JCI The Journal of Clinical Investigation

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J Clin Invest. 1988;81(1):142-148. https://doi.org/10.1172/JCI113285.

Research Article

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Experimental Chemotherapy-induced Skin Necrosis in Swine

Mechanistic Studies of Anthracycline Antibiotic Toxicity and Protection with a Radical Dimer Compound

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Abstract

The reactivity of antitumor anthracycline and mitomycin C antibiotics with the oxomorpholinyl radical dimers, bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (TM3) and bi(3,5-dimethyl-5-hydroxymethyl-2-oxomorpholin-3-yl) (DHM3), was studied in vitro. The oxomorpholinyl radical reduced daunorubicin to a quinone methide intermediate that reacted with solvent to form 7-deoxydaunorubicinone. The solvolysis reaction followed first order kinetics, and the reactivity rate constants (k_2) measured for seven anthracycline analogues ranged from 2×10^{-2} s^{-1} to 8.0×10^{-4} s^{-1}. The chemical reactivity of each anthracycline quinone methide correlated with the total skin toxicity caused by the respective parent anthracycline following injection into swine skin.

Microscopic examination of experimental lesions in swine skin resemble those observed in humans after inadvertant chemotherapy extravasation. Hydrocortisone sodium succinate was not effective for the treatment of doxorubicin-induced skin necrosis, whereas DHM3 was effective for the treatment of skin necrosis caused by all seven anthracyclines and by the quinone containing antibiotic, mitomycin C.

Introduction

Cytotoxic drug treatment of human cancer is associated with a spectrum of host organ toxicities. Skin injury resulting from extravasation of necrotizing chemotherapeutic agents accounts for 1–5% of all chemotherapy-related toxicities (1). Extravasation of doxorubicin, a widely used anthracycline antibiotic, is a well-recognized complication of its use with an estimated incidence of 0.5 to 6% (2–4). Mitomycin C and the vinca alkaloids are also necrotizing antitumor drugs that cause tissue injury after extravasation (5, 6). Although infrequent, chemotherapy extravasation may result in significant local and systemic morbidity (7). Clinical experience, uncontrolled clinical trials, and studies in animal models have resulted in different treatment recommendations for chemotherapy extravasation (1, 3–4, 6–10). For example, the Oncology Nursing Society (9), the most recent edition of a medical oncology textbook (10), and the official manufacturer's product labeling[1] currently recommend corticosteroids to treat anthracycline extravasation. However, there is no proven mechanistic rationale for this practice and the empirical use of corticosteroids has been recently challenged by the results of animal studies (11–14) and clinical experience (6). In spite of extensive laboratory investigation, the molecular mechanism of anthracycline antibiotic cellular toxicity is not fully understood (15). The anthracyclines have been shown to intercalate into DNA (16), form reactive intermediates capable of covalent binding to macromolecules (17) and generate free radical species (18). However, as is true for most of the biological effects of the anthracyclines, it is not clear which of these mechanisms accounts for the skin damage caused by these drugs.

The oxomorpholinyl radical dimers bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (TM3)[2] and bi(3,5-dimethyl-5-hydroxymethyl-2-oxomorpholin-3-yl) (DHM3) react with doxorubicin or daunorubicin to produce the inactive 7-deoxyaglycone metabolite (19). TM3 was shown to be an effective protectant of systemic anthracycline toxicity (20), and more recently, DHM3 was shown to be effective against acute anthracycline toxicity produced in two animal models (21–22). Because of the anatomic similarities between pig and human skin (23–24), chemotherapy-induced skin injury was studied in a swine model and, in this model, DHM3 was found to be an effective treatment for doxorubicin-induced skin necrosis (22).

In the present study, the capacity of the oxomorpholinyl dimers, TM3 and DHM3, to chemically reduce seven anthracycline antibiotic analogues and mitomycin C is examined. The kinetically determined chemical reactivity of the anthracyclines following reduction with the oxomorpholinyl dimers is shown to correlate with the biological effect of skin injury caused by each anthracycline analogue. Finally, the utility of DHM3 for the prevention of skin injury resulting from anthracycline and mitomycin C antibiotic antitumor agents is shown, and the superiority of DHM3 to that of hydrocortisone for the treatment of doxorubicin-induced skin injury is demonstrated.

Methods

Drugs. Clinical grade doxorubicin HCl, daunorubicin HCl, aclarubicin, menogaril, mitomycin C, vinblastine, mitoxantrone, and bulk powder 5-iminodaunorubicin were obtained from the Drug Synthesis

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Presented in part at the 77th Annual Meeting of the American Association for Cancer Research, 8 May 1986.

