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ORIGINAL ARTICLE

**Michael Pearlman · David Jendiroba
Lance Pagliaro · Afsaneh Keyhani · Baoshun Liu
Emil J. Freireich · Elizabeth Travis**

Dexrazoxane's protection of jejunal crypt cells in the jejunum of C3Hf/Kam mice from doxorubicin-induced toxicity

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Abstract Dexrazoxane (DEX) is used clinically to reduce doxorubicin-induced cardiotoxicity. Because DEX inhibits anthracycline-induced toxicity, we set out to investigate DEX's ability to reduce the incidence and severity of gastrointestinal toxicity associated with anthracycline administration in C3Hf/Kam mice. Doxorubicin and idarubicin, two commonly used anthracyclines, were each examined in combination with DEX. A jejunal crypt survival assay demonstrated that DEX increased crypt survival from 40% (doxorubicin 22.5 mg/kg) to 63% at a DEX/doxorubucin dose ratio of 10:1 ($P < 0.05$). When doxorubicin was increased to a dose of 27.5 mg/kg, crypt survival increased from 18% to 40% at a DEX:Dox ratio of 5:1 ($P < 0.05$). At ratios of 10:1 and 20:1, DEX had no protective effect on idarubicin-induced crypt cell toxicity. Our findings support the use of DEX to prevent or ameliorate mucositis in patients receiving anthracycline-based therapy and the use of DEX with high-dose doxorubicin to treat refractory disease.

Keywords Dexrazoxane · Mucositis · Gastrointestinal toxicity

M. Pearlman · D. Jendiroba · A. Keyhani · B. Liu
E. J. Freireich (✉)
Departments of Leukemia and Special Medical Educations Program-Unit 55, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA
E-mail: efeirei@mdanderson.org
Tel.: +1-713-7922660
Fax: +1-713-7941812

L. Pagliaro
Department of Genitourinary Oncology-Unit 427, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA

E. Travis
Department of Radiation Oncology-Unit 66, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA

Introduction

Gastrointestinal toxicity is induced by many types of chemotherapy, especially anthracyclines, and by radiation, and it complicates bone marrow and peripheral blood stem cell transplantation. Rapidly proliferating cells are most affected, and mucositis is often the most debilitating manifestation of gastrointestinal toxicity. Doxorubicin and idarubicin are anthracyclines commonly associated with mucositis. Patients who take either drug may suffer such severe discomfort that oral intake is insufficient to maintain adequate nutritional status, thus contributing to the morbidity and mortality of the disease the drug is intended to cure. Because mucositis treatment is only palliative and often inadequate, new preventive therapeutic strategies are under development.

One drug with potential as a mucositis preventative is dexrazoxane (DEX). In one clinical study, Basser et al. observed that stomatitis and vomiting are less common when DEX is also administered [2]. Elimination half-life, maximum plasma concentration, and volume of distribution were not significantly affected by DEX. Hence pharmacokinetic differences could not explain the observations reported. In a preclinical investigation, Wang et al. found that razoxane (RZ), DEX's racemate, protects animals receiving ordinarily lethal doses of daunorubicin and that this protection is probably due to RZ's ability to protect the mucosa of the small bowel [15]. Inhibition of anthracycline-induced nephrotoxicity by RZ has also been reported [4].

Swain et al. reported that DEX used in combination with a FAC (5-fluorouracil, doxorubicin, cyclophosphamide) significantly reduces gastrointestinal toxicity due to FAC. Toxicity was diminished at all intestinal levels: nausea, vomiting, dysphagia, esophagitis, and stomatitis were all reduced [12]. Other clinical studies have suggested that DEX can prevent cytotoxicity caused by anthracyclines in general [5, 13, 14]. Non-cardiac toxicities for doxorubicin or epirubicin given

with or without DEX have been examined previously [8, 9, 10, 11]. However, no systematic study has specifically demonstrated the efficacy of DEX in preventing or ameliorating gastrointestinal cytotoxicity induced by doxorubicin in particular. We investigated the ability of DEX to protect C3Hf/Kam mice from gastrointestinal cytotoxicity induced by intraperitoneal injection of doxorubicin or idarubicin.

