

# Treatment of Anthracycline Extravasation with Dexrazoxane<sup>1</sup>

Seppo W. Langer,<sup>2</sup> Maxwell Sehested, and Peter Buhl Jensen

The Laboratory of Experimental Medical Oncology, The Finsen and Laboratory Centers, National University Hospital, DK-2100 Copenhagen, Denmark

## ABSTRACT

Accidental extravasation of anthracyclines is a feared complication. Present treatment consists of local cooling and extensive surgical debridement, which often results in severe morbidity. All clinically important anthracyclines are topoisomerase II poisons that are antagonized by topoisomerase II catalytic inhibitors such as dexrazoxane. Therefore, we investigated whether dexrazoxane protects against extravasation lesions caused by anthracyclines. B6D2F1 mice received s.c. daunorubicin, doxorubicin, or idarubicin followed by systemic treatment with dexrazoxane or saline. One single systemic dose of dexrazoxane immediately after s.c. administration of doxorubicin, daunorubicin, or idarubicin reduced the tissue lesions (expressed as area under the curve of wound size times duration) by 96% ( $P < 0.0001$ ), 70% ( $P < 0.0001$ ), and 87% ( $P = 0.0004$ ), respectively. Moreover, the treatment resulted in a statistically significant reduction in the fraction of mice with wounds as well as the duration of wounds. The induction of wounds was dose-dependent, as was the degree of protection by dexrazoxane. Dexrazoxane could be administered up to 3 h after the anthracycline without loss of protection. Triple-dosage of dexrazoxane tended to be more effective than a single injection. Dexrazoxane had no effect on lesions induced by hydrogen peroxide. This is the first report of use of a topoisomerase II catalytic inhibitor such as dexrazoxane in the treatment of anthracycline extravasation injuries. These convincing preclinical data represent a novel nontoxic approach that can easily be implemented into the clinical handling of accidental extravasation of anthracyclines.

## INTRODUCTION

The anthracyclines, *e.g.*, daunorubicin, idarubicin, epirubicin, and doxorubicin, are widely used cytotoxic drugs in the

treatment of hematological malignancies and solid tumors. Although new drugs are promising, the anthracycline-based treatments become still more important, *e.g.*, in breast cancer. These agents are especially prone to cause severe tissue damage on extravasation (1). Accidental extravasation has been estimated to occur in 0.5–6% of all patients receiving chemotherapy (1–3). The local toxicity is characterized by immediate pain, erythema, and swelling at the extravasation site (4, 5). The ulceration may not appear for several days or even weeks and may continue to worsen for months, probably because of drug diffusion into adjacent tissue. Indeed, it has been demonstrated that doxorubicin can persist in the tissue for at least a month (6). Whereas small ulcerations may heal, large ulcerations require surgical excision.

During the last two decades, a large number of nonsurgical treatment modalities have been investigated experimentally. However, only a few have gained access to clinical evaluation. Local injection or topical administration of corticosteroids has produced contradictory results and may even worsen the lesion. In fact, inflammation does not seem to be a part of the pathophysiology (7–9). The effect of local sodium bicarbonate (10–12) has been investigated in animal experiments with varying results as have local sodium thiosulfate (13, 14), hyaluronidase (8, 15), and  $\beta$ -adrenergic agonists and antagonists (16). The effect of intensive and prolonged local application of DMSO has been addressed in two clinical trials (17, 18), both of which had no study control. Dorr and Alberts (19) found that topical DMSO had no effect on the incidence or size of ulcers in mice given doxorubicin intradermally. IL<sup>3</sup> treatment with bi(3,5-dimethyl-5-hydroxymethyl-2-oxomorpholin-3-yl) of intradermal doxorubicin extravasation in a swine has shown some benefit (20, 21). However, no published studies have confirmed this since 1988. Experiments with antidotes against idarubicin-induced lesions have not been published.

At present, the treatment of choice is an early surgical approach with extensive debridement of the involved area followed by skin grafting (1, 22, 23). The extension of surgery may be guided by fluorescence microscopy (24). Whereas none of the above mentioned treatments appear to be widely used, local cooling with ice lasting from 1 h to 3 days or longer is a frequently used initial treatment (1).

The topo II poisons, *e.g.*, doxorubicin, daunorubicin, idarubicin, epirubicin, etoposide, and teniposide, act on the so-called cleavable complex in the catalytic cycle of the essential nuclear enzyme topo II, thereby prolonging the transient stage, where the enzyme has locked the gate DNA molecule with a strand break. The drug action becomes lethal because of the accumulation of DNA strand breaks (see Ref. 25 for recent review). In contrast, catalytic inhibitors, such as DEX and aclarubicin, inhibit other steps of the catalytic cycle of the

Received 3/2/00; revised 6/5/00; accepted 6/5/00.

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<sup>1</sup> Supported by grants from the Danish Cancer Society and the H:S Research Council. Patent pending, PA 1999 00355, "Treatment of extravasation of anthracyclines with the topoisomerase II catalytic inhibitor ICRF-187."