Received for publication 23 October 1986 and in revised form 13 August 1987.

The Journal of Clinical Investigation, Inc.
Volume 81, January 1988, 142–148

1. Package insert product information (revised December 1985), Adria Laboratories, Columbus, OH.

2. *Abbreviations used in this paper:* AUC, area under curve; DHM3, bi(3,5-dimethyl-5-hydroxymethyl-2-oxomorpholin-3-yl; TM3, bi(3,5,5-trimethyl-2-oxomorpholin-3-yl).

Branch, Developmental Therapeutics Program, National Cancer Institute, Bethesda, MD. Clinical grade 4-demethoxydaunorubicin and 4'-epidoxorubicin was the generous gift of Adria Laboratories, Columbus, OH. Each drug was reconstituted with sterile water to a final drug concentration of 3.4 μmol/ml. This concentration is equivalent to 2.0 mg/ml doxorubicin HCl; the final concentration recommended by the manufacturer for intravenous administration to patients. DHM3 was synthesized by condensation of 2-amino-2-methyl-1,3-propanediol with ethyl pyruvate followed by a photoreduction reaction to yield the final product, DHM3, a crystalline, white powder. In solution, DHM3 is oxidized by molecular oxygen; therefore, the DHM3 solution was prepared by dissolving the powder in water that had been purged with N_2 gas for 5 min. Solubilization was facilitated by brief sonication to give a final concentration of 10 mg/ml (31.6 μmol/ml). The solution was kept cold and protected from air and light. The synthesis of TM3 has been described previously (25). Hydrocortisone sodium succinate (Solu-Cortef; 50 mg/ml, 105 μmol/ml, Upjohn Laboratories, Kalamazoo, MI) was obtained from commercial sources.

Chemical reaction kinetic determinations. Oxomorpholinyl dimer, DHM3, (1.38 mg, 4.37 μmol) was dissolved in 0.5 ml of oxygen-free methanol and quickly transferred via syringe to the spectrophotometric compartment of a two-compartment Pyrex cell with attachment for connection to a vacuum line with an Ultra Torr Union. The solvent was promptly removed under vacuum. Daunorubicin HCl (11.9 mg, 21.1 μmol) was dissolved in 100 ml of an aqueous solution of Tris (24.2 mg) and Tris HCl (31.6 mg), and 2.6 ml of this solution was introduced into the other compartment of the cell. The solution was freeze (liquid N_2)-pump (2×10^{-6} Torr)-thaw degassed through three cycles, sealed, and placed in a thermostated cell holder at 10.0±0.1°C for 20 min before mixing. Full spectra between 320 and 800 nm were recorded every 10 s with a diode array spectrophotometer (8450 UV-visible; Hewlett-Packard Co., Palo Alto, CA) for the first 125 s and then every 20 s. From the absorbance change at 620 nm attributed to the quinone methide intermediate (26) over the first 85 s (50% reaction of daunorubicin), a nonlinear least-squares analysis was used to determine a first order rate constant for bond homolysis of DHM3 (k_1) and a first order rate constant (k_2) for the production of 7-deoxydaunorubicinone from the quinone methide intermediate. The rate constant (k_1) for bond homolysis of DHM3 was also determined at 25°C by trapping with Ferrin (Fe^{3+}-1,10-phenanthroline complex). Using an independently determined value of $k_1 = 3.4 \times 10^{-3}$ s^{-1} for bond homolysis of TM3, the reactivity of TM3 with doxorubicin, daunorubicin, 4-demethoxydaunorubicin, aclarubicin, and menogaril, and the k_2 rate constant for each respective compound in methanol solvent was determined at 25°C in a similar fashion as previously described (26).

For the measurement of the rate constant (k_2) for tautomerization of the quinone methide of 5-iminodaunomycin, TM3 was replaced by sodium dithionite since the rate of reduction by TM3 was too slow for spectroscopic observation of the quinone methide intermediate. Reactivity of TM3 with mitomycin C was examined in chloroform solvent under anaerobic conditions.

Animals. Inbred female weanling miniature white swine weighing approximately 12 kg were obtained from the Veterinary Resources Branch, National Institutes of Health, Bethesda, MD. The development of this strain of swine has been described previously (27). Each animal was housed in a 6 × 4 foot metabolic cage and was fed standard sow chow, 1.5 lb twice daily.

