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Dose-dependent skin ulcers in mice treated with DNA binding antitumor antibiotics*

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Summary. The DNA-binding agents daunomycin (DAUNO), mithramycin (MITH), dactinomycin (ACT-D), amsacrine (mAMSA) and esorubicin (ESO) were tested for local vesicant potential in a quantitative intradermal mouse skin model. Only MITH, which adlineates but doses not intercalate DNA, did not produce dose-dependent skin ulcerations in the mouse. The anthracycline antibiotics DAUNO and ESO produced the largest skin ulcers when administered intradermally at clinically relevant doses (adjusted on the basis of comparable body surface areas). Numerous local pharmacologic adjuvants were tested for activity to decrease skin ulceration patterns in mice given one of the DNA intercalators. Inactive local adjuvants included heat, cold, saline, hyaluronidase, glucorticosteroids and isoproternol. Only one adjuvant, topical dimethylsulfoxide (DMSO), was found to reduce DAUNO skin lesions. A single topical DMSO application significantly decreased ulceration size to almost half of control levels. However, it was ineffective for the other intercalating agents. These results show that the DNA intercalators DAUNO, ESO and ACT-D are potent vesicants in a mammalian skin model. These vesicant agents must be administered cautiously to prevent extravasation. No single local adjuvant treatment can be recommended for extravasation of these drugs in the clinic. One significant exception is DAUNO, where topical DMSO may reduce clinical toxicities.

Introduction

Local toxicities caused by extravasation of vesicant cancer chemotherapeutic agents can produce severe soft tissue damage [16, 29]. Many of these vesicant compounds are classified as DNA-binding and/or -intercalating agents. These drugs include daunorubicin (DAUNO), dactinomycin (ACT-D), amsacrine (mAMSA), esorubicin or 4'-deoxydoxorubicin (ESO) and plicamicin (mithramycin, MITH). Some related agents have been reported to cause severe necrosis if inadvertently extravasated. For example, doxorubicin (Adriamycin or DOX) is a well-known clinical vesicant [3, 28, 29] which is best managed locally using topical cooling [9]. DAUNO has also been reported to cause vesicant skin reactions if extravasted in both animals [2] and man [10, 14, 23]. There are no controlled studies available which document efficacy of local adjuvants to DAUNO extravasations. Similarly, mAMSA [15, 22], ACT-D [2, 11], ESO [21] and MITH are all reported to be clinical vesicants, but definitive case reports or controlled experimental documentation are lacking. Therefore, we have applied an established murine skin toxicity model to evaluate these agents (1) for their vesicant potential, (2) to establish a dose-response relationship for skin ulceration, and (3) to compare various pharmacologic and nonpharmacologic adjuvants as local treatments to ameliorate skin ulcers.

Materials

ACT-D (Cosmegen, Merck Sharp Dohme), DAUNO (Cerubidine, Ives Pharmaceutical), ESO (esorubicin, Farmitalia) and MITH (Mithracin, Miles Laboratories) were dissolved in 0.89% unpreserved sodium chloride immediately prior to injection. mAMSA (National Cancer Institute) was dissolved in *N,N*-dimethylacetamide for subsequent dilution into 0.0355 *M* L-Lactic acid diluent (supplied with the clinical formulation). All drug injections were performed at a final volume of 0.05 ml.

Methods

Female BALB/c mice (Jackson Laboratories, Boston Harbor, MA) weighing 20–25 g were used for these experiments. A standard intradermal (i.d.) testing protocol was used [16]. Groups of five mice were used for each treatment group. Animals were dehaired 24 h prior to injection with topical applications of the depilatory, NEET lotion (Whitehall Laboratories). Drug injections were made into a 3 × 3 cm hairfree site on the dorsum of the mouse. All injections were made i.d. using a 25-gauge needle (bevel up) and a 1.0-ml tuberculin syringe.