<sup>2</sup> To whom requests for reprints should be addressed, at the Laboratory of Experimental Medical Oncology, The Finsen Center 5074, National University Hospital, 9 Blegdamsvej, DK-2100 Copenhagen, Denmark. Phone: 45-35454949; Fax: 45-35456966; E-mail: swlanger@dadlnet.dk.

<sup>3</sup> The abbreviations used are: IL, intralésional; topo II, DNA topoisomerase II; AUC, area under the curve; DEX, dexrazoxane.

enzyme (26). Thus, the catalytic inhibitors can block the action of the topo II poisons (27–29). Moreover, DEX is also a potent metal ion chelator that protects against the free radical toxicity induced by anthracycline-iron complexes. It is licensed in any part of the world as a cardioprotectant in doxorubicin-based chemotherapy.

The present study is the first to pursue the interaction between the topo II poisons and the catalytic inhibitors as a means of treatment of accidental extravasation. We here demonstrate a highly significant inhibition of daunorubicin-, doxorubicin-, and idarubicin-induced tissue lesions in mice by systemic DEX administration.

## MATERIALS AND METHODS

**Materials.** The following commercial available drugs were used: DEX hydrochloride (ICRF-187; Cardioxane, Chiron, Amsterdam, the Netherlands); daunorubicin hydrochloride (Cerubidin, Rhône-Poulenc Rorer, Holte, Denmark); doxorubicin hydrochloride (Adriamycin, Pharmacia & Upjohn, Copenhagen, Denmark); idarubicin hydrochloride (Zavedos, Pharmacia & Upjohn, Copenhagen, Denmark); hydrogen peroxide (Perhydrol, Merck, Darmstadt, Germany); fentanyl-fluanisone (Hypnorm, Janssen-Cilag, Birkerød, Denmark); and midazolam (Dormicum, Roche, Hvidovre, Denmark).

Female B6D2F1 hybrid mice (M&B A/S, Ry, Denmark) were kept in a controlled environment with *ad libitum* access to water and food. All mice went through a pre-experiment acclimatization period of at least 1 week. The weight range was 19–21 g at the start of the experiments. Each mouse was ear-marked for individual identification. Animal handling and experiments were carried out in accordance to directives for animal handling and welfare depicted in Refs. 30 and 31.

**Anesthesia.** A standard solution containing one part fentanyl-fluanisone, one part midazolam, and two parts isotonic saline in an i.p. dose of 0.1 ml/10 g (32) was used in all experiments.

**Injection Technique.** Hair was removed with an electrical shaving device. s.c. injection was carried out using a Hamilton syringe (Bonaduz AG, Bonaduz, Switzerland) with a 0.05-ml fixed volume deposit and a 27-gauge, 3/4-inch needle. The injection site was ~1 cm above the root of the tail after retraction of the loose dorsal skin. All mice received one s.c. injection. DEX was injected i.p. or i.v. after dilution in isotonic saline in ~0.2 ml with a 27-gauge, 3/4-inch needle.

**Observation.** The two longest perpendicular wound diameters were measured daily with a ruler. We defined a wound as a tissue lesion of at least 2 mm<sup>2</sup> with disruption of the epidermis. Healing was defined as healing of epidermis and complete regrowth of hair in a wound area. The mice were euthanized after healing of all wounds.

**Data Handling and Statistical Methods.** The areas of the wounds were calculated as the product of the two longest perpendicular diameters in mm. The wound area times duration, *i.e.*, the AUC, was calculated for individual mice as well as the mean AUC for whole groups of mice. AUCs, time to occurrence of wounds in mice with wounds, and duration of wounds in wounded mice were compared using the Mann-Whitney test. Fisher's exact *t* test was used to compare the fraction of mice

with wounds. The Student-Newman-Keul's multiple comparisons test was used to compare multiple AUCs.

**Experiments.** The details of the various experiments are depicted in Table 1. We conducted 28 experiments with daunorubicin, 8 with idarubicin, 28 with doxorubicin, and 7 with hydrogen peroxide.

## RESULTS

We observed no wound infections or DEX-related deaths. Fifteen (2.4%) mice died in relation to the anesthetic procedure. The tissue lesions did not alter the behavior of the mice. They were spontaneously mobile and fed normally throughout the observation periods. All wounds healed completely during the observation period. Regrown hair was white in all anthracycline-induced lesions, which is in contrast to the normal black hair of this mouse strain. The regrown hair after hydrogen peroxide was grayish white. Neither s.c. DEX nor saline induced vitiligo (data not shown).