Anesthesia and antibiotics. Each animal was anesthetized by intramuscular injections of ketamine (20 mg/kg), xylazine (20 mg/kg), and atropine (1.0 mg). After endotracheal intubation, anesthesia was maintained with a mixture of nitrous oxide, halothane, and oxygen gas. If at any time throughout the experiment the skin lesions appeared to be involved with a superinfection, oxytetracycline (Liquamycin; Pfizer Inc., New York) was administered intramuscularly until there was complete resolution of the infection.

Drug administration and lesion measurement. All chemotherapeutic drugs were administered intradermally with a 25-gauge needle along the midlateral aspect of each side of the swine (eight injection sites per side). After the injection of 1.0 ml of drug solution, (3.4 μmol/ml) the syringe was removed from the hub, leaving the needle in place. After 15 min, 1.0 ml DHM3 (31.6 μmol/ml) or hydrocortisone (105 μmol/ml) was injected through the same needle and the needle was then removed. Alternating adjacent injection sites served as controls, receiving 1.0 ml sterile water instead of DHM3 or hydrocortisone. All lesions were measured by a single observer with metric calipers three times weekly for 2 wk, followed by weekly measurements thereafter. The areas of skin ulceration and induration were estimated by the product of the cross-perpendicular diameters. The total toxicity of drug-induced skin ulceration-vs.-time curve (AUC) was determined by the "cut and weigh" method and was expressed in squared millimeters × days (12, 22). Each swine was observed for 8–10 wk.

Histopathology. Incisional biopsies were performed on representative lesions 6 and 20 d after doxorubicin intradermal injections, and 9 and 24 d after DHM3 injections. Upon termination of the experiment on day 71, incisional biopsies were performed on representative healing lesions that had received water, DHM3, or hydrocortisone treatment after doxorubicin injection. Samples were placed in 10% buffered formalin and processed for examination by standard light microscopy (Pathco, Inc., Potomac, MD).

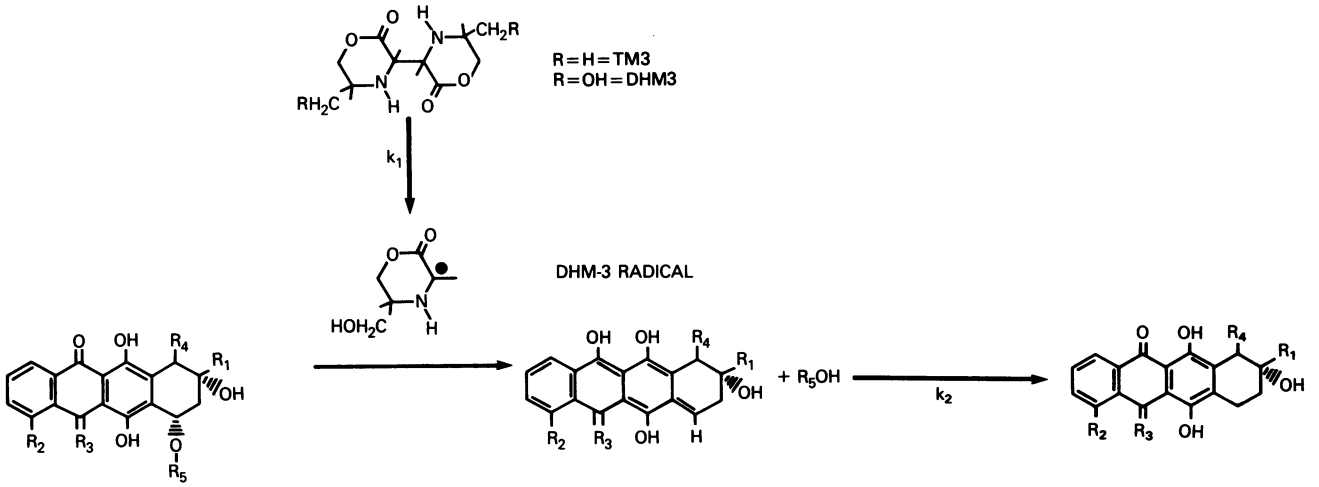
Statistical analysis. The skin toxicity data were analyzed by the two-tailed t test for independent means, and linear regression for toxicity versus k_2 was determined by the least squares method (28).