Materials and methods

Mice

Male C3Hf/Kam mice, 11–13 weeks of age, were bred and maintained in a specific pathogen-free mouse facility approved by the American Association for Accreditation of Laboratory Animal Care and in accordance with current regulations and standards of the United States Department of Agriculture and Department of Health and Human Services, National Institutes of Health. Mice were housed two to five per cage under a 12-h light/dark cycle at about 23°C and fed autoclaved feed and sterile acidified water ad libitum.

Dexrazoxane

DEX was generously supplied by Pharmacia-Upjohn (Kalamazoo, Mich.) in a lyophilized form. When needed, it was solubilized with 0.1% sodium lactate at a concentration of 25 mg/ml.

Doxorubicin and idarubicin

Doxorubicin and idarubicin were purchased from Pharmacia-Upjohn. Each drug was injected into the peritoneal cavity of C3Hf/Kam mice with a 26.5-gauge needle.

Jejunal microcolony assay

The jejunal microcolony assay of Withers and Elkind [16] was used to quantify crypt survival, a surrogate for protection against mucositis. Briefly, groups of five mice were given a single injection of DEX 4 h before or after an anthracycline. A pretreatment time of 4 h was selected based on a terminal elimination half-life ranging from 1.8 to 3.3 h in a clinical cancer study [7]. The molar ratios of DEX to doxorubicin used were 20:1, 10:1, 5:1, 2:1, and 1:1, and the molar ratios of DEX to idarubicin were 20:1 and 10:1. Doxorubicin was injected at doses of 22.5 or 27.5 mg/kg, and idarubicin at doses of 10 or 12 mg/kg. One group of five mice was given doxorubicin, another group was given idarubicin, another group was given DEX, and a fourth group was left untreated (sham injections) and served as a control. All mice were killed 96 h after treatment with the anthracycline, and a 2.5-cm segment of jejunum was removed and fixed according to standard histological techniques. The number of surviving crypts was scored on 5-μm Histolyn & Eosin-stained transverse histological sections of jejunum from each mouse, using the criterion of at least ten surviving cells as indicative of a surviving crypt. Crypts in three to five circumferences per mouse were counted and averaged.

Statistical analysis

ANOVA and Student's *t*-test were used to assess differences in jejunal crypt survival. Differences were considered significant for P values < 0.05.

Results

Doxorubicin

Doxorubicin caused significant damage to the jejunal crypts. After treatment with 22.5 mg/kg of doxorubicin, 40% of the crypts survived (Fig. 1), while after treatment with 27.5 mg/kg, only 18% of the crypts survived (Fig. 2; $P < 0.05$).

Idarubicin

Idarubicin was very toxic in our model as demonstrated by only 5% of the crypts surviving after treatment with both the 10 mg/kg and the 12 mg/kg doses (Fig. 3).

Dexrazoxane

DEX alone at concentrations from 137.5 to 687.5 mg/kg had no effect on intestinal crypt viability (data not shown).

Dexrazoxane plus doxorubicin

When DEX was administered at ratios of 5:1 and 10:1, 4 h prior to 22.5 mg/kg of doxorubicin, the crypt survival rates were 54% and 63%, respectively (ANOVA, $P < 0.05$; Fig. 1A). When the dose of doxorubicin was increased to 27.5 mg/kg (Fig. 2), crypt survival rates were significantly lower than those obtained using doxorubicin plus DEX at 22.5 mg/kg (1:1, 2:1, 5:1, and 10:1:24%, 29%, 40%, and 30%, respectively; ANOVA, $P < 0.05$).

In contrast, when DEX doses were increased to a ratio of 20:1, at a doxorubicin dose of 22.5 mg/kg, there was potentiation of doxorubicin cytotoxicity against jejunal crypts (Fig. 1A). Crypt survival decreased from 40% for doxorubicin alone to 13% for DEX plus doxorubicin ($P = 0.003$). At a doxorubicin dose of 27.5 mg/kg and at a ratio of 20:1 (Fig. 2), DEX again potentiated doxorubicin's cytotoxic activity against crypt cells ($P = 0.17$).

