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Amelioration of doxorubicin-induced skin necrosis in mice by butylated hydroxytoluene

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Summary. *The effect of butylated hydroxytoluene (BHT) on doxorubicin (Adriamycin)-induced skin ulcers was investigated in mice. The skin lesions produced by a single intradermal (ID) injection of doxorubicin (0.05 mg; 1 mg/ml) reached maximum size between 5 and 10 days after injection of ADR. Different concentrations of BHT were administered by different routes and at different times in relation to the injection of doxorubicin. The most effective dose of BHT was 4 mg/animal. The topical application of BHT immediately following doxorubicin injection reduced the area of the ulcer by 57%; the immediate ID injection of BHT reduced the size of the ulcer by 84%. Additional studies are required to determine whether BHT will be a clinically useful modifier of the toxicity associated with doxorubicin extravasation in cancer patients.*

Introduction

Although doxorubicin (Adriamycin, ADR) is a widely used anticancer drug having a broad spectrum of anticancer activity [5], the drug has several clinical toxicities [4]. The typical dose-limiting toxicities are myelosuppression (acutely) and cardiotoxicity (chronically). However, extravasation of doxorubicin into adjacent soft tissues frequently occurs during IV infusion of the drug to cancer patients [2, 19]. Although the local effects of doxorubicin are not life-threatening, they can be severe [22]. The extravasation can result in progressive tissue necrosis with ultimate formation of ulcers which may take several months to heal [18], or may require surgical debridement and skin grafts [6].

Although the exact cause of doxorubicin-induced ulcers is unknown, several studies have indicated that it is possible to decrease the size of the ulcer by such chemicals as DMSO [12, 24], β-adrenergic compounds [13], and α-tocopherol [24]. Several free radical scavengers and membrane-perturbing agents have been shown to decrease the amount of doxorubicin-induced skin necrosis [12, 24], and we have previously shown that pretreatment of animals with BHT reduced the acute and chronic toxicity of doxorubicin (the decreased mortality was paralleled by the prevention of the formation of lipid peroxides) [11]. Butylated hydroxytoluene is a widely used antioxidant in the food and petroleum industries and presumably functions as a scavenger of free radicals to prevent the autoxidation of unsaturated lipids [1, 17]. Numerous groups of investigators have examined the role of BHT in modifying the toxicity and carcinogenesis of a variety of exogenous chemicals [1, 8, 25] and radiation [8], as well as interference with the replication of viruses [7]. In addition, BHT has been reported to protect against photosensitization reactions [20] and to alter membrane fluidity [9]. Since BHT possesses many of the chemical and biological characteristics associated with other agents which have been demonstrated to protect against doxorubicin-induced skin ulcers, we initiated a study to determine the effect of BHT on the ulcerogenic potential of doxorubicin. In this paper we describe the effect of topical and ID administration of BHT on skin ulcers induced by doxorubicin, and demonstrate that BHT significantly decreased the toxicity of doxorubicin in the mouse skin model.

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Materials and methods

The effect of BHT on the ulcerogenic activity of doxorubicin was investigated in mice, which have been shown to provide a quantitative, dose-dependent model system [14]. Groups of five male Swiss mice (Southern Animal Farms) each weighing 25 g underwent removal of dorsal hair (3 × 3 cm) 24 h before injection of doxorubicin. The hair was removed with two applications of the topical depilatory agent Neet lotion. The animals received an ID injection of 0.05 mg doxorubicin (1 mg/ml in 0.9% sodium chloride solution). The BHT was dissolved at a concentration calculated to ensure that an injection of 0.05 ml would result in the desired amount of BHT. The BHT was dissolved in purified olive oil and was injected ID or applied topically at a concentration of 1, 2, 4, 8, or 16 mg/mouse immediately after the injection of doxorubicin. In addition, the effects of the BHT solvent vehicle, olive oil, were also tested. All doxorubicin solutions were applied by tunneling a 25-gauge needle ID for several mm to create a loculate ID bleb, after which the 0.05 ml soluiton was delivered to the center of the hair-free dorsal skin area. Following demonstration of the maximum protective effect of BHT at a dose of 4 mg/animal, the effect of route of administration of BHT and the time of administration of BHT in relation to the doxorubicin injection were studied.

The area of the injection site was inspected periodically, and the widest diameters along the perpendicular and parallel axes to the long axis of the animal were measured with a micrometer. Three lesion parameters: ulcer, erythema, and induration were assessed. The following toxicity parameters were evaluated [14]: (a) total area of ulceration expressed as the area under a toxicity-time curve [AUC]; (b) peak area of ulceration; and (c) duration of ulcer. Statistical significance

was assessed at the 0.05 level according to Student's *t*-test. The results of the treatment with BHT were compared to those in which the mice received olive oil after the injection of doxorubicin.

The doxorubicin was a gift of Adria Laboratories and BHT was purchased from Eastman Kodak Chemical Co. Other chemicals were obtained from Sigma Chemical Co.