Results

Chemical reactivity of oxomorpholinyl dimers with anthracycline antibiotics and mitomycin C. When a 10-fold excess of DHM3 was added to a solution of 200 μM daunorubicin in buffered (pH 8.0), oxygen-free water at 10°C there was a rapid and irreversible formation of the aglycone, 7-deoxydaunorubicinone. This reaction is shown in Fig. 1 where the end-product 7-deoxydaunorubicinone, the structure of which was established by spectral and chromatographic comparison with an authentic sample, is the aglycone derived from daunorubicin (compound B). As determined from experimentally derived absorbance data at 10°C, the reaction followed consecutive first-order kinetics with a rate constant (k_1) of 6×10^{-4} s^{-1} for bond homolysis of DHM3 and a rate constant (k_2) of 2×10^{-2} s^{-1} for the production of 7-deoxydaunorubicinone from a quinone methide intermediate (Fig. 1). At 25°C, $k_1 = 6 \times 10^{-3}$ s^{-1}. The rate constant (k_1), which determines the rate of production of the quinone methide intermediate, indicated that DHM3 has approximately the same reactivity with daunorubicin in water as does the related oxomorpholinyl dimer TM3 in methanol solvent ($k_1 = 3.4 \times 10^{-3}$ s^{-1}, at 25°C, [26]). Thus, TM3 was used to study the reactivity of the oxomorpholinyl dimers with seven different anthracycline analogues. As shown in Fig. 1, specific substitutions on the anthracycline molecule result in different rate constants (k_2) for the production of the aglycone from each respective analogue through the quinone methide intermediate. The rate constants (k_2) ranged from 2×10^{-2} s^{-1} for 5-iminodaunorubicin to 8.0×10^{-4} s^{-1} for aclarubicin.

When TM3 was added to a chloroform solution containing mitomycin C, spectroscopic analysis showed an efficient reaction as indicated by the disappearance of the mitomycin C absorption at 358 nm and increase in absorbance at 312 nm with a sharp isosbestic point at 324 nm (data not shown).

Drug-induced skin necrosis in swine. The intradermal injection of doxorubicin in swine produced a predictable and reproducible skin ulceration that has been fully described previously (22).



Compound	R_1	R_2	R_3	R_4	R_5	$k_2(s^{-1})$
A. Doxorubicin	$COCH_2OH$	OCH_3	O	H_2	Daunosamine	1.1×10^{-2}
B. Daunorubicin	$COCH_3$	OCH_3	O	H_2	Daunosamine	1.3×10^{-2}
C. 4-Demethoxydaunorubicin	$COCH_3$	H	O	H_2	Daunosamine	9.0×10^{-3}
D. 4'-Epidoxorubicin	COCHOH	OCH_3	O	H_2	4-Epidaunosamine	1.0×10^{-2}*
E. 5-Iminodaunorubicin	$COCH_3$	OCH_3	NH	H_2	Daunosamine	2×10^{-2}‡
F. Aclarubicin	C_2H_5	OH	O	$COOCH_3$	Rhodosamine-Deoxyfucose-L-Cinerulose	8.0×10^{-4}
G. Menogaril	(7-Con-O-methylnogarol)					2.5×10^{-3}

Figure 1. In vitro reaction of anthracycline antibiotics with the substituted oxomorpholinyl dimers, TM3 and DHM3, to form the 7-deoxyanthracycline aglycone. The anthracycline analogues were dissolved in methanol and reacted with TM3 under anaerobic conditions as described in Methods. The rate constant, k_2, describes the tautomerization of the quinone methide intermediate to the aglycone product. * The rate constant for 4'-epidoxorubicin was not measured, but assumed equal to that of doxorubicin (34). ‡ The rate constant for 5-iminodaunorubicin was measured by replacing TM3 with sodium dithionite in the reaction.

Intradermal injection of 1.0 ml (3.4 μmol/ml) of several cytotoxic antitumor agents resulted in skin injury similar to doxorubicin, but the extent and time course of the injury varied with the drug (Table I). Mitomycin C caused the greatest skin injury (40% greater than for doxorubicin) while the anthracycline analogues aclarubicin and menogaril caused similar lesions that were one-third as severe as doxorubicin.

Since the rate constants (k_2) reflect the reactivity of the quinone methide intermediate for each anthracycline analogue and since this intermediate has been proposed as a species capable of covalently binding to biological macromolecules (26), we compared the rate constant (k_2) to the total toxicity (Table I) for each of the anthracycline analogues studied. As shown in Fig. 2, the reactivity of each analogue, determined in a defined in vitro chemical system, correlated with the degree of in vivo skin toxicity caused by that analogue.

DHM3 effectively reduced the skin necrosis caused by the anthracycline analogues and mitomycin C (Table I). Skin toxicity caused by vinblastine or mitoxantrone was not reduced by DHM3.

