared with that of control mice (Fig. 5D, E; Table 1). In addition, pretreatment with DEX slightly decreased carboplatin uptake in liver by 40% (Fig. 5B, E; Table 1).

Gemcitabine pharmacokinetics

The gemcitabine pharmacokinetic study was also carried out in CD-1 mice using a protocol similar to that previously described. The time-concentration curves are shown in Fig. 6. Slight but significant differences in plasma pharmacokinetics of gemcitabine were observed between control and mice pretreated with DEX (Fig. 6A). Pharmacokinetic analysis indicated that plasma AUC was decreased with DEX pretreatment (Fig. 6E; Table 2). No significant differences in liver drug concentrations were found between control and mice pretreated with DEX (Fig. 6B). However, DEX

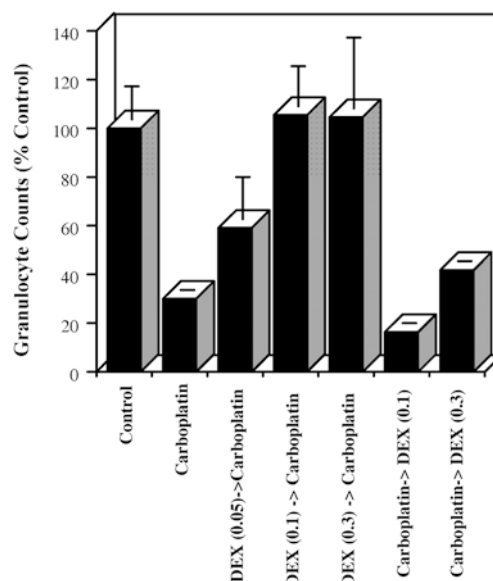


Fig. 4 Pretreatment but not posttreatment with DEX prevents hematopoietic toxicity of carboplatin chemotherapy in CD-1 mice. Animals were randomly divided into multiple treatment and control groups (ten mice per group). Various doses of DEX (s.c., 0.05, 0.1 and 0.3 mg/mouse per day for 5 days) were given prior to a single i.p. dose of carboplatin (360 mg/m²) or postchemotherapy. The data presented are nadirs of granulocyte counts on day 7, expressed as percentage of untreated control

Fig. 5A–E Pharmacokinetics of carboplatin in CD-1 mice. Animals were pretreated with DEX (s.c., 0.1 mg/mouse per day for 5 days) or saline (as controls) and given a single i.v. dose of carboplatin (180 mg/m²). Plasma and tissue samples were collected at various times up to 24 h. Carboplatin was analyzed by HPLC. **A** Plasma, **B** liver, **C** spleen, **D** bone marrow, **E** comparison of AUCs of carboplatin in plasma and various tissues (B.M. bone marrow)