Doxorubicin-induced lesions were similar in sizes and duration, whether they were induced by s.c. injection of 2 or 3 mg/kg. Thus, these two dose levels are pooled (Table 1). The interexperimental variation in the mean AUCs in the seven experiments with daunorubicin, 3 mg/kg s.c., plus saline i.p. was very small ( $P > 0.05$ ). Neither were the mean AUCs different in the seven experiments where daunorubicin, 3 mg/kg s.c., were accompanied by DEX, 250 mg/kg i.p., at  $t = 0$  (Fig. 1). The same notable reproducibility was present in experiments with doxorubicin, 2 or 3 mg/kg s.c., plus saline i.p. or plus DEX, 250 mg/kg i.p. Furthermore, because the DEX treatment resulted in statistically significant reductions in the mean AUC in all of the above-mentioned experiments (individual Mann-Whitney tests all  $P < 0.01$ ), the individual data were pooled into two "basic" schedules with  $n = 61$  and 58 (doxorubicin), and  $n = 56$  and 55 mice (daunorubicin), respectively, as depicted in Table 1.

A single i.p. injection of DEX, 250 mg/kg, administered immediately after a s.c. deposit of 3 mg/kg daunorubicin reduced the mean AUC by 70% ( $P < 0.0001$ ; Figs. 2 and 3). The fraction of mice with wounds was reduced from 96% to 78% ( $P = 0.0041$ ). In mice with detectable wounds, the mean time to wounds was delayed by 76% from 5.5 to 9.7 days ( $P < 0.0001$ ). Furthermore, DEX reduced the mean duration of wounds by 35% from 26.6 to 17.4 days ( $P < 0.0001$ ).

In the case of doxorubicin (Figs. 2 and 4), the mean AUC after 2 or 3 mg/kg s.c. was reduced by 96% ( $P < 0.0001$ ) with DEX, 250 mg/kg i.p., at  $t = 0$ . The fraction of mice with wounds was reduced from 77 to 14% ( $P < 0.0001$ ); the duration of wounds shortened with 28% ( $P = 0.0035$ ). There was no delay in the time to appearance of wounds.

At a dose of 0.75 mg/kg idarubicin s.c., treatment with a single i.p. injection of 250 mg/kg DEX reduced the AUC by 87% ( $P = 0.0004$ ) and the frequency of mice with wounds from 83 to 28% ( $P = 0.0020$ ; Fig. 2). The mean time to appearance of wounds was delayed by 52% from 6.7 to 10.2 days ( $P = 0.0193$ ), and the mean duration of wounds was decreased by 26% from 21.2 to 15.6 days ( $P = 0.0054$ ).

In both daunorubicin- and doxorubicin-induced wounds, there was no statistically significant difference between the

Table 1 Results of the treatment of SC daunorubicin-, idarubicin-, doxorubicin-, and hydrogen peroxide-induced wounds in mice<sup>a</sup>