The effect of several pharmacological interventions on the course of the doxorubicin-induced skin injury is shown in Fig. 3 and Table II. When DHM3 (31.6 μmol) was injected into the same site 15 min after doxorubicin (3.4 μmol), the peak area of the ulcer was reduced 77%, the total toxicity was reduced 95%,

Table I. Protection against Chemotherapy-induced Skin Injury

Drug	Total toxicity ($mm^2 \times d$)		
	H_2O	DHM3	Difference
			%
Mitomycin C	22,271	9,252	−58
Doxorubicin	16,014	4,646	−71
Daunorubicin	14,517	5,310	−63
4-Demethoxydaunorubicin	9,994	2,451	−75
4'-Epidoxorubicin	12,564	2,031	−84
5-Iminodaunorubicin	16,712	6,450	−61
Aclarubicin	5,900	1,742	−70
Menogaril	5,265	2,087	−60
Vinblastine	12,946	16,101	+24
Mitoxantrone	14,187	16,063	+13

After the intradermal injection of 1.0 ml chemotherapeutic drug (3.4 μmol), either 1.0 ml sterile H_2O or DHM3 (31.6 μmol) was injected through the same needle 15 min later. All lesions were measured three times weekly for 2 wk and then weekly. The area of skin ulceration was estimated by the product of the cross-perpendicular diameters. The total toxicity is the AUC for the skin ulceration vs. time curve. Data are the mean of two experiments.

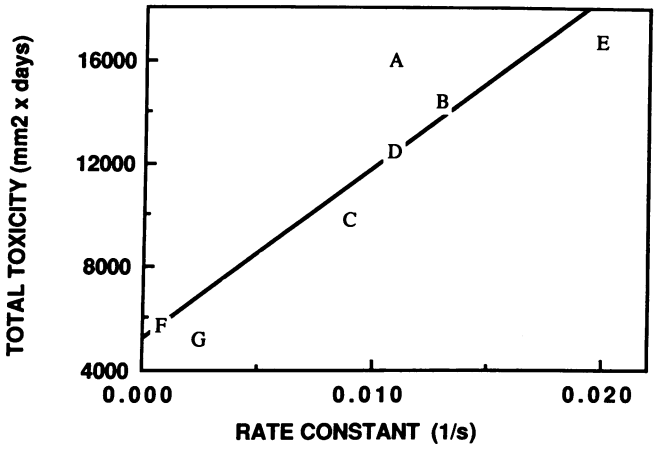


Figure 2. Correlation between skin toxicity after intradermal anthracycline injection in vivo and the reactivity of anthracyclines in vitro. The curve was derived from data presented in Table I and Fig. 1. The letters (*A–G*) correspond to the anthracycline analogues shown in Fig. 1. Each data point represents the mean of two skin ulceration sites. $r^2 = 0.85$, $P < 0.005$

Table II. Protection against Doxorubicin-induced Skin Injury

	H_2O	Hydrocortisone	DHM3
Maximal lesion size (mm^2)			
Ulcer	159	234	36*
Induration	1048	1169	714
Healing time (d)			
Ulcer	>70	>70	35*
Induration	>70	>70	50*
Total toxicity ($mm^2 \times d$)			
Ulcer	10,742	12,726	570*
Induration	45,536	45,992	14,800*

After the intradermal injection of 1.0 ml doxorubicin (3.4 μmol), 1.0 ml sterile water, hydrocortisone sodium succinate (105 μmol), or DHM3 (31.6 μmol) was injected through the same needle 15 min later. All lesions were measured three times weekly for 2 wk and then weekly. The areas of skin ulceration and induration were estimated by the product of the cross-perpendicular diameters. The total toxicity is the AUC for the skin ulceration vs. time curve. Data are the mean of three experiments.

* P value < 0.025 compared to H_2O treatment.