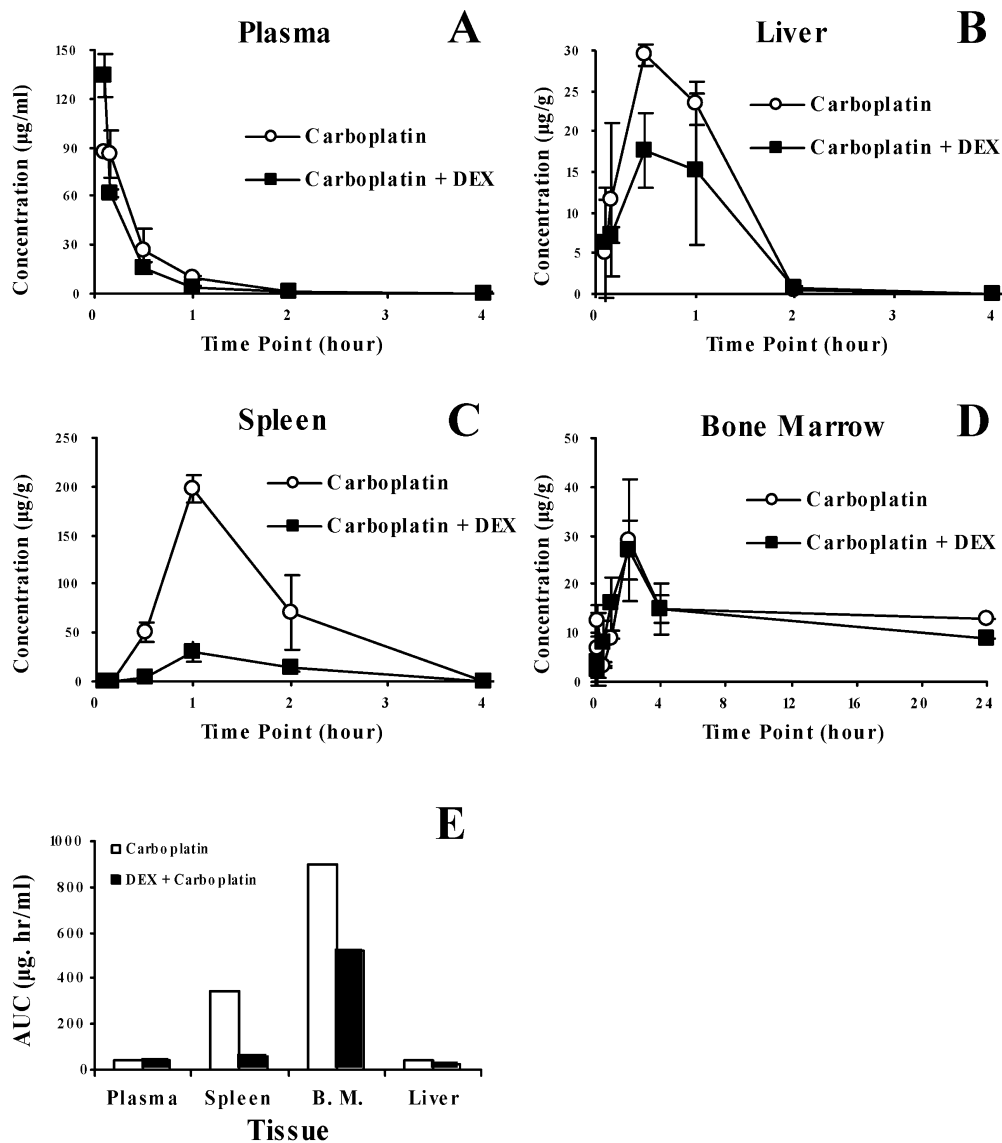


Table 1 Pharmacokinetic parameters of carboplatin in CD-1 mice following pretreatment with DEX or saline. A one-compartmental model was fitted to the data for plasma and tumor time-concentration curves and a first-order absorption, one-compartmental model was fitted for the data for spleen, bone marrow and liver time-concentration curves

Parameter	Plasma		Spleen		Bone marrow		Liver	
	Saline	DEX	Saline	DEX	Saline	DEX	Saline	DEX
AUC (µg·h/ml)	44.18	42.69	344.11	56.72	901.60	517.16	38.71	25.02
T _{1/2} (h)	0.09	0.13	0.75	0.82	28.47	15.38	0.39	0.39
C _{max} (µg/ml)	352.62	227.58	118.73	17.79	20.14	20.61	25.44	16.55
CL (ml/g/h)	1.36	1.41	0.17	1.06	0.07	0.12	1.55	2.40
V _{ss} (ml/g)	0.17	0.26	0.19	1.25	2.75	2.57	0.87	1.35

Table 2 Pharmacokinetic parameters for gemcitabine in CD-1 mice following pretreatment with DEX or saline

Parameter	Plasma		Spleen		Bone marrow		Liver	
	Saline	DEX	Saline	DEX	Saline	DEX	Saline	DEX
AUC (µg·h/ml)	552.32	319.96	375.21	135.50	18.35	7.03	123.68	124.80
T _{1/2} (h)	1.44	0.87	1.32	0.49	0.79	0.31	0.54	0.52
C _{max} (µg/ml)	264.13	253.68	196.84	193.29	16.12	15.82	158.43	166.94
CL (ml/g/h)	0.29	0.50	0.43	1.18	8.72	22.76	1.29	1.28
V _{ss} (ml/g)	16.64	10.81	0.81	0.83	9.93	10.12	1.01	0.96

markedly decreased gemcitabine concentrations in spleen and bone marrow ($P < 0.01$; Fig. 6C, D). There was a significant decrease in AUC and an increase in the CL in mice pretreated with DEX ($P < 0.01$; Fig. 6E; Table 2). The AUCs of gemcitabine in spleen and bone marrow from animals pretreated with DEX were approximately 36% and 38% those of control mice, respectively ($P < 0.01$; Fig. 6E; Table 2).

Discussion

Major problems of cancer chemotherapy are host toxicity and drug resistance. The general consensus at present is that hematological support with high-dose cytotoxic therapy does not allow clinically meaningful improvements in patient survival. This issue is directly associated with the purpose of the present study. The current practice in the clinic is to rescue key organs or tissues from toxicity, which is frequently ineffective and expensive. The rationale for developing chemoprotective approaches is to alter the microenvironment of critical tissues/organs that are susceptible to unwanted toxicity from chemotherapeutic agents. This approach may hold significant potential to prevent chemotherapy-induced toxicity.