Treatment		<i>n</i> <sup>b</sup>	AUC	FW	TTW	DW
Daunorubicin (mg/kg s.c.)						
3	Saline i.p.	56 <sup>c</sup>	1260 (±72)	96	5.5 (±0.2)	26.6 (±0.3)
3	DEX, 250 mg/kg i.p.	55 <sup>c</sup>	373 (±48)	78	9.7 (±0.5)	17.4 (±0.6)
3	DEX, 62.5 mg/kg i.p.	9	662 (±223)	89	8.1 (±0.5)	19.4 (±1.3)
3	DEX, 125 mg/kg i.p.	16 <sup>c</sup>	692 (±115)	88	8.4 (±0.6)	20.6 (±0.8)
3	DEX, 375 mg/kg i.p.	4	57 (±57)	25	4.0 (±0.0)	23.0 (±0.0)
3	DEX, 62.5 mg/kg i.p. at <i>t</i> = 0, <i>t</i> = +3h, and <i>t</i> = +6h	9	0	0		
3	DEX, 125 mg/kg i.p. at <i>t</i> = 0, <i>t</i> = +3h, and <i>t</i> = +6h	9	112 (±37)	22	16.5 (±0.5)	18.0 (±0.0)
3	DEX, 250 mg/kg i.p. day 0, 1, and 2	8	128 (±34)	75	17.7 (±1.0)	13.3 (±1.2)
3	DEX, 250 mg/kg i.p. at <i>t</i> = +3h	7	420 (±206)	43	7.0 (±0.6)	21.0 (±4.0)
3	DEX, 250 mg/kg i.p. at <i>t</i> = +6h	7	927 (±163)	100	6.7 (±0.6)	24.9 (±0.8)
3	DEX, 250 mg/kg i.v.	9	479 (±141)	67	9.2 (±1.2)	19.3 (±1.3)
1	Saline i.p.	16 <sup>c</sup>	263 (±56)	88	6.9 (±0.7)	22.6 (±1.1)
1	DEX, 250 mg/kg i.p.	16 <sup>c</sup>	28 (±22)	13	10.5 (±3.5)	16.0 (±4.0)
Idarubicin (mg/kg s.c.)						
0.75	Saline i.p.	18 <sup>c</sup>	308 (±58)	83	6.7 (±0.4)	21.2 (±0.7)
0.75	DEX, 250 mg/kg i.p.	18 <sup>c</sup>	42 (±19)	28	10.2 (±0.5)	15.6 (±2.7)
0.05	Saline i.p.	9	0	0		
0.05	DEX, 250 mg/kg i.p.	9	4 (±4)	11	12.0 (±0.0)	13.0 (±0.0)
0.25	Saline i.p.	9	51 (±22)	44	5.5 (±0.9)	24.8 (±1.4)
0.25	DEX, 250 mg/kg i.p.	9	38 (±38)	11	6.0 (±0.0)	20.0 (±0.0)
1.50	Saline i.p.	9	353 (±97)	78	7.1 (±0.6)	19.4 (±2.0)
1.50	DEX, 250 mg/kg i.p.	8	183 (±54)	75	7.7 (±0.2)	17.8 (±0.9)
Doxorubicin (mg/kg s.c.)						
2 and 3	Saline i.p.	61 <sup>c</sup>	467 (±61)	77	9.3 (±0.5)	20.5 (±0.7)
2 and 3	DEX, 250 mg/kg i.p.	58 <sup>c</sup>	17 (±7)	14	8.6 (±1.8)	14.8 (±1.9)
3	DEX, 62.5 mg/kg i.p.	9	21 (±14)	22	12.0 (±2.0)	17.5 (±1.5)
2 and 3	DEX, 125 mg/kg i.p.	16 <sup>c</sup>	32 (±23)	13	10.2 (±1.2)	17.0 (±0.9)
2	DEX, 375 mg/kg i.p.	7	0	0		
3	DEX, 62.5 mg/kg i.p. at <i>t</i> = 0, +3, and +6h	9	0	0		
3	DEX, 125 mg/kg i.p. at <i>t</i> = 0, +3, and +6h	9	34 (±23)	22	14.0 (±1.0)	14.0 (±2.0)
3	DEX, 125 mg/kg i.p. at <i>t</i> = 0, +3, and +6h	9	34 (±23)	22	14.0 (±1.0)	14.0 (±2.0)
3	DEX, 250 mg/kg i.p. on day 0, 1, and 2	9	0	0		
2 and 3	DEX, 250 mg/kg i.p. at <i>t</i> = +3h	18 <sup>c</sup>	62 (±37)	28	7.2 (±1.0)	19.0 (±1.5)
2 and 3	DEX, 250 mg/kg i.p. at <i>t</i> = +6h	18 <sup>c</sup>	77 (±20)	50	9.3 (±1.1)	18.2 (±0.8)
3	DEX, mg/kg i.p. 250 I.V.	9	17 (±11)	22	10.5 (±0.5)	12.0 (±1.0)
3	Mixed with DEX, 30 mg/kg	9	0	0		
3	Mixed with DEX, 250 mg/kg	9	169 (±47)	78	6.6 (±2.0)	18.3 (±1.6)
1	Saline i.p.	9	0	0		
1	DEX, 250 mg/kg i.p.	9	0	0		
Hydrogen peroxide (s.c.)						
1%	Saline i.p.	4	0	0		
3%	Saline i.p.	9	95 (±65)	22	10.5 (±3.5)	14.5 (±2.5)
3%	DEX, 250 mg/kg i.p.	9	46 (±15)	11	9.0 (±0.0)	15.0 (±0.5)
6%	Saline i.p.	9	407 (±103)	100	5.7 (±0.4)	16.1 (±0.5)
6%	DEX, 250 mg/kg i.p.	9	350 (±80)	78	5.9 (±0.4)	15.9 (±0.8)
10%	Saline i.p.	6	616 (±51)	100	5.0 (±0.4)	17.4 (±0.5)
10%	DEX, 250 mg/kg i.p.	8	668 (±81)	100	5.0 (±0.3)	17.0 (±0.4)

<sup>a</sup> If nothing else is stated, the treatment (dexrazoxane or saline) was administered at *t* = 0. Data are no. or mean (±SE).

<sup>b</sup> *n*, number of mice; FW, fraction of mice with wounds (%); TTW, mean time to appearance of wound (days); DW, mean duration of wound (days).

<sup>c</sup> Pooled data (see "Results" for details).

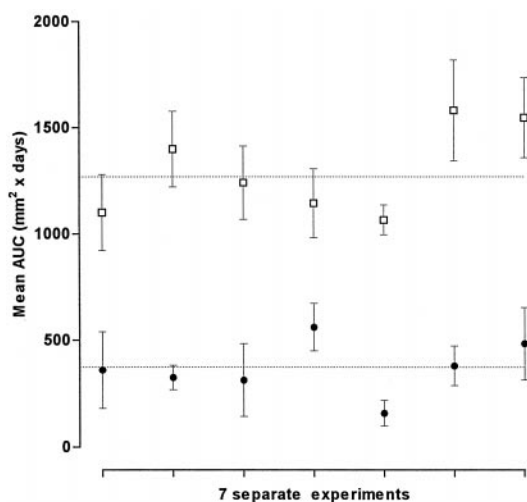
protection provided by i.p. injection of DEX compared with i.v. administration.