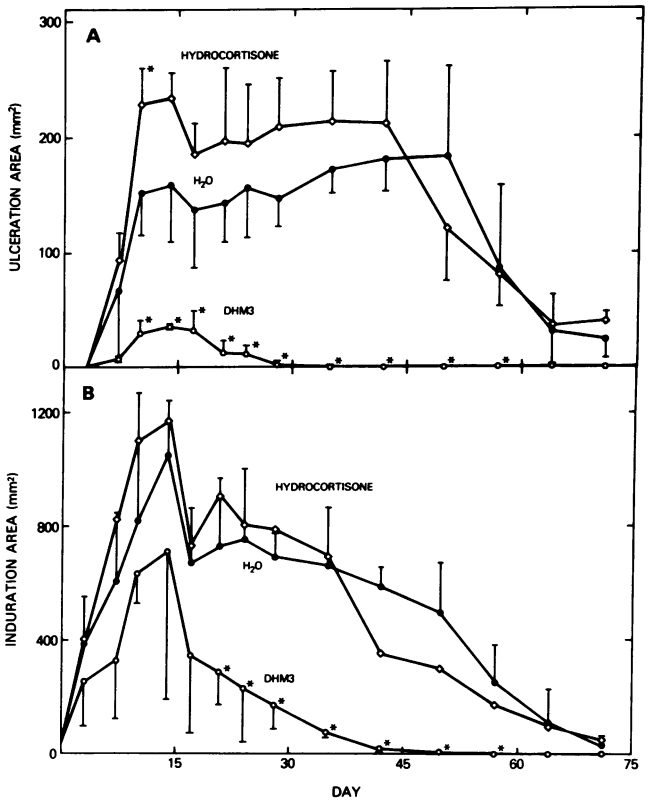


Figure 3. Time course of doxorubicin-induced skin injury. (*A*) Skin ulceration. (*B*) Skin induration. Following the intradermal injection of doxorubicin, 3.4 μmol in 1.0 ml sterile H_2O, each site received 1.0 ml of either sterile H_2O, hydrocortisone sodium succinate (105 μmol), or DHM3 (31.6 μmol) as described in Methods. Each lesion area was estimated by the cross-perpendicular diameters. Each point represents the mean±SD of triplicate experiments. * $P < 0.05$, determined by the two-tailed t test for independent means.

and healing was completed in less than one-half the time when compared to controls. In contrast, injection of hydrocortisone sodium succinate (105 μmol) 15 min after doxorubicin failed to provide any protection against subsequent development of skin ulceration. In fact, hydrocortisone treatment appeared to worsen the extent of skin injury; although this was statistically significant for only one time point (day 12, Fig. 3 *A*). The effect of DHM3 and hydrocortisone on the surrounding skin induration paralleled that observed for the ulceration (Fig. 3 *B*, Table II).

Histopathological studies. The acute and chronic histopathological appearance of doxorubicin-induced skin injury was determined from representative biopsies. 6 d after intradermal injection of doxorubicin, microscopic examination of an incisional biopsy of the lesion showed focal epidermal necrosis with early ulceration (Fig. 4 *A*). There was neutrophilic infiltration of the necrotic epidermis and underlying superficial dermis with moderate dermal vascular congestion. At 20 d, tissue necrosis was more extensive involving the full thickness of dermis, subcutaneous fat, and muscle (Fig. 4 *B*). There was partial reepithelialization of the ulceration with focal residual ulcer containing overlying crusting. Lymphocytic infiltration was in the superficial dermis and neutrophilic infiltration was in the dermis beneath the residual ulceration. The intradermal injection of DHM3 alone caused no macroscopic skin injury. Microscopic examination of incisional biopsies of DHM3 injection sites taken 9 and 24 d after intradermal injection showed normal appearing epidermis, dermis, and subdermal structures (Fig. 4 *D*).

At the termination of the experiment, 10 wk after doxorubicin injection, histopathological examination of biopsies from the doxorubicin injection sites treated with water or hydrocortisone showed scar tissue replacing the full dermal thickness (Fig. 4 *C*, *E*). In contrast, the doxorubicin injection site treated with DHM3 showed minimal deep dermal fibrosis with preservation of normal collagen pattern throughout most of the dermis (Fig. 4 *F*).