For proof of principle, we employed murine models in the present study to determine the biological effects on carboplatin/gemcitabine and pharmacokinetic mechanisms of pretreatment with DEX. The results from the present study demonstrated at least four points. First, in a dose-dependent manner, pretreatment, but not post-treatment, with DEX significantly reduced the mortality from carboplatin therapy in CD-1 mice. Second, in a dose-dependent manner, DEX pretreatment, but not post-treatment, significantly decreased carboplatin-induced hematotoxicity in CD-1 mice, which may be responsible for the reduction of carboplatin-associated mortality. Third, pretreatment with DEX significantly decreased the carboplatin concentrations in spleen and bone marrow. Fourth, pretreatment with DEX also significantly decreased the gemcitabine concentrations in spleen and bone marrow as seen with carboplatin, although the two compounds have relatively different patterns of tissue distribution. These results, along with our previous data [8, 9, 10, 19], provide a basis for further investigating the effect of DEX as a chemoprotectant of cancer chemotherapeutics in human clinical trials.

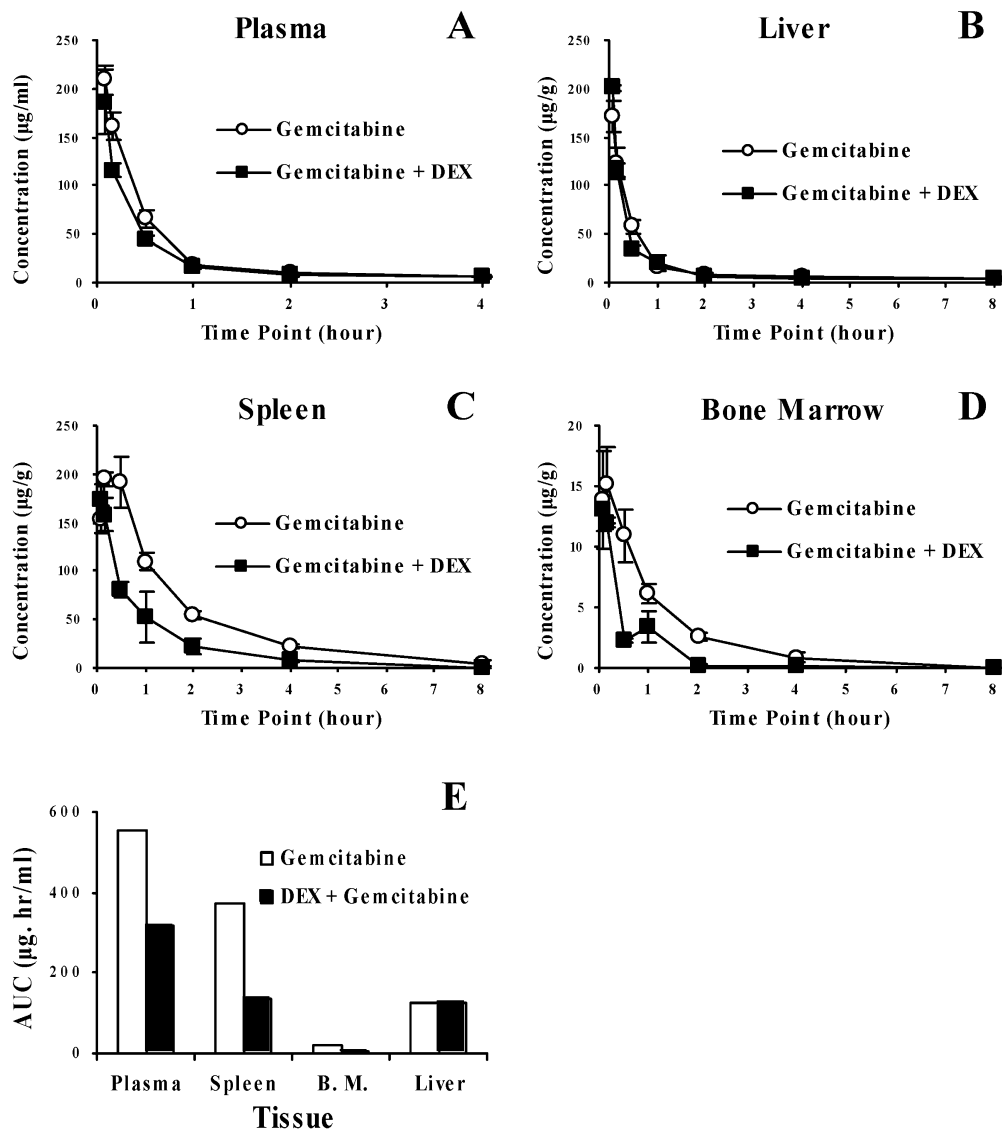
To illustrate the protective effects of pretreatment with DEX on chemotherapy-induced toxicity, we used CD-1 mice in the present survival study. Our results demonstrated that pretreatment with DEX significantly reduced the mortality from carboplatin administered alone or in combination with gemcitabine. These results are consistent with our previous findings with cortisone acetate [8, 9, 10]. In our previous studies, using a clinically relevant murine tumor model, we demonstrated that pretreatment with corticosteroids reduces carboplatin-induced mortality from 80–90% to 10–20% [10].