Reduction of the daunorubicin dose from 3 mg/kg to 1 mg/kg resulted in significantly smaller AUCs ( $P < 0.0001$ ), with no difference in frequency of wounds, time to, or duration of wounds. i.p. treatment with DEX also resulted in a statistically significant reduction in AUC ( $P < 0.0001$ ) at the low daunorubicin dose. Tissue lesions induced by idarubicin correlated to the dose in exactly the same manner. Doxorubicin injected s.c. in doses  $< 2$  mg/kg did not produce any wounds.

In daunorubicin-induced lesions, the reduction in AUC

decreased from 70 to 45% ( $P = 0.0175$ ), when the DEX dose was reduced from 250 mg/kg i.p. to 125 mg/kg. In contrast, the protection against doxorubicin injuries was evenly effective at all doses of DEX.

Three or even 6 h of delay in administration of DEX did not impair the degree of protection against doxorubicin lesions when compared with the effect obtained by treatment at *t* = 0. Similarly, when DEX was administered 3 h after the injection of daunorubicin, the protection was no different from the protection obtained by immediate DEX administration. However, the protection was lost if the delay was 6 h (Fig. 5).



**Fig. 1** The induction of wounds by s.c. daunorubicin and the protection from such lesions with i.p. DEX is highly reproducible. The mean AUC from seven independent experiments with daunorubicin, 3 mg/kg s.c.,  $\pm$  DEX, 250 mg/kg i.p., at  $t = 0$ . The interexperimental variation was not statistically different in the treated (plus DEX;  $P > 0.05$ ) or the controls (no DEX;  $P > 0.05$ ). Treatment with DEX resulted in statistically lower mean AUCs ( $P < 0.001$ ) in all experiments.  $\square$ , no DEX;  $\bullet$ , plus DEX;  $\dots$ , mean; bars, SE.

Treatment with DEX, 250 mg/kg i.p., 3 days in a row completely prevented both doxorubicin- and daunorubicin-induced lesions. Moreover, triple-treatment with DEX, 62.5 mg/kg or 125 mg/kg i.p., administered 0, 3, and 6 h after injection of daunorubicin or doxorubicin resulted in at least the same degree of protection as a single injection of 250 mg/kg DEX i.p.

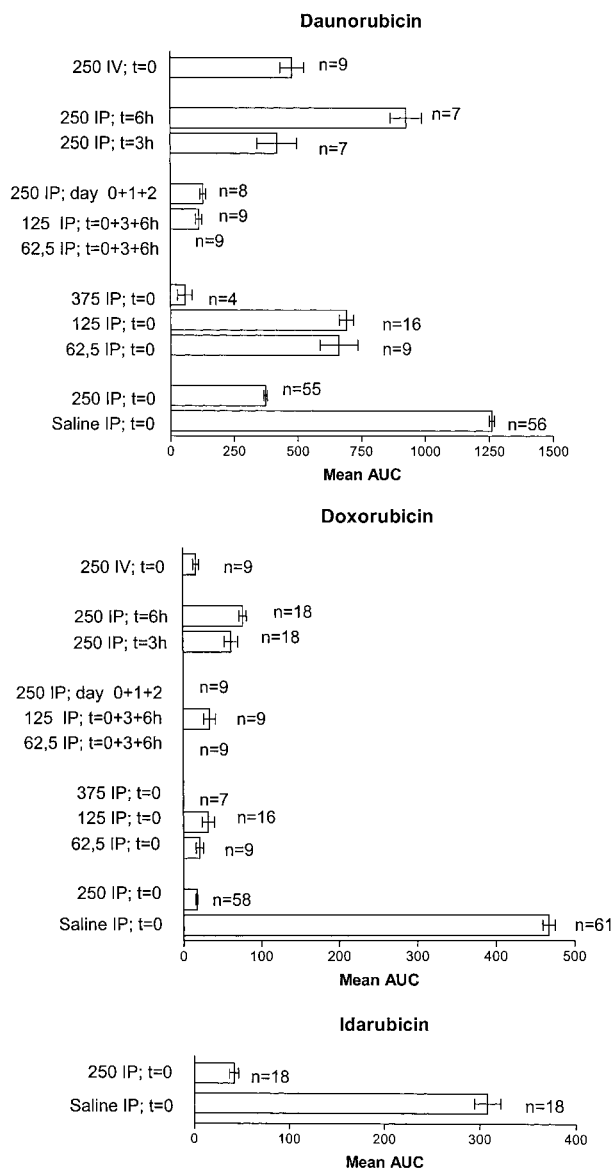
The dose of DEX correlated inversely with the protection against wounds in the two experiments in which DEX and doxorubicin were mixed before s.c. injection. Thus, s.c. injection of a mixture of DEX, 30 mg/kg, and doxorubicin, 3 mg/kg, resulted in complete protection against wounds. However, the increment of the dose of DEX to 250 mg/kg in the same volume resulted in the appearance of wounds in seven of nine treated mice.

