Journal of Surgical Oncology 1999;72:14–17

Use of Subatmospheric Pressure to Prevent Doxorubicin Extravasation Ulcers in a Swine Model

MICHAEL J. MORYKWAS, PhD,* AMANDA KENNEDY, JAMES P. ARGENTA, AND LOUIS C. ARGENTA, MD

Department of Plastic and Reconstructive Surgery, Wake Forest University School of Medicine, Winston-Salem, North Carolina

Application of subatmospheric pressure to sites injected with doxorubicin prevented ulcer formation in treated sites (0 ulcers/16 sites) compared to control wounds (10 ulcers/16 sites) in a pig model.

Background and Objectives: Extravasation of doxorubicin hydrochloride (Adriamycin) frequently causes chronic ulcers, which usually progress and expose underlying structures such as tendons and bone. The exact mechanism of action that causes cell death and the chronic ulcers is unknown.

Methods: Eight sites were injected intradermally with doxorubicin on each of 4 pigs. Four sites on each animal served as untreated controls. The remaining four sites were exposed to 125 mm Hg subatmospheric pressure applied 1 h after injection. The sites were observed on a three times per week schedule. Sites that did not develop ulcers were re-injected up to a total of four injections. The animals were observed for 5 weeks.

Results: Ten of sixteen control sites developed ulcers. No subatmospheric pressure treated sites developed ulcers. The incidence of ulcer formation was significantly less for treated wounds compared to control wounds at $P < 0.001$ by Fisher's exact test.

Conclusions: This physical modality appears to successfully prevent ulcer formation after doxorubicin injection.

J. Surg. Oncol. 1999;72:14–17.

KEY WORDS: doxorubicin; extravasation; vacuum; swine model; wound healing

INTRODUCTION

Doxorubicin hydrochloride (Adriamycin), an anthracycline antibiotic, is a potent antineoplastic agent used to treat solid tumors and hematologic cancers. An infrequent but potentially severe complication of treatment with doxorubicin is skin and tissue necrosis secondary to extravasation. These ulcers frequently progress and expose deeper structures, such as tendons, ligaments, and joints than would be expected by the initial appearance of the ulcer [1]. Complete prevention of extravasation in the affected patient population is difficult due to the mobility and fragile nature of the veins, the often limited number of injection sites, superior vena cava syndrome (with associated elevated venous pressure), and lymphedema secondary to surgery and radiation in the axilla [2–4]. Extravasation is usually accompanied by a burning or stinging sensation or local swelling, so infusions should

Michael J. Morykwas and Louis C. Argenta are coinventors of the device and technology described in this paper. Wake Forest University has licensed Kinetic Concepts, Inc. (San Antonio, TX) to make and market the device. Wake Forest University and the above-named authors receive royalties from the sale and use of the device.

* Correspondence to: Michael J. Morykwas, PhD, Department of Plastic and Reconstructive Surgery, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1075. E-mail: mmorykwa@wfubmc.edu.

Accepted 8 June 1999

be discontinued immediately when one of these signs is noted. The presence of one of these signs often allows for definitive identification of the time of injury.

Once introduced into the local tissues, anthracyclines intercalate into DNA, indirectly inhibit the activity of topoisomerase II, form reactive intermediates that can covalently bind to macromolecules, may generate free radicals, and may induce apoptosis [5–11]. However, which of these actions, singly or in combination, causes skin necrosis is not known. As a result of the uncertainty of the mechanism of injury, a single definitive treatment has not been identified. Both physical and chemical treatments have been advanced, with varying and sometimes contradictory results depending on the animal model and clinical setting [2,12–19].

On the basis of the uncertainty of the mechanism of action of doxorubicin and a successful history of treatment of a variety of envenomation injuries by application of subatmospheric pressure, we designed the present study to determine the efficacy of local application of subatmospheric pressure to intradermal doxorubicin injection sites in the pig model.

MATERIALS AND METHODS

Four 20-kg Chester pigs were obtained and housed for 1 week before the study began, to allow the animals to acclimate to the housing conditions. On the day of injection, the animals were sedated with an intramuscular cocktail of ketamine:acepromazine (22/1.1 mg/kg). The backs of the animals were shaved. One milliliter of a 2 mg/ml solution of doxorubicin hydrochloride was injected intradermally with a 25-gauge needle into the tissue 3 cm lateral to the spine [12–16]. A gloved finger was placed over the injection site for 30 sec to prevent any fluid from escaping from the injection site. Eight sites were injected, four on each side of the animal. A minimum of 5 cm separated each injection site within groups, and a minimum of 8 cm between groups. The anterior four sites were untreated controls and the posterior four sites were exposed to subatmospheric pressure. After the initial injections, the epidermis was perforated by two holes created with a 2-mm biopsy punch, although no tissue was removed. This was performed to allow a path for egress of the injected doxorubicin solution. The epidermis was not additionally perforated following any subsequent injections.

The four anterior sites were covered with gauze. The four posterior sites were covered with a subatmospheric pressure dressing (The V.A.C.™; Kinetic Concepts, San Antonio TX). The sites and dressing were covered with an adhesive thin film dressing to create an air-tight seal. The backs of the animals were covered with a protective plastic saddle, and the saddle was covered with a tubular cotton bandage. The tubing for the subatmospheric pressure dressing was wound several times around the neck band of the tubular dressing to minimize disruptive forces on the dressing. The tubing was then suspended from the top of the cage and attached to a microprocessor controlled V.A.C.™ pump. For 1 h after injection, subatmospheric pressure (125 mm Hg) was continuously applied to the posterior injection sites.