Results

The ID injection of doxorubicin (0.05 ml; 1 mg/ml) produced necrotic skin ulcers on the backs of the mice. The skin lesions reached a maximum size of ulceration approximately 5 days after the ID administration of doxorubicin and healed slowly over a period of 3 weeks. The appearance and kinetics of the

Table 1. Effect of butylated hydroxytoluene on doxorubicin-induced skin necrosis

Dose of BHT[a] (mg/animal)	Time of administration of antidote	Total ulceration AUC ($cm^2 \times$ day)	Maximum ulceration area (cm^2)[c]	Duration of ulcer (days)
0 (saline; ID)	Immediately after ADR	1.015	0.081	21.0
0 (olive oil; ID)	Immediately after ADR	1.105	0.078	21.4
1 (ID)	Immediately after ADR	0.945	0.084	19.5
2 (ID)	Immediately after ADR	0.428	0.043	21.8
4 (topical)	Immediately after ADR	0.370	0.035	19.8
	30 min before ADR	0.722	0.057	19.2
	30 min after ADR	0.684	0.052	20.4
4 (ID)	Immediately after ADR	0.152[b]	0.021[b]	10.0
	30 min before ADR	0.310	0.032	16.2
	30 min after ADR	0.282[b]	0.026[b]	14.4
4 (SC)	Immediately after ADR	0.85	0.071	19.8
4 (IG)	Immediately after ADR	0.92	0.078	21.4
8 (ID)	Immediately after ADR	0.132[b]	0.020[b]	9.6
16 (ID)	Immediately after ADR	0.168[b]	0.022[b]	13.2

[a] ID, intradermally; SC, subcutaneous; IG, intragastric
[b] Denotes $P < 0.05$ difference (treatment over control)
[c] The maximum area of the ulcers was reached on day 5