To test for a dose-response relationship with these agents, drug doses were selected to approximate human clinical dose ranges in mg/m^2 of body surface area [12]. Intradermal ACT-D doses were 3.0 μg, 5.0 μg, 7.5 μg, 15 μg and 30 μg/mouse (0.043–4.3 mg/m^2). DAUNO doses were 0.01 mg, 0.05 mg, 0.1 mg, 0.4 mg and 0.5 mg (1.4–70 mg/m^2). ESO doses were 0.02 mg, 0.2 mg and 0.5 mg/mouse (2.9–70 mg/m^2). mAMSA doses were 0.07 mg, 0.7 mg,

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Table 1. Effects of local adjuvants on areas under ulceration-time curves (cm^2 days) after intradermal injection of DNA-binding anticancer drugs (dose per mouse under drug name)

Local adjuvant	ACT-D (7.5 μg)	mAMSA[a] (1.2 mg)	DAUNO (0.4 mg)	ESO (0.2 mg)
None (control)	0.90 (1.3)	4.67 (1.15)	42.27 (19.26)	9.95 (1.76)
Adenosine[b]	NT	NT	NT	10.54 (6.06)
Asorbic acid[c]	1.54 (1.49)	NT	NT	NT
Sodium bicarbonate[d]	1.13 (1.31)	6.29 (3.31)	41.19 (9.62)	NT
Cold	2.61 (2.51)	9.14 (2.90)	53.37 (17.62)	13.80 (3.04)
DMSO[e]	0.45 (0.50)	2.19 (1.12)**	29.85 (6.99)	7.56 (2.91)
Heat	8.09 (7/48)*	18.45 (2.19)*	52.82 (4.80)	11.63 (4.80)
Heparin[f]	NT	11.24 (2.93)*	49.92 (11.71)	NT
Hyaluronidase[g]	2.39 (2.24)	4.43 (0.78)	36.72 (13.49)	8.96 (5.93)
Hydrocortisone[h]	1.83 (0.94)	8.80 (3.25)	31.12 (5.42)	9.35 (4.89)
Isoproterenol[i]	NT	NT	44.06 (6.78)	11.01 (8.49)
N-Acetylcysteine[j]	NT	12.65 (4.68)*	NT	NT
Propranolol[k]	NT	NT	30.39 (5.70)	NT
Sodium chloride	1.35 (1.41)	6.19 (1.71)	29.50 (6.03)	13.58 (2.40)
Sodium thiosulfate[l]	1.48 (1.79)	NT	NT	NT

All results expressed as means from five mice (SD in parentheses).
NT, not tested

* Increased ulceration over control group, $P < 0.05$ by Student-Newman-Keuls (SNK) multiple-range test following analysis of variance
** Reduced ulceration over control group, $P < 0.05$ by SNK multiple-range test following analysis of variance

[a] mAMSA diluent consisted of 1.5 ml anhydrous *N,N*-dimethylacetamide combined with 13.5 ml 0.0353 *M* L-lactic acid
[b] Syma Chemical Co., St. Louis, MO; 3.16 *M* solution in water, 42 mg/mouse
[c] Eli Lilly and Co., Indianapolis, IN; 50 mg/ml solution; 2.5 mg/mouse
[d] Sodium bicarbonate 8.4%, International Medication Systems, El Monte, CA; 0.05 MEq/mouse
[e] Bakers Reagent Grade, 99%, full strength, topically applied once to dorsum, approximately 0.3 ml/mouse
[f] Lipo-Hepin, Riker Laboratories St. Paul, MN (Riker Labs); 1000 units/ml
[g] Wydase, Wyeth Laboratories, Pittsburgh, PA; 10 units/mouse
[h] A-Cort, Abbott Laboratories, Chicago, IL; 2.5 mg/mouse
[i] Isuprel, Winthrop Laboratories, New York, NY; 10 μg/mouse
[j] Mucomyst, Mead Johnson Laboratories, Evansville, IN; 10 mg/mouse
[k] Inderal, Ayerst Laboratories, New York, NY; 100 μg/mouse
[l] From 10% U.S.P. Solution; Torrigian Laboratories, Queens Village, NY; 0.17 *M* solution in water, 2.0 mg/mouse

1.2 mg and 1.75 mg/mouse (10–250 mg/m^2). MITH was evaluated at doses of 0.05 cg, 0.1 cg, 0.5 cg, 1.0 cg and 5.0 cg/mouse (7.1–714 cg/m^2).

