Modulation of Experimental Doxorubicin Skin Toxicity by β-Adrenergic Compounds

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ABSTRACT

Doxorubicin [Adriamycin (ADM), a potent intercalating antineoplastic agent, occasionally causes severe local skin toxicity if extravasated during administration. Previous experiments using intradermal (i.d.) ADM in BALB/c mice have shown limited antidotal activity for local i.d. corticosteroids in preventing ADM-induced ulceration and no effect for a number of other compounds except β-adrenergic agonists and antagonists. Three sequences of i.d. administration of ADM were evaluated in this study: a single dose immediately after 0.05 or 0.5 mg ADM; 8 daily doses of isoproterenol (ISO) or 0.9% NaCl solution, 0.05 ml after ADM; and 5 days of pre-ADM to ostensibly "up" or "down"-regulate β-receptor number (with propranolol and ISO, respectively). The results demonstrate consistent antidotal activity for ISO and propranolol as single antidotal injections. Terbutaline, a β2-specific agonist, was not effective as an antidote. Continuous daily ISO did not improve results, whereas continuous i.d. NaCl solution significantly increased skin lesion size and duration. ISO pretreatment significantly decreased subsequent ADM-induced ulceration, while propranolol pretreatment was not different from control. The results confirm a role for β-adrenergics in the management of experimental ADM skin ulceration and suggest that local toxicity is mediated through specific β-receptors (possibly β1) in the mouse skin.

INTRODUCTION

Doxorubicin (ADM)3 is an important anticancer drug (2). It possesses both broad-spectrum anticancer activity and protein clinical toxicities (1). While the typical dose-limiting toxicities are, acutely, myelosuppression and, chronically, cardiomyopathy (1, 2), ADM-induced skin ulceration comprises an infrequent but very severe complication of inadvertent extravasation of the drug (24, 25). Several clinical reports have described in detail the indolent course of severe ADM-induced skin lesions. Medical management of extravasations is empirical, and large lesions must be widely excised to prevent further local expansion and involvement of important deep tissues such as nerves and tendons underlying extravasated areas (3).

Several experimental rodent models have been developed to describe the pathogenesis of ulceration (26), inflammation (27), and the effects of local pharmacological antidotes. Local corticosteroids, which are empirically recommended clinical antidotes, proved to be of limited (7) or no benefit in reducing ADM-induced ulceration (5) or inflammation (27). A number of other compounds used at clinically achievable dose levels have also been proven to be ineffective in reducing experimental ADM-induced ulceration and inflammation. The list of ineffective compounds includes the following: local anesthetics and hyaluronidase (5, 6); antihistamines, including H1 and H2 blockers (6, 27); p-chlorophenylamine, an antiserotonin drug; the nonsteroidal antiinflammatory drugs, aspirin, ibuprofen, and indomethacin (27); heparin; sodium bicarbonate (to locally deactivate ADM); the oil-soluble antioxidant, α-tocopherol (vitamin E); and N-acetylcysteine (Mucomyst), a sulfhydryl donor and free radical scavenger (6).

We chose to study β-adrenergic compounds as potential antidotes to ADM-induced ulceration because Bristow et al. (4) had described complete prevention of ADM-induced cardiomyopathy in rabbits given the combination of high-dose H1 and H2 antihistamines and α- and β-adrenergic blockers and since β blockade with phenolamine was ineffective in one series (5). In a previous report, we described a significant reduction in experimental ADM-induced skin ulceration in mice given either the β-agonist isoproterenol or the β-antagonist propranolol (6). This report describes in greater detail the modulation of i.d. ADM skin ulceration in mice by different β-adrenergic compounds in a variety of dosing sequences.

MATERIALS AND METHODS

The methods for this study have been described previously (8). The procedure involves the total removal of hair from approximately a 3-sq cm area on the dorsum of adult female BALB/c mice (The Jackson Laboratory, Bar Harbor, Maine). Animals are divided into treatment groups of 4 and 5 each in separate cages identified by number only until study termination. Animals receive a standard solid laboratory feed. One day after dorsal hair removal (using Neet lotion, topical depilatory agent), animals are given i.d. injections of either 0.05 or 0.5 mg ADM (doxorubicin-HCl; Adria Laboratories, Wilmington, Del.) each diluted to 0.05 ml in 0.9% NaCl solution (preservative-free). These ADM doses approximate 7.1 to 71 mg/sq m (12).

Antidotes for this study included isoproterenol HCl (Isuprel injection 0.5%; Winthrop Laboratories, New York, N. Y.), propranolol HCl (Inderal injection; Ayerst Laboratories, New York, N. Y.), terbutaline sulfate (Brethine sterile aqueous solution; Geigy Pharmaceuticals Corp., Ardsley, N. Y.), and 0.9% NaCl.