These studies also demonstrated that corticosteroid administered after chemotherapy does not improve survival rates [8, 9]. In the present study, we further demonstrated that pretreatment with DEX had a hematopoietic protection effect on carboplatin-based chemotherapy in mice; this effect was dose- and schedule-dependent. Several previous studies from various groups have demonstrated that pretreatment with corticosteroid protects experimental animals from chemotherapy-induced hematopoietic toxicity [6, 7, 8, 9, 10]. For example, Joyce and Chervenick [6] demonstrated that pretreatment of mice with a single dose of corticosteroid reduces bone marrow depletion of granulocyte-macrophage colony-forming units (CFU-GM) and protects mice from intravenous bacterial challenge following a sublethal dose of chemotherapy with cyclophosphamide. They also examined postchemotherapy bone marrow CFU-GM for sensitivity to high specific activity ^3H -thymidine and concluded that a lower fraction of residual CFU-GM is in S-phase after treatment with corticosteroids and cyclophosphamide, compared to treatment with cyclophosphamide alone [6]. Our previous studies confirmed this observation and further demonstrated that CFU-GM taken from mice treated with corticosteroids are resistant to cisplatin in vitro [8, 9]. In a separate study, Kriegler et al. found that pretreatment of DEX protects against toxicities of 5-fluorouracil and methotrexate [7].

Both our previous observations and those of others [6, 7, 8, 9, 10] have suggested that corticosteroid reduction of hematopoietic toxicity is in part due to induction of stem cell resistance to DNA interactive agents at the cellular level. However, our present studies suggest that pharmacokinetic factors are involved in the process. The decreases in spleen and bone marrow uptake of carboplatin and gemcitabine in mice pretreated with DEX may partly explain the mechanisms responsible for reduced hematotoxicity of carboplatin/gemcitabine. The reasons for decreased tissue uptake are not clear but may be associated with drug redistribution and decrease in spleen tissue mass and cell density following DEX treatment. Matsukado et al. demonstrated that DEX decreases brain uptake of carboplatin [31]. Whether the same mechanism applies to decreased bone marrow uptake remains to be seen. In addition, it should be pointed out that we extrapolated the AUCs to infinity based on the last measured time point. In this case, some differences in AUCs in plasma and bone marrow (Figs. 5 and 6) between DEX-treated and untreated groups may not be statistically significant if a different extrapolation model were used, e.g., comparing $\text{AUC}_{0-24\text{ h}}$ rather than $\text{AUC}_{0-\infty}$.

A concern is that the effect of DEX on drug levels could also occur in tumors and hence reduce the anti-tumor effect of carboplatin and/or gemcitabine. Recent data generated in our laboratory with xenograft models demonstrate that DEX could in fact enhance the therapeutic effects of carboplatin and gemcitabine [32]. We have also demonstrated that pretreatment of DEX

Fig. 6A–E Pharmacokinetics of gemcitabine in CD-1 mice. Animals were pretreated with DEX (0.1 mg/mouse per day for 5 days) or saline (as controls) and given a single i.v. dose [^3H]-gemcitabine (500 mg/m 2). **A** Plasma, **B** liver, **C** spleen, **D** bone marrow, **E** comparison of AUC of gemcitabine in plasma and various tissues (*B.M.* bone marrow)



significantly alters carboplatin and gemcitabine pharmacokinetics in nude mice bearing human cancer xenografts, which may be associated with its effects on antitumor activity of carboplatin and gemcitabine [32]. Since there is no appreciable overlap of side effects between carboplatin and gemcitabine, the combination of these two drugs may offer clinical benefits to cancer patients. In the present study, we demonstrated that pretreatment with DEX may be a promising approach to preventing carboplatin-associated bone marrow toxicity and to reducing tissue levels of both drugs in spleen and bone marrow. Therefore, the present study provides a potential new avenue for therapeutic use of DEX as a chemoprotectant in the treatment of human cancers.

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