DEX was also administered 4 h after the doxorubicin doses of 22.5 and 27.5 mg/kg at a ratio of 10:1. Protection was still observed for the 22.5 mg/kg dose but to a decreased extent when compared to the results of DEX administered 4 h before doxorubicin (63% vs 50%; before vs after doxorubicin, respectively). There was no observed protection for the doxorubicin dose of 27.5 mg/kg (30% vs 16%; before vs after doxorubicin, respectively).

Dexrazoxane plus idarubicin

In contrast to DEX's ability to protect against doxorubicin's cytotoxicity, little or no interference was

observed when DEX was administered 4 h before idarubicin. The addition of DEX at ratios of 10:1 and 20:1 resulted in little or no protection against the toxic effects of either concentration of idarubicin on crypt survival (Fig. 3), and there was no statistically significant difference in crypt survival (4.2% and 4.6%; $P = 0.56$ and $P = 0.13$, respectively). DEX to idarubicin ratios less than 10:1 were not included because there was no difference in toxicity from the higher ratios.

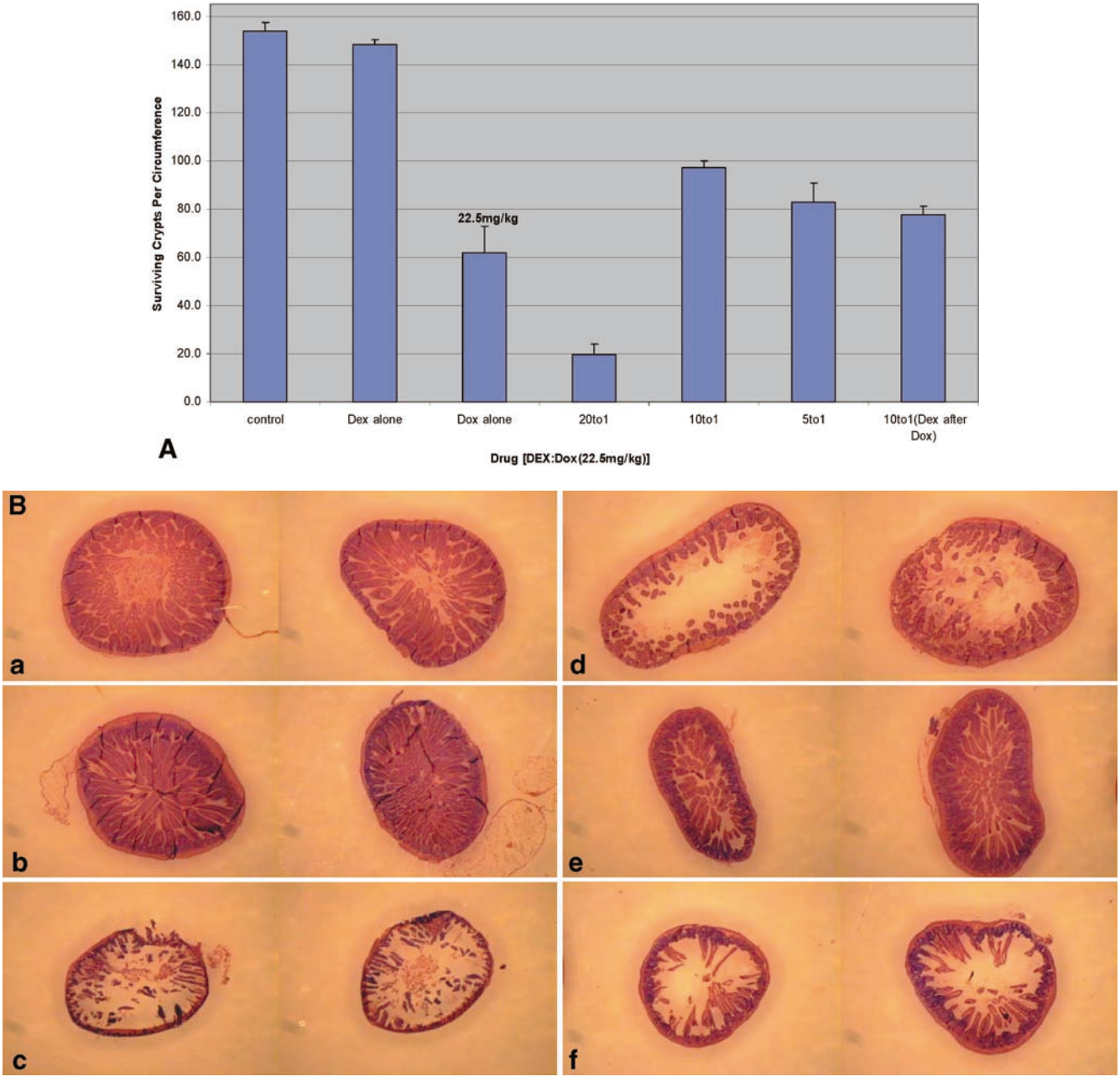