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3 The abbreviations used are: ADM, Adriamycin; AUC, calculated area of ulceration; ISO, single dose of isoproterenol; C-ISO, 7 daily injections of isoproterenol; C-NaCl, 7 daily injections of 0.9% NaCl solution; P-ISO, 5 days of isoproterenol pretreatment; P-PRO, 5 days of propranolol pretreatment; PRO, single dose of propranolol; TERB, single dose of terbutaline; LDHC, single dose of low-dose hydrocortisone.

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solution (0.9% sodium chloride injection; Travenol Laboratories, Deerfield, Ill.). Control injections (i.d.) were performed in separate animals for each antidote tested. Antidote injections were diluted to 0.05 ml final volume in NaCl solution. All injections involved tunneling a 25-gauge needle i.d. for several mm to create a loculated i.d. bleb as 0.05 ml was delivered into the center of the hair-free dorsal skin area.

The area of injection was inspected daily, and the widest perpendicular diameters of lesions were measured by micrometer (Mitutoyo, Japan). Three lesion parameters, induration, erythema, and ulceration, were assessed. Only ulceration was used in the statistical data analysis. For each animal, analysis included calculation of the total area under the lesion size × time plot (AUC in sq cm days), the peak lesion size (sq cm), and the cumulative time to (a) peak lesion size, (b) death, or (c) complete healing (all in days). All treatment groups were followed until there was complete resolution of all toxicities.

The treatment design included 3 sequences of antidote administration. The first sequence followed our earlier method of i.d. ADM, 0.5 or 0.05 mg in 0.05 ml followed immediately by a single 0.05-ml i.d. antidote injection into the ADM bleb area from a different angle. Care was taken not to inject directly into the ADM bleb. The second sequence involved 7 daily i.d. injections following the i.d. ADM in an attempt to improve protection afforded by ISO as a single injection. The dose for this series was 2.0 µg isoproterenol (C-ISO) in 0.05 ml. A separate group of animals received 7 daily i.d. injections of NaCl solution, 0.05 ml (C-NaCl), as a control procedure. The final sequence of antidote administration evaluated 5 days of i.d. treatment given prior to the i.d. ADM challenge on Day 6. The doses used for this pretreatment study were 2.0 µg isoproterenol (P-ISO) and 10 µg propranolol (P-PRO). No treatment was given on the day of ADM injection or thereafter.

Statistical analysis was performed by computer. Independent t test procedures were performed to compare the following: (a) single-dose isoproterenol (ISO), propranolol (PRO), terbutaline (TERB), and low-dose hydrocortisone (LDHC) to the NaCl solution-treated controls (NaCl); (b) C-NaCl to C-ISO; and (c) P-ISO to P-PRO for each ADM dose (0.05 or 0.5 mg). A separate statistical analysis using analysis of variance was performed on the following groups of treatments and controls for each ADM dose (0.5 or 0.05 mg) as follows: (a) NaCl, ISO, TERB, PRO, P-ISO, P-PRO, and LDHC; (b) PRO, ISO, TERB, P-ISO, P-PRO, C-ISO, and C-NaCl. Following analysis of variance procedure, a multiple range test, the Student Newman-Keuls procedure, was performed (at the 0.05 level). This statistical procedure segregates different treatment groups into homogeneous subsets where the means of the first and last groups differ by less than a critical value (for significance) for a subset of that size. Thus, treatments significantly different from one another are found as unique members of separate subsets.

RESULTS

Charts 1 and 2 display the mean total ulceration results (AUC) for animals given 0.5 and 0.05 mg of i.d. ADM, respectively. Statistical analysis by t test of the 3 ulceration toxicity parameters (Table 1) reveals several significant differences (p < 0.05) between NaCl solution (control) animals (NaCl, C-NaCl) and the various adrenergic-type antidotes. In animals receiving 0.5 mg i.d. ADM, the most effective local antidote given as a single dose was ISO (10 µg) which significantly reduced both the peak lesion size and ulceration (AUC). The continuous administration of isoproterenol for 7 days (C-ISO) was not significantly better than when given as a single post-ADM injection. However, the continuous (7 day) i.d. administration of 0.05 ml NaCl to the lesion area significantly increased local ADM-induced ulceration compared to C-ISO. Pretreatment of 0.5-mg ADM injection areas with propranolol (P-PRO) also significantly increased the peak lesion areas as compared to the results with isoproterenol pretreatment. Statistical analysis at this higher ADM dose level using the nonparametric Student Newman-Keuls multiple-range procedure (Table 1) also showed the peak lesion and total toxicity AUC’s for P-PRO to be significantly greater than that resulting from all other β-adrenergic treatments. There were no differences in the time to the peak lesion area for any of the treatments at this ADM dose level.

With a 10-fold i.d. ADM dose reduction to 0.05 mg, propran-
olol was the most effective single-dose treatment (no ulcerative toxicity produced) (Table 1). Isoproterenol was not effective to a significant level at this ADM dose level (Chart 3). Hydrocortisone sodium succinate, which was ineffective against the larger ADM dose, decreased ulceration and peak lesion size associated with the 0.05-mg ADM dose.