For local adjuvant studies, various pharmacologic and nonpharmacologic agents were tested against the intercalating agents (Table 1). Each adjuvant was given immediately after, and directly adjacent to, the DNA-binding drug. No adjuvants were injected directly into the vesicant drug site to prevent chemical admixture or loss of vesicant from the skin bleb. Thus, the vesicant and adjuvant solutions were separated by only a thin membrane of skin and muscle tissue to facilitate local diffusion of the two solutions. Topical heat and cold were applied as reported elsewhere [8] to achieve intradermal skin temperatures of 8°–10 °C for cooling, and 41°–43 °C for heating.

Ulceration was measured thrice weekly with calipers. The widest perpendicular widths were measured and converted to a simple area. The area under an ulceration-time curve (AUC ulceration) in cm^2 · days was integrated by a computer to yield AUC ulceration for each mouse. Statistical comparisons of AUC ulceration between groups involved an initial analysis of variance followed by a Student-Newman-Keuls (SNK) multiple-range test performed at a 0.05 level.

Results

As seen in Fig. 1, all agents except MITH produced skin ulcers and demonstrated a proportional dose-response effect. For ACT-D and ESO, the slope of the ulceration area-dose line was roughly linear over the range of doses tested. For ACT-D, ulceration areas did not plateau even at the highest dose tested (4.3 mg/m^2). Initial tests with the special mAMSA diluents showed that 0.05 ml *N,N*-dimethylacetamide produced no ulcerations in four of five mice, while 0.05 ml lactic acid produced minor ulcerations in two of five mice. For mice with diluent reactions, the cumulative ulceration toxicities were very small (AUCs < 0.03 cm^2 · days) and the lesions healed completely within 3 days. In contrast, mAMSA had a rather steep dose-response curve over a 2 log dose range. Additionally, there was evidence for a threshold mAMSA i.d. dose requirement of 0.7 mg/mouse (Fig. 1, top right panel). Similarly, the DAUNO dose-response curve demonstrated a sharp increase in slope with i.d. doses greater than 0.1 mg. However, all DAUNO doses greater than 0.5 mg/mouse (70 mg/m^2) caused death in four of five animals; 40%–60% lethality was produced at the 0.4 mg dose/mouse. MITH, at all doses tested, did not produce skin ulcers after i.d. injection in the mice. Figure 1 also shows