The animals were sedated at 48-h intervals and the wounds examined for evidence of tissue necrosis and ulcer formation. Sites that did not develop an ulcer by 7 days after the initial injection were reinjected with an additional 1 ml of a 2 mg/ml solution of doxorubicin. Sites that did not develop an ulcer by 14 days after the initial injection were reinjected with 2 ml of a 2-mg/ml solution of doxorubicin. Similarly, sites that did not develop an ulcer 21 days after the first injection were reinjected with 2 ml of a 2-mg/ml solution of doxorubicin. The animals were observed for an additional 14 days (35 total days).

The ulcers were counted and the size of each ulcer was measured at weekly intervals for 5 weeks. Statistical analysis consisted of Fisher's exact test for comparison of number and size of ulcers present in control group vs. the subatmospheric pressure treated group, with significance accepted at $P \leq 0.01$.

RESULTS

For nontreated control sites, 10 ulcers developed over the course of the study at the 16 injection sites. Ulcer size ranged from 15.7 to 471.3 mm^2, with a mean size of 175.3 $mm^2 \pm 156.8$ mm^2, as measured 7 days after the first recorded disruption in epithelium. Two ulcers were present 7 days after the initial injection. An additional 2 ulcers had developed by day 14 after initial injection (1 additional injection of 2 mg doxorubicin). Three additional ulcers had developed by day 21 after initial injection (one additional injection of 2 mg and one of 4 mg of doxorubicin). The final 3 ulcers developed by 28 days after the initial injection (one additional injection of 1 mg and two injections of 4 mg of oxorubicin). No additional ulcers had developed by day 35 following the initial injection.

For the subatmospheric pressure–treated injection sites, no ulcers developed at any time during the course of the study.

Statistical analysis by Fisher's exact test was significant at $P < 0.001$ for comparison of the number of ulcers present at the control sites ($n = 10$) vs. the number present at the treated sites ($n = 0$). Similarly, the ulcers in the control group (173.3 ± 156.8 mm^2) were significantly larger than the ulcers in the treated group (0 mm^2) at $P < 0.001$ (Fisher's exact test).

DISCUSSION

Ulcers that develop secondary to extravasation of doxorubicin hydrochloride often progress and expose

deeper structures, greatly increasing the severity of the injury. The ulcers do not reach their maximum size for several weeks, and the rate of healing of the established ulcers is extremely slow. A wide variety of treatment regimens has been proposed, including both physical and (bio)chemical interventions. Physical interventions to prevent ulcer formation include elevation of the extremity and intermittent application of local cooling, which both decreases the local metabolic rate and inhibits the action of doxorubicin by an unknown mechanism [20]. Surgical excision has been proposed for both ulcer prevention and treatment of established ulcers [21]. The exact mechanisms of action by which doxorubicin causes the local tissue necrosis is unknown, thus (bio)chemical interventions have focused on both trying to reduce local inflammation (e.g., by administration of steroids or dapsone) and prevention and scavenging free radicals (e.g., by administration of DMSO) [2,9,10,12–19,21].

Because of the uncertainty of the mechanism of action, this study was designed to use a physical modality to remove the doxorubicin before an ulcer could become established. The modality examined the effects of the application of subatmospheric pressure to sites of intradermal doxorubicin injections in a young pig model. The similarity of the dermal microarchitecture of young pig skin to human skin and the tight-skinned nature of pigs makes them the model of choice for investigations of cutaneous lesions [22]. Additionally, the skin of young pigs is closest to human skin in regard to diffusion properties of the commonly used animal models [23]. A V.A.C.™ wound healing device was used to apply a controlled (125 mm Hg) vacuum to the injection sites [24,25]. According to basic laws of diffusion, molecules migrate to create a homogeneous concentration of the molecules. We postulated that by applying a continuous vacuum to the surface of the skin, migration of the molecules of doxorubicin through the epidermis into the vacuum space would be enhanced. Thus, the concentration of the doxorubicin in the tissues would be below the threshold required to develop necrotic lesions.

The vacuum was applied to the injection sites 1 h after the sites were injected. This was done to simulate the projected time it would take from notification that an extravasation incident had occurred to procure a V.A.C.™ pump from hospital supplies, apply the V.A.C.™ dressing, and apply subatmospheric pressure to the site. Several of the sites had to be injected more than once in this model to develop an ulcer. While the majority of the control site did develop ulcers (10 of 16), none of the subatmospheric pressure–treated sites did. The fact that not all control sites developed ulcers after the initial injection seems to be a limitation of the model, although previous reports of intradermal injection of 1 mg of doxorubicin did cause ulceration and were the basis of our model.

It does appear that the physical modality of application of subatmospheric pressure to sites deliberately injected with doxorubicin was successful at preventing ulcer formation. The mechanism of action is most likely due to the enhanced diffusion gradient that is formed, which causes the doxorubicin molecules to migrate out of the underlying tissues through the epidermis into the vacuum dressing. Creation of holes through the