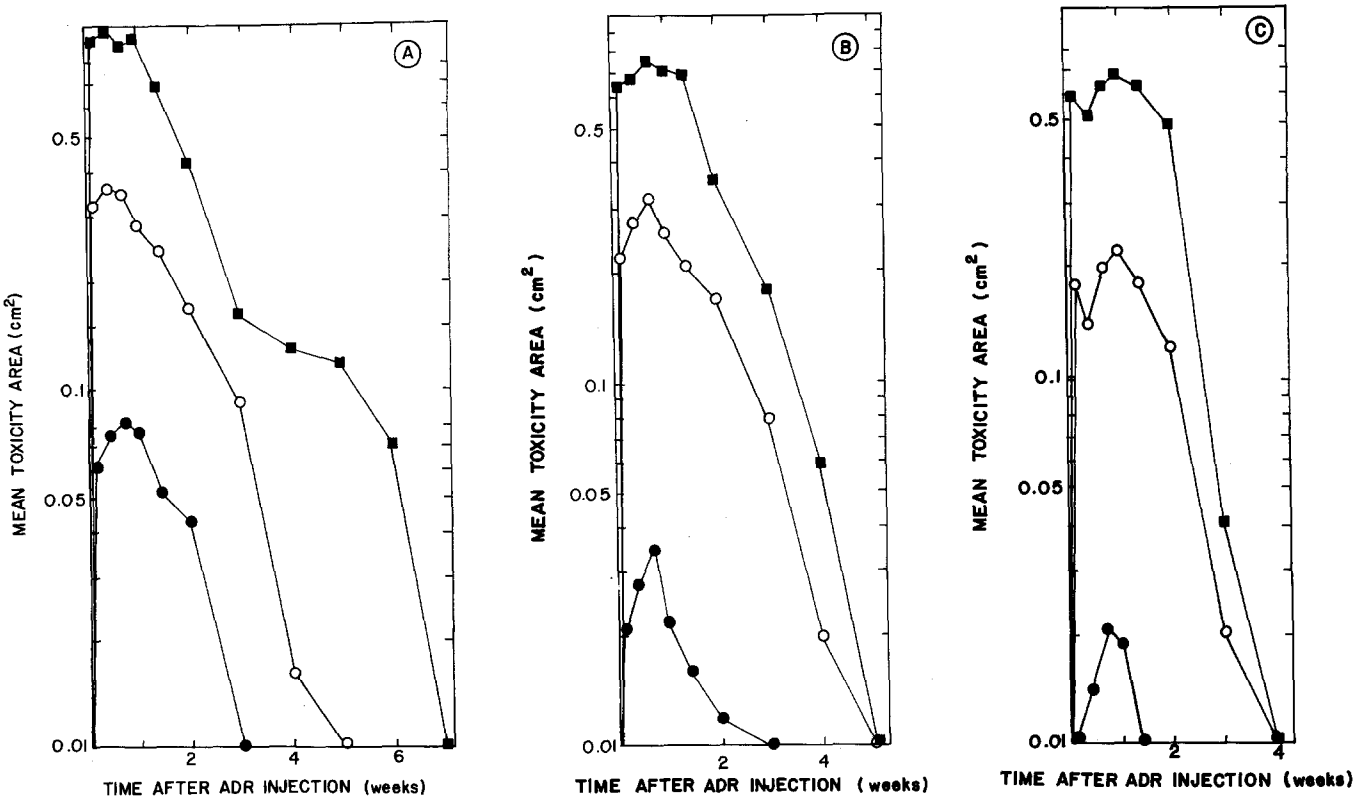

Fig. 1. Effect of butylated hydroxytoluene on doxorubicin-induced skin toxicity. The parameters investigated were ulceration (●), erythema (○), and induration (■). **A** adriamycin alone, **B** adriamycin plus topically applied BHT, **C** adriamycin plus ID-injected BHT. The values represent the average values obtained from five animals. The standard deviation was less than ± 15%

skin lesions were similar to those described previously [14]. However, in the present study the total area of the skin lesions was greater than that reported previously [13, 14].

The effect of various concentrations of ID-administered BHT on doxorubicin-induced skin necrosis is summarized in Table 1. A BHT dose of 4 mg/animal was found to provide the optimum protection against the development of skin lesions. The kinetics of ulcer formation was also determined (Fig. 1). The administration of doxorubicin produced an ulcer with a mean area of approximately 0.08 cm^2 on its peak toxicity day (day 5) (Fig. 1A). Pretreatment with either topically applied (Fig. 1B) or ID injected (Fig. 1C) BHT was effective in reducing the size of the necrotic lesion. A single topical application of BHT reduced the peak ulcer area from 0.08 to 0.035 cm^2, and a single ID injection of BHT reduced the peak ulcer area to 0.0125 cm^2. Topical application or ID injection of olive oil had essentially no effect on the mean ulcer diameter at any time during the study.

Discussion

Doxorubicin has been shown to have numerous biological activities, and the exact relationships of these activities to either its therapeutic or toxic effects are, at present, unknown. The extravasation of doxorubicin in the clinical setting typically involves SC delivery of the drug, and can be treated with compresses, sodium bicarbonate, steroids or surgery [2, 3, 6, 18, 22]. However, these clinical treatment modalities were frequently initiated without the benefit of quantitative experimental information. In an effort to overcome this limitation several attempts have been made to develop animal models which provide a quantitative measure of doxorubicin toxicity. Although animal models have been developed which provide quantitation of doxorubicin toxicity [14], the doxorubicin must be administered ID at higher drug concentrations than typically seen in the clinical situation, and the possible limitations of these models have been discussed [14]. Although animal studies have suggested a lack of protection by compresses, sodium bicarbonate, and steroids [3, 10, 14, 21], a protective role has been suggested for some free radical scavenging agents and β-adrenergic compounds. A single ID injection of BHT (4 mg/animal) immediately after the injection of doxorubicin (0.05 mg/animal) significantly reduced the skin toxicity associated with doxorubicin. There appeared to be no additional advantage to increasing the dose of BHT four-fold. In addition, the systemic administration of BHT (intragastrically; 40 mg/kg) was not effective in significantly decreasing the skin toxicity associated with doxorubicin.

The free radical scavengers α-tocopherol and DMSO have been shown to reduce the size of skin ulcers in rats [12, 24]. However, other studies have suggested no protective role for α-tocopherol and *N*-acetylcysteine [13]. The results reported here, using the free radical scavenging agent BHT, further support the possible involvement of free radical-mediated processes in doxorubicin-induced skin necrosis.

Although BHT is a widely used free radical scavenger in the food and petroleum industry [1], its antioxidant properties may be independent of its antiulcerogenic properties. BHT has numerous biological activities [1], and several of these may operate simultaneously to decrease the skin toxicity associated with doxorubicin. For example, BHT is known to bind to and alter the activity of microsomal P450 enzyme systems [23], and these enzyme systems have been demonstrated to reduce doxorubicin to the semiquinone free radical, which may undergo redox cycling to generate reactive oxygen intermediates [15]. In additon, the chromophoric nature of doxorubicin suggests the possibility of photosensitization-type reactions [16], and BHT has been demonstrated to protect against certain photosensitization reactions [20]. The BHT-induced alterations in membrane fluidity [9] may play an important role in modifying the biological effects of doxorubicin. It is possible that BHT may enhance the absorption of doxorubicin and result in a decreased concentration at the site of injection.

The results suggest the need for additional animal studies with BHT to allow a fuller evaluation of its potential clinical usefulness. Indeed, BHT may have several advantages over other modifiers of ADR skin toxicity. These include: (a) BHT is a relatively nontoxic compound [1]; (b) BHT has been shown to protect against acute and chronic ADR-induced cardiotoxicity [11]; (c) BHT does not decrease the antitumor effectiveness of ADR [11]; (d) BHT has anticarcinogeneic properties [25] and may be useful in decreasing the tumorigenic potential of ADR.

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