As with the higher dose of ADM, the continuous 7-day i.d. NaCl solution treatment (C-NaCl) was significantly more toxic than either continuous isoproterenol (C-ISO) or the single NaCl treatment (Table 1; Chart 4). Propranolol pretreatment (P-PRO) was significantly more toxic than pretreatment for 5 days with isoproterenol (i.e., no toxicity noted for P-ISO) in terms of both total toxicity and peak toxicity (Chart 5). Terbutaline sulfate which is thought to be a β₂-selective agonist (23) was ineffective against the lower ADM dose (Chart 3). With the higher ADM dose, 3 of 5 terbutaline-treated animals experienced early treatment deaths. All had large lesions at the time of death but were not necropsied.

Several of the antidotes resulted in significant time delays to the onset of a peak lesion following 0.05-mg ADM (Table 1). Most notable was the prolonged delay to the onset of a maximal lesion associated with C-NaCl (12.8 days) as compared to C-ISO (2.8 days).

**DISCUSSION**

We have described previously a mouse model for i.d. ADM-induced skin toxicity in which an ADM dose-dependent relationship is operant for skin lesion size and time to healing (8). Subsequent reports by our group and others have described the limited protection from ADM-induced skin ulceration af-
forded by low-dose, local corticosteroid injection (which at higher doses were toxic locally) (7) and a lack of efficacy for a variety of experimental pharmacological antidotes (5, 6, 27). The results of the present studies would seem to confirm the efficacy of local β-adrenergic interventions in reducing ulceration following i.d. ADM.

Because the pharmacologically opposite β-agonist (ISO, 10 μg) and a β-antagonist (PRO, 100 μg) were effective as antidotes (8), 2 different adrenergic administration sequences were devised. The first involved a daily i.d. antidote injection, C-ISO (2.0 μg) immediately after the ADM for 7 consecutive days. A control group received an equivalent sequence of 0.9% NaCl solution injections. The results showed no significant improvement for the repeated injection schedules over the single isoproterenol injection. In fact, the continued daily injection of NaCl solution as a local ADM diluent (C-NaCl) markedly increased the local skin toxicity resulting from both i.d. ADM dose levels. While others have shown that the dilution of the ADM infusion solution can reduce experimentally induced ADM skin ulceration (5, 26) local NaCl solution dilution in this series appeared to be detrimental.

Somberg et al. (28) have described a significant propranolol-induced reduction in the myocardial uptake of doxorubicin in isolated perfused cat hearts. This occurred in the absence of a change in myocardial blood flow and without decreasing contractility. β-Adrenergic receptors have been identified in mammalian epidermis and appear to involve an isoproterenol-sensitive propranolol-blocked adenyl cyclase system (11, 30). We have studied this putative β-receptor-ADM interaction by pretreating the mouse skin with isopropenol (P-ISO) to decrease or "down-regulate" β-receptors and with propranolol (P-PRO) to increase or "up-regulate" β-receptors. Down-regulation by continuous β-stimulation has been demonstrated following chronic isoproterenol administration in radioligand-binding studies of rat pineal gland (17) and frog erythrocytes (21) and, clinically, in mononuclear leukocytes (29), in polymorphonuclear leukocytes (13), and in decreased bronchodilation following chronic catecholamine stimulation in asthmatics (16, 22). Long-term terbutaline, which was ineffective in our system, did not induce β-adrenergic receptor resistance in one clinical study (18). Conversely, continuous β-blockade-producing chemical denervation has been shown in an experimental chronic propranolol rat ventricle study (14).

The results of the P-ISO and P-PRO treatments seen in this study are important in this regard. The P-ISO treatment completely prevented skin ulceration resulting from 0.05 mg i.d. ADM, a result markedly better than pretreatment with propranolol (P-PRO). However, chronic propranolol pretreatment did not increase toxicity significantly when compared to 0.9% NaCl solution-treated controls.

These results show that β-adrenergic drugs can modulate experimental ADM-induced skin ulceration. The effect does not appear to be β-specific because of the relative ineffectiveness of a single β-specific agonist, terbutaline. Also, while both β-receptor subtypes appear to coexist in most tissues studied to date (19), the β2 receptor has been the predominant type found in mammalian epidermis (10) as well as the smooth muscle of the lung and bronchi. On the other hand, the β1-receptor is more common in adipose tissue and, in the heart, a site of major ADM toxicity. In regard to the heart, Herman et al. (15) found that racemic (DL) propranolol was the most effective of several antiarrhythmic drugs in suppressing arrhythmias induced in hamsters by a related anthracycline, daunomycin. However, classic β-receptor blockade in their model did not satisfactorily explain the antiarrhythmic activity, since specific receptor blockade using compounds without the membrane-depressant actions of dexpropranolol (and the racemate DL-propranolol) did not reduce daunomycin arrhythmias. Further experiments should be directed to specific β-receptor radioligand binding studies with skin and heart tissue using high-affinity antagonists such as [3H]dihydroxybenzympindolol or [3H]dihydroalprenolol (20) in the presence and absence of clinically achievable levels of ADM.

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