Fig. 1 **A** Using the assay of Withers and Elkind, DEX was administered 4 h before doxorubicin (22.5 mg/kg) at the ratios indicated. DEX at 550 mg/kg was used as a control dose. *(DEX after Dox)* means that DEX was administered 4 h after doxorubicin. **B** Microscopic cross-sections of the jejunum of C3H/Hfk mice exposed to DEX, doxorubicin or control (each horizontal pair is two cross-sections from the same mouse): *a)* control group—nothing administered, *b)* DEX administered at 687.5 mg/kg, *c)* doxorubicin administered at 22.5 mg/kg, *d)* DEX combined with doxorubicin at a ratio of 5:1 (22.5 mg/kg doxorubicin), *e)* DEX combined with doxorubicin at a ratio of 10:1, *f)* DEX combined with doxorubicin at ratio of 20:1

Discussion

Our results, along with previous clinical and in vitro evidence, suggest that DEX would be effective in preventing or ameliorating mucositis in patients receiving anthracycline-based therapy and its use in combination with high-dose doxorubicin to treat refractory disease.

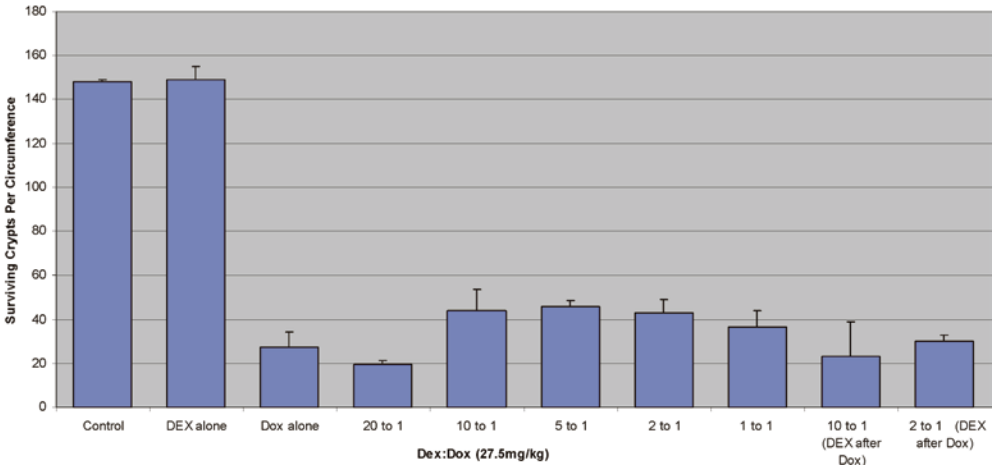

Fig. 2 Using the assay of Withers and Elkind, DEX was administered 4 h before doxorubicin (27.5 mg/kg) at the ratios indicated. *(DEX after Dox)* means that DEX was administered 4 h after doxorubicin