Hydrogen peroxide s.c. clearly produced dose-dependent lesions. Wounds induced by a solution of 3% were less frequent and had a smaller mean AUC than wounds induced by a solution of 6% ( $P = 0.0237$ ) and 10% ( $P < 0.0001$ ). However, treatment with DEX, 250 mg/kg i.p., had no effect on the AUC at any of the three dose levels ( $P > 0.05$  at all levels).

## DISCUSSION

This is the first report on the experimental amelioration of s.c. injuries caused by anthracycline DEX. We have demonstrated that the protection depends on the dose of DEX as well as on the time and frequency of administration.

DEX is registered as a cardioprotective agent (Zinecard, Cardioxane) against anthracycline-induced cardiotoxicity. A hypothesis for this indication has been that DEX, as an analogue of the cation binder EDTA, protects against free radical damage by binding and thus concealing iron from oxygen (33, 34). How-



**Fig. 2** The protection against daunorubicin-induced lesions is more dose- and time-dependent than against injuries caused by s.c. doxorubicin. However, note the marked protection obtained by triple treatment with DEX. The histograms compare the mean AUCs of different schedules of DEX after s.c. injection of 3 mg/kg daunorubicin, 0.75 mg/kg idarubicin, and 2 or 3 mg/kg doxorubicin, respectively. Mean AUC,  $\text{mm}^2 \times \text{days}$ ;  $n$ , number of mice; bars, SE. Except for the bars representing saline, the legends depict the dose, route of administration, and timing of DEX.

ever, we have recently demonstrated that cells with acquired resistance to DEX carry mutations in topo II $\alpha$  (an isoform of topo II), which are in different sites than those induced by topo II poisons, such as daunorubicin and etoposide. We confirmed that these mutations were functional using humanized topo II $\alpha$  in human yeast (35, 36). Accordingly, DEX is most likely a specific topo II agent. Whether the true mechanism underlying the demonstrated amelioration of soft tissue injuries is attribut-

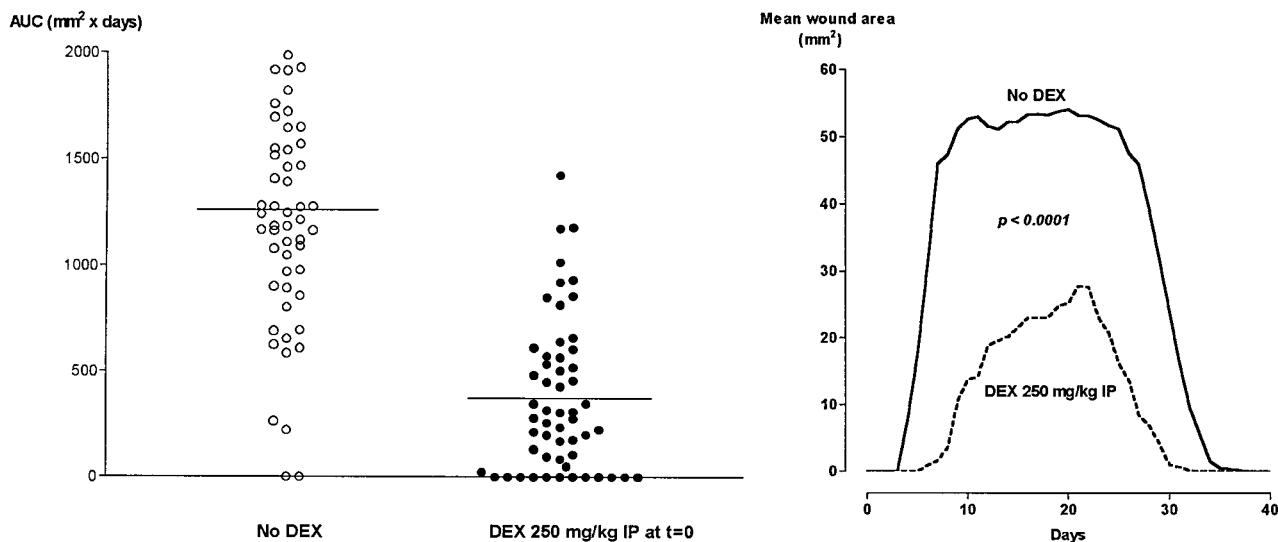


Fig. 3 A single systemic injection of DEX significantly reduces the wounds induced by s.c. daunorubicin. *Left*, scatter plot showing the distribution of the AUCs of individual mice after 3 mg/kg daunorubicin s.c. followed by saline i.p. (○;  $n = 56$ ) or 250 mg/kg DEX i.p. at  $t = 0$  (●;  $n = 55$ ). —, mean AUCs. *Right*, mean wound area versus time of the same data as in the left graph. The difference in AUCs is highly significant. Moreover, the curves reveal the delay in the appearance and the shorter duration of wounds.