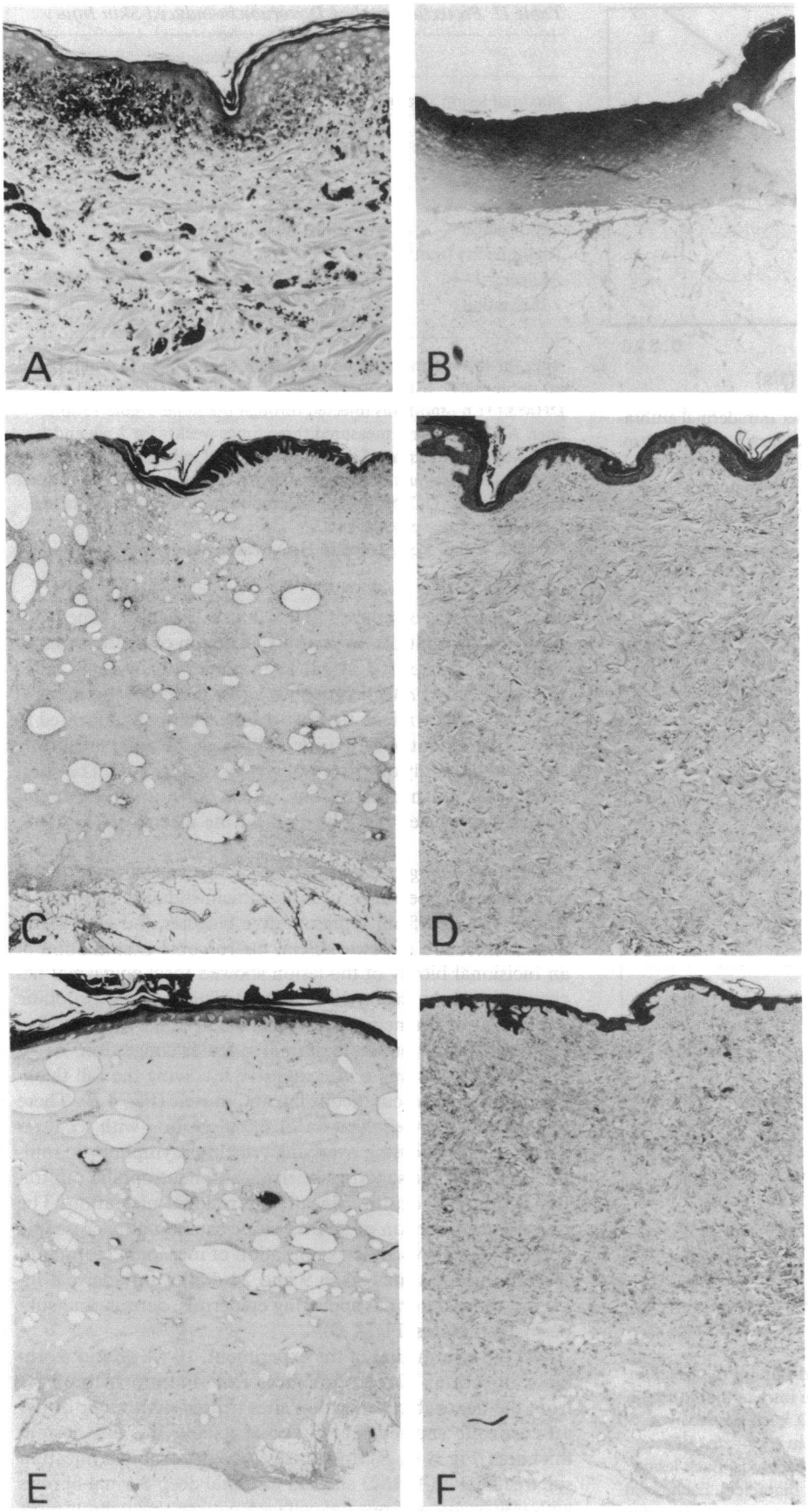


Figure 4. Hematoxylin-eosin stained microscopic sections from skin biopsies. (*A*) Doxorubicin injection site on day 6, showing necrosis of epidermis, neutrophils in necrotic epidermis and subjacent dermis, and congested dermal blood vessels (× 130). (*B*) Doxorubicin injection site on day 20, showing extensive necrosis of the full thickness of dermis and subcutaneous fat with reepithelialization of most of the ulcer (× 11.5). (*C*) Doxorubicin injection site (treated with H_2O) on day 71, showing extensive dermal fibrosis and fatty infiltration (× 11.5). (*D*) DHM3 injection site on day 24, showing normal epidermis and dermis (× 54). (*E*) Doxorubicin injection site (treated with hydrocortisone) on day 71, showing extensive dermal fibrosis and fatty infiltration (× 11.5). (*F*) Doxorubicin injection site (treated with DHM3) on day 71, showing minimal fibrosis in the deep dermis (× 38).

Discussion

Our study demonstrates the utility of the swine model for examining chemotherapy-induced skin injury wherein early and late histopathological features of doxorubicin skin injury in the pig are similar to those described from human skin biopsies (7, 29). It follows that the results of various pharmacologic interventions designed to reduce or protect against antitumor drug-induced skin injury in the swine model would reliably predict the effects of these interventions in humans.