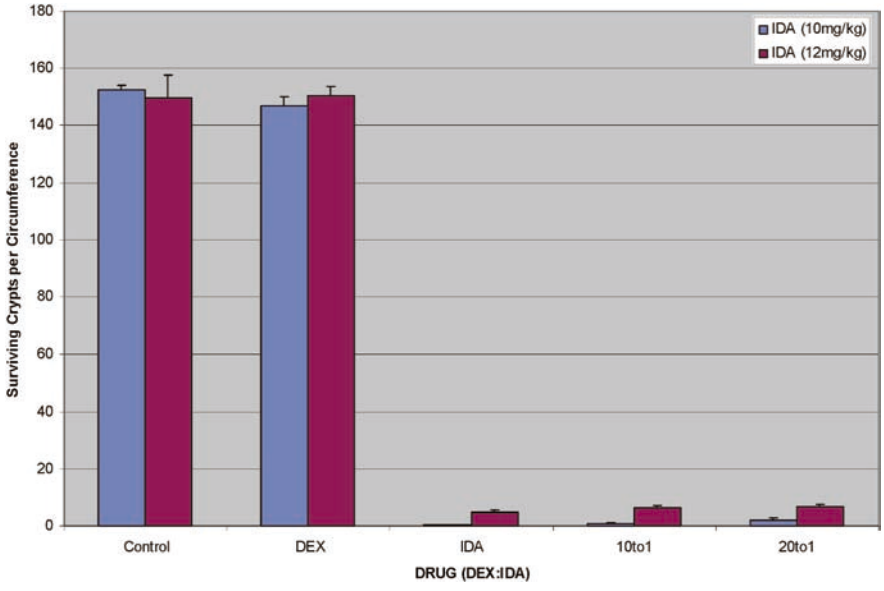

Fig. 3 Using the assay of Withers and Elkind, DEX was administered 4 h before idarubicin at the ratios depicted. Two administered doses of idarubicin are shown, 10 and 12 mg/kg

We systematically evaluated DEX's ability to protect against anthracycline-induced gastrointestinal toxicity. DEX decreased doxorubicin-induced toxicity up to a ratio of 10:1 and then began to plateau. At a higher ratio, DEX appeared to synergize with doxorubicin, resulting in increased toxicity to the jejunum (Figs. 1A and 2).

In contrast, DEX had no protective effect on idarubicin-induced jejunal crypt cell toxicity at ratios of 10:1 and 20:1. These results agree with our findings from myeloid cell experiments [6]. In those experiments, DEX did not antagonize the cytotoxicity of idarubicin in any of the experimental schedules, consistent with our findings on jejunal cytotoxicity. This evidence indicates that doxorubicin and idarubicin are cytotoxic via different mechanisms of action. This may be due to the difference in the structure of idarubicin and doxorubicin. Doxorubicin has four rings linked by a glycosidic bond to an amino sugar, daunosamine, and has an alpha-ketol group on its side chain. Idarubicin has the same structure except that it lacks the alpha-ketol group. Hasinoff has demonstrated that anthracyclines with an alpha-ketol group are oxidized by a Fe^{3+}-ADR-925 (ADR-925 is a DEX hydrolysis intermediate) complex that inhibits the production of oxygen radicals, and thereby decreases their antitumor efficacy [3]. Since, idarubicin does not possess an alpha-ketol group, its mechanism of cytotoxicity is probably not dependent on oxygen radicals.

We have also found that, in a myeloid cell culture system, DEX in combination with doxorubicin produces additive toxicity [6]. It is difficult to compare different model systems, but these differing results suggest that there are multiple mechanisms by which combinations of DEX and doxorubicin produce opposite effects. These mechanisms remain unclear.

We demonstrated that DEX protects the jejunum, as well as the heart, from doxorubicin-induced toxicity, but we have also demonstrated that DEX, in combination with anthracyclines and other agents, potentiates their antitumor activity against myeloid leukemic cells. The mechanism of protection could be the result of heavy-metal chelation or of the inhibition of DNA topoisomerase II [1]. The mechanism by which DEX accomplishes

inhibition or enhanced cytotoxicity will be addressed in future studies.

We demonstrated that DEX significantly protected the jejunum of C3Hf/Kam mice from doxorubicin-induced gastrointestinal toxicity but had no inhibitory effect on jejunal cytotoxicity exerted by idarubicin. These findings suggest that DEX could reduce the incidence and severity of gastrointestinal toxicity associated with doxorubicin or daunorubicin administration. Further clinical study of DEX in novel drug combinations whose efficacy is limited by mucositis and gastrointestinal toxicity is warranted. Based on other findings, it may also have additional therapeutic benefits such as in the treatment of iron overload, bleomycin-induced pulmonary toxicity, AIDS-related Kaposi's sarcoma, and the enhancement of platinum anticancer activity [14].

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