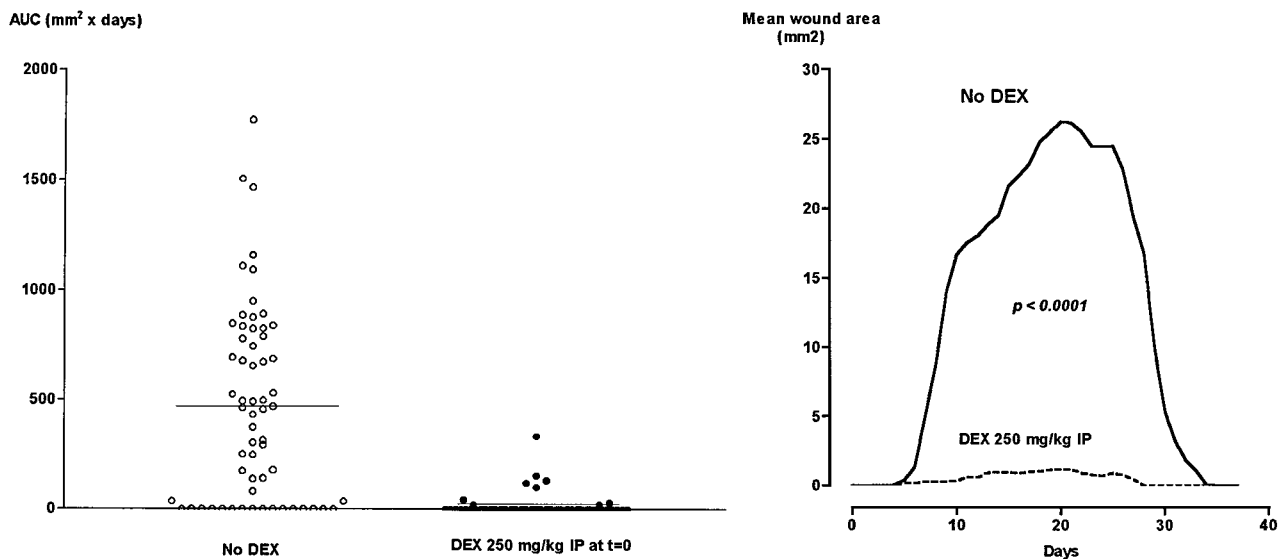


Fig. 4 A single systemic injection of DEX significantly reduces the wounds induced by s.c. doxorubicin. *Left*, scatter plot showing the distribution of the AUCs of individual mice after 2 or 3 mg/kg doxorubicin s.c. followed by saline i.p. (○;  $n = 56$ ) or 250 mg/kg DEX i.p. at  $t = 0$  (●;  $n = 55$ ). —, mean AUCs. *Right*, mean wound area versus time of the same data as in the left graph. The difference in AUCs is highly significant.

able to an effect on the catalytic cycle of topo II, scavenging of free radical damage, a combined effect, or even a third mechanism is beyond the scope of this study. Therefore, the fact that DEX had no effect on the appearance of lesions induced by hydrogen peroxide, which supposedly result from the formation of toxic hydroxyl radicals, should not lead to premature conclusions about the mechanism. Although the present results support the topo II interaction as the inhibitory mechanism, we have not measured the topo II levels in the extravasation area to support or reject this hypothesis.

The systemic "standard" dose of DEX of 250 mg/kg used in here is a safe dose, which corresponds to one-half to one-third of the bolus i.p. LD<sub>10</sub> in the mouse strain. Comparing the i.v. LD<sub>10</sub> dose of daunorubicin with the systemic dose of DEX yields a daunorubicin:DEX ratio of 1:15. The s.c. dose of daunorubicin used in our experiments was one-fifth of the i.v. LD<sub>10</sub>. Doxorubicin produced identical lesion sizes and wound duration at 2 and 3 mg/kg s.c. corresponding to one-twelfth to one-eighth of the i.v. LD<sub>10</sub> in this mouse strain. Accordingly, the doxorubicin:DEX ratio was 1:10. The dose of idarubicin was



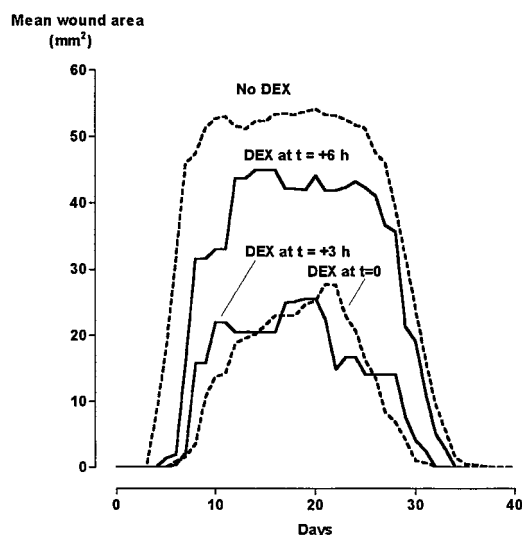


Fig. 5 DEX protection against s.c. daunorubicin is time-dependent. DEX can be administered 3 h after daunorubicin. However, the protection is lost if DEX treatment is given 6 h after daunorubicin. In contrast, the protection against injuries induced by doxorubicin is still statistically significant after 6 h of delay.