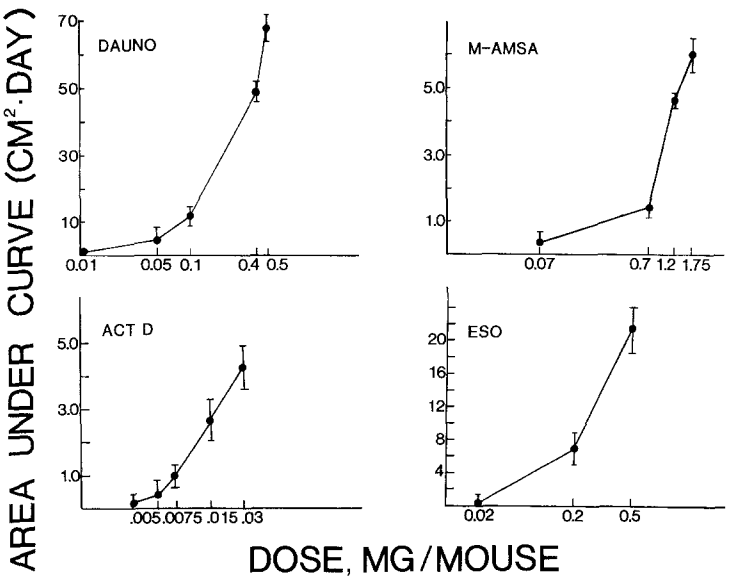

Fig. 1. The dose-response relationships are plotted for the area under the ulceration-time curve (AUC ulceration) over a range of doses of intradermal DNA-binding agents (logarithmic scale). Each point represents the mean AUC for five mice followed until all signs of skin toxicity are resolved. Drug doses are listed as the total amount injected on day 1 in each BALB/c mouse. *DAUNO*, daunomycin; *M-AMSA*, mithramycin (plicamicin); ACT D, dactinomycin; *ESO*, esorubicin (4'-deoxydoxorubicin). *Vertical bars* indicate the SD

that the anthracycline analogs DAUNO and ESO produced the largest murine skin lesions at clinically relevant doses (adjusted on a comparable body surface area basis).

Table 1 illustrates the summary results of local adjuvant studies with DNA-intercalating agents. For mAMSA, the local adjuvants heparin, topical heat and *N*-acetylcysteine (NAC) significantly enhanced ulceration ($P< 0.05$ by SNK multiple-range test). Other, ineffective adjuvants, such as normal saline hyaluronidase, hydrocortisone, sodium bicarbonate and topical cold, did not significantly alter ulceration levels after mAMSA.

DAUNO intervention studies showed that normal saline and sodium bicarbonate significantly enhanced ulceration ($P< 0.05$). Conversely, the adjuvants hyaluronidase, isoproterenol, propranolol, topical cold and topical heat did not change ulceration. Dimethylsulfoxide (DMSO) was shown to decrease DAUNO ulceration significantly. Thus, after a 0.4 mg dose of mAMSA, ulceration areas dropped from 49.1 cm^2 · days to 29.9 cm^2 · days ($P< 0.05$).

ESO was tested against normal saline, hyaluronidase isoproternol, hydrocortisone, adenosine, topical heat and topical cold. Again, these adjuvants did not significantly alter ESO ulceration patterns. ACT-D was evaluated with the local adjuvants normal saline, hyaluronidase, hydrocortisone, 0.17*M* sodium thiosulfate, ascorbic acid, cold and DMSO. Once more, these adjuvants demonstrated no significant changes from control, except that topical heat significantly increased ACT-D skin ulcerations ($P< 0.05$).

Discussion

We have shown in this study that each of the DNA-intercalating agents studied produce potent vesicant ulcers in mice. MITH, which adlineates but does not intercalate into DNA, did not produce consistent ulceration in our mouse model even at total i.d. doses approximating lethal human clinical doses. In contrast, good dose responses for DAUNO, ESO, ACT-D and mASMA were obtained in our mouse model.

For most of the DNA intercalating drugs, clinical reports have corroborated the fact that local reactions do occur following extravasation. These reactions typically include phlebitis, as well as rare are full-thickness skin necrosis if the drug is extravasated in large or concentrated amounts. For mAMSA, one clinical report has described frank local necrosis [12]. More typically, only moderate to severe local phlebitis is seen with this agent [17]. However, in this study, we have clearly shown that mAMSA can cause dose-dependent skin ulcers. This was also seen in a previous mAMSA toxicology study in guinea pigs and rabbits [2]. The current results more clearly document the dose-response skin toxicity pattern with mAMSA and establish an apparent threshold for ulceration at 0.7 mg/mouse or 100 mg/m^2 (Fig. 1). This very closely approximates the maximally-tolerated human antileukemic dose of mAMSA [17]. Unfortunately, none of the adjuvants tested significantly decreased mAMSA's ulcerative effects (Table 1).

There are several case reports of DAUNO extravasations in the literature [10, 14, 23]. In addition, literature from the drug manufacturer has advocated the use of hydrocortisone, ice packs and DMSO for managing DAUNO extravasations [1]. However, as shown in this study, hydrocortisone and cold did not change DAUNO ulceration patterns. In contrast, DMSO did significantly decrease ulceration. A similar antidotal role for DMSO with DOX is also reported in pigs and rats [5, 27, 30], although this was not confirmed in subsequent trials in mice [7, 30] and rats [31].