In a well-defined in vitro chemical system the oxomorpholinyl radical dimers, TM3 and DHM3, were potent reducing agents of the anthracycline antibiotic, daunorubicin, resulting in the production of the pharmacologically inactive 7-deoxydaunorubicinone. This finding predicted that these radical dimers could be used in vivo to inactivate the parent anthracyclines and thereby ameliorate acute toxicity. As predicted from this in vitro chemical reaction, where a 10-fold molar excess of TM3 reductively inactivated anthracyclines to deoxyaglycone metabolites, a 10-fold excess of DHM3 was an effective antidote for anthracycline-induced skin necrosis in vivo. Whereas the skin was not analyzed for doxorubicin and its metabolites, previous studies from this laboratory have shown that DHM3 caused a decrease in parent doxorubicin and an increase in 7-deoxydoxorubicinone concentrations in vivo (21). The observation of mitomycin C reactivity with TM3 led to the postulate that these compounds would also interact in vivo and our results confirmed this prediction. In contrast, there is no chemical basis for chemical reactivity of the oxomorpholinyl dimers with the vinca alkaloids nor with the substituted anthracenediones; and, as predicted, there was no DHM3 protection of vinblastine nor mitoxantrone-induced skin necrosis.

The current recommendation for the use of glucocorticoids to treat anthracycline-induced skin necrosis is based on one clinical study (3) and conflicting data from several animal studies (8, 11–14). However, there is no mechanistic rationale for the corticosteroids to be an anthracycline antidote. Even though we observed moderate polymorphonuclear infiltration of the dermis 6 d following the injection of doxorubicin, it is likely that this represents an acute response reaction rather than a cause of tissue injury. By directly comparing the effect of hydrocortisone to controls, we were unable to show any protective activity of this glucocorticoid against anthracycline-induced skin necrosis.

Our data provide additional insight into the mechanism of anthracycline-induced tissue injury. While the antracyclines have been shown to intercalate DNA base pairs, and to inhibit DNA and RNA synthesis (15); there are anthracycline analogs that appear to act by different mechanisms (30). In addition, the anthracycline antibiotics are also capable of undergoing enzymatic reduction to form free radical species which may then cause damage to cellular macromolecules (18, 31–33). At the present time, there is no evidence that either DNA intercalation or free radical generation is directly responsible for the tissue injury resulting from anthracycline extravasation.

Since the anthracyclines and mitomycin C are capable of undergoing bioreductive activation (15, 34), the formation and reaction of quinone methide intermediates has been proposed as an important process in drug mechanism (35). The measured rate constant (k_2) for the tautomerization of the quinone methide intermediate formed from the reduction of each anthracycline analogue is a measure of the nucleophilicity of the respective quinone methide intermediate. The rate constants (k_2) measured in this study established an order of quinone methide nucleophilicity and show a strong correlation with the total toxicity observed in the pig skin necrosis model (Fig. 2). This correlation is best shown in the case of 5-iminodaunorubicin, a compound not easily reduced to produce oxygen free radicals (33), but one that forms the most reactive quinone methide intermediate and was also found to be the most toxic analogue in skin. In contrast, the least reactive quinone methides result from reduction of menogaril and aclarubicin, and these compounds were the least toxic compounds in skin. All of which suggests that the bioreductively formed quinone methide nucleophilic reactivity with biological macromolecules is the basis of the anthracycline cellular damage.

Correlation of skin toxicity with anthracycline quinone methide reactivity must be rationalized with the antidotal activity of DHM3 that forces rapid formation of the quinone methide. The antidotal activity of DHM3 most likely results from reductive glycosidic cleavage in a compartment (such as the extracellular space) where the quinone methide is presumably innocuous, being formed before association of the anthracycline with a critical intracellular macromolecule. Formation of the quinone methide at these locations would simply yield the pharmacologically inactive 7-deoxyaglycone anthracycline (21). Whether the nucleophilicity of the quinone methide intermediate within the intracellular compartment is responsible for other toxicity or antitumor action of the anthracycline antibiotics is the subject of current investigation.

Acknowledgments

We thank Dr. Donna Mathews and Dr. James Harwell of the Veterinary Resources Branch, Division of Research Services, NIH, for excellent veterinary support services and we thank Dr. Anthony D. Barone for investigation of TM3 reactivity with mitomycin C. We also thank Raymond Bork for technical assistance, Dr. Jerry Collins for statistical advice, Dr. Angelo Russo for review of the manuscript, and Beverly Sisco for preparation of this manuscript.

This work was supported in part by the National Cancer Institute, grant CA-24665.

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