one-third of the i.v. LD<sub>10</sub>. Thus, the anthracycline:DEX ratios are comparable with those being used in studies of anthracycline-induced cardiac toxicity in animals (37). Similarly, the ratios are comparable with the recommended doxorubicin:DEX ratio of 1:20, when DEX is used to prevent doxorubicin-induced cardiomyopathy in humans (38).

We chose the s.c. model to mimic the actual clinical extravasation situation as closely as possible, although it has been proposed that injections beneath the rodent skin muscle layer, panniculus carnosus, cause irregular ulcerative lesions (39). However, in our experiments, we convincingly demonstrated that the interexperimental variation of the duration and extension of the s.c. lesions induced by anthracyclines as well as by hydrogen peroxide control groups (no DEX) did not differ statistically. This is an indication of s.c. injection as a reliable administration method in this kind of experiment, at least in the used mouse strain.

The antagonistic effect of DEX on the toxicity of idarubicin, doxorubicin, and daunorubicin obviously poses a risk of counteracting the antitumor effect of any drug infused before the extravasation. However, the main model demonstrated here is a single-shot administration, and the longest duration of treatment in our experiments was 3 days. The plasma half-life of DEX and the intracellularly generated hydrolysis product ADR-925 is  $3.2 \pm 0.9$  h and  $\sim 28$  h, respectively (40). Consequently, it is unlikely that any antagonistic effect of DEX should extend to subsequent courses of anthracycline-based chemotherapy. In addition, the ratios of anthracycline:DEX are very close to the clinically recommended ratio in multiple courses of doxorubicin + DEX therapy. Finally, it should be brought into mind that the morbidity and risk of postponing subsequent cycles of chemotherapy because of surgical management, including skin grafting, might be a greater threat to the life of the patients in question.

We have demonstrated that the protection obtained by triple-

dose DEX is superior to single-dose. Still, there is an unexplained imbalance between the protective effect of the relatively short-lived DEX and the long persistence of anthracyclines in tissues. However, we do lack knowledge about the protective mechanism and thus are not presently able to explain this.

The reason for using systemic administration of DEX instead of local or IL injection is 2-fold. Firstly, there has been a report of vesicant properties of DEX by itself (41), although two nonvesicant extravasations of DEX have been reported (42). However, DEX is generally considered a skin irritant (38). Nonetheless, in pilot animal studies, we have been able to demonstrate a significant protection by IL injection of DEX, which is not merely caused by dilution of anthracycline (results not shown). In the present study, we also demonstrate that mixing of small doses (30 mg/kg) of DEX and doxorubicin before s.c. injection produce only very small tissue lesions. An increment of the DEX dose to 250 mg/kg in the mixture results in much greater AUCs. Thus, further exploration of the efficacy and tolerability of IL injections of DEX is warranted. Secondly, systemic treatment with an antidote could be very useful in soft tissue extravasation from centrally placed venous access devices. Brothers *et al.* (43) demonstrated leakage in 6.4% of 300 s.c. infusion ports of which 83% were used in cancer chemotherapeutic treatments. Curran and Luce (44) assessed 36 extravasations of doxorubicin from venous access devices and found that nine (25%) required surgical debridement or excision. Accordingly, the increased use of such devices should not induce a false sense of safety against extravasation accidents. An effective antidote that is rapidly and easily administered in a peripheral vein in case of suspicion of leakage would be advantageous.

The convincing preclinical data presented here has already led to a change of clinical practice in our institution. Thus, a mandatory venous access device during anthracycline therapy is now optional, which cuts treatment costs and patient discomfort. Furthermore, the acute treatment of accidental extravasation of anthracycline extravasation now consists of termination of the infusion of DEX, 1000 mg/m<sup>2</sup> i.v. (in a separate infusion), within 6 h after the extravasation and on day 2, and 500 mg/m<sup>2</sup> i.v. on day 3. In addition, acute surgical evaluation is performed. Additional studies will show whether this schedule should be changed. It is our belief that the use of DEX in accidental anthracycline extravasations will result in fewer and less extensive surgical procedures as well as in a reduction in the numbers of reoperations.

## ACKNOWLEDGMENTS

We are indebted to Annette Nielsen for her expert technical support.

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Seppo W. Langer, Maxwell Sehested and Peter Buhl Jensen

*Clin Cancer Res* 2000;6:3680-3686.

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