In a previous report on local venous reactions to ESO [21], one clinical extravasation was documented. A topical ice pack was empirically used and no lesion resulted. However, one should use caution in evaluating isolated clinical cases, since, as Larson has pointed out, only about one-third of documented vesicant drug extravasations will ever lead to ulceration, even without local treatment [20]. Nonetheless, because ESO is quite similar to the parent anthracycline doxorubicin, one may assume that it will cause a similar necrosis pattern. This was indeed seen in our mouse model. For ESO, none of the pharmacologic antidotes tested changed the ulceration patterns compared to control. This included topical cooling, which is extermely effective for managing DOX skin ulcers [9].

ACT-D has also been shown to cause both phlebitis

and irritation in clinical series [11, 25]. Like doxorubicin [24], ACT-D is also known to produce synergistic skin toxicity with ionizing radiation [4]. In our mouse model, we showed a dose-dependent relationship for ACT-D ulceration. Unfortunately, none of the pharmacologic adjuvants changed ACT-D ulceration patterns compared to untreated controls.

MITH was the only DNA-binding agent tested that did not produce consistent ulceration in our mouse model. In the past, several reviews have placed MITH in the vesicant drug category [6, 16] despite the lack of clinical reports of severe local necrosis upon extravasation [19]. MITH was previously found to be negative or only mildly irritating following subcutaneous injection in guinea pigs or intramuscular injection in rabbits [26]. MITH is also known to bind to DNA primarily by adlineation outside the DNA helix and not by intercalation between base pairs [13, 32]. This nonintercalative type of DNA association may also help to explain MITH's diveregence from the other intercalators in terms of local ulceration potential as seen in the current study.

In summary, we have shown that in a controlled mouse skin toxicity model, the DNA-intercalating agents DAUNO, ESO, mASMA and ACT-D each posses potent, dose-dependent vesicant potential. The nonintercalating antibiotic MITH did not produce such skin lesions. Pharmacologic adjuvants tested locally to reduce intercalator ulcers predominantly lacked antidotal activity. The sole exception was the case of DAUNO, for which a single topical DMSO application significantly decreased ulceration. Based on these results extreme caution should be used in administering each of these intercalating anticancer agents. The use of topical DMSO may be helpful in ameliorating skin toxicity from inadvertent clinical extravasations of DAUNO.

References

1. Anonymous (1981) How to prevent tissue damage should extravasation occur with cerubidine™. Ives Laboratories, New York
2. Barr RD, Benton SG, Belbeck SW (1981) Soft tissue necrosis induced by extravasated cancer chemotherapeutic agents. J Natl Cancer Inst 66(6): 1129–1133
3. Bowers DG, Lynch JB (1977) Adriamycin extravasation. Plastic Reconstruct Surg 61(1): 86–92
4. D'Angio GJ, Farber S, Maddock CL (1959) Potentiation of X-ray effects by actinomycin D. Radiology 73: 175–177
5. Desai MH, Teres D (1982) Prevention of doxorubicin-induced skin ulcers in the rat and pig with dimethyl sulfoxide (DMSO). Cancer Treat Rep 66: 1371–1374
6. Dorr RT (1981) Extravasation of vesicant antineoplastics: clinical and experimental findings. Ariz Med 38: 271–275
7. Dorr RT, Alberts DS (1983) Failure of DMSO and Vitamin E to prevent doxorubicin skin ulceration in the mouse. Cancer Treat Rep 67: 499–501
8. Dorr RT, Alberts DS, Chen H-SG (1980) Experimental model of doxorubicin extravasation in the mouse. J Pharmacol Meth 4: 237–250
9. Dorr RT, Alberts DS, Stone A (1985) Cold protection and heat enhancement of doxorubicin skin toxicity in the mouse. Cancer Treat Rep 69: 431–437
10. Dragon LH, Braine HG (1979) Necrosis of the hand after daunorubicin infusion distal to an arteriovenous fistula. Ann Intern Med 91: 58–59
11. Frei E (1974) The clinical use of actinomycin. Cancer Chemother Rep Pt. 1, 58: 49–54
12. Freireich EJ, Gehan EA, Rall DP, Schmidt LW, Skipper HE (1966) Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother Rep 50: 219–244
13. Gause GF (1975) In: Antibiotics III: Mechanisms of action of antimicrobial and antitumor agents (Corcoran JW and Hahn FE, eds), Springer, Berlin Heidelberg New York, pp 197–202
14. Greene W, Huffman D, Wiernik PH, Schimpff S, Benjamin R, Bachur N (1972) High-dose daunorubicin therapy for acute nonlymphocytic leukemia: correlation of response and toxicity with pharmacokinetics and intracellular daunorubicin reductase activity. Cancer 30: 1419–1427
15. Henry MC, Port CD, Levine BS (1980) Preclinical toxicologic evaluation of 4'(9-acridinylamino) methanesulfon-M-anisidide (AMSA) in mice, dogs, and monkeys. Cancer Treat Rep 64: 855–860
16. Ignoffo RJ, Friedman MA (1980) Therapy of local toxicities caused by extravasation of cancer chemotherapeutic drugs. Cancer Treat Rev 7: 17–27
17. Issell BF (1980) Amsacrine (AMSA). Cancer Treat Rev 7: 73–83
18. Jenkins J, Corden BJ (1983) Vesicant activity of chemotherapeutic agents. Cancer Treat Rep 67: 409
19. Kennedy BJ (1970) Metabolic and toxic effects of mithramycin during tumor therapy. Am J Med 49: 494–503
20. Larson DL (1982) Treatment of tissue extravasation by antitumor agents. Cancer 49: 1796–1799
21. Lee KM, Dorr RT, Robertone A (1987) High incidence of local venous reactions to esorubicin. Invest New Drugs 5: 43–47
22. Legha SW, Gutterman JU, Hall SW, Benjamin RS, Burgess MA, Valdivieso M, Bodey GP (1978) Phase I clinical investigation of 4'-(9-acridinylamino) methanesulfon-M-anisidide (NSC 249992), a new acridine derivative. Cancer Res 38: 3712–3716
23. Lippman M, Zager R, Henderson ES (1972) High-dose daunorubicin (NSC-83142) in the treatment of advanced acute myelogenous leukemia. Cancer Chemother Rep, Pt. 1, 56: 755–760
24. Mayer EG, Poulter CA, Aristizabel SA (1976) Complications of irradiation related to apparent drug potentiation by Adriamycin. Inst J Radiation Oncol Biol Phys 1: 1179–1188
25. Moore GE, Di Pablo JA, Kondo T (1958) The chemotherapeutic effects and complications of actinomycin D in patients with advanced cancer. Cancer 11: 1204–1214
26. Morrison RK, Brown DE, Oleson JJ (1967) A toxicologic study of mithramycin. Toxicol Appl Pharmacol 11: 468–481
27. Olver IN, Schwarz MA (1983) Use of dimethyl sulfoxide in limiting tissue damage caused by extravasations of doxorubicin. Cancer Treat Rep 67: 407–408
28. Reilly JJ, Neifeld JP, Rosenberg SA (1977) Clinical course and management of accidental adriamycin extravasation. Cancer 40: 2053–2056
29. Rudolph R, Stein RS, Patillo RA (1976) Skin ulcers due to adriamycin. Cancer 39: 1094–1087
30. Svingen BA, Powis G, Appel PL, Scott M (1981) Protection against adriamycin-induced skin necrosis in the rat by dimethylsulfoxide and alpha tocopherol. Cancer Res 41: 3395–3399
31. Wolgemuth RL, Myers CA, Luce JK, Imond AR, Carrano RA (1982) Doxorubicin extravasation ulceration: animal model development and testing of potential antidotes. Proc AACR 23: 171
32. Yarbro JW, Wollhein M, Kennedy BJ (1965) Differential inhibition of RNA synthesis by mithramycin. Clin Res 13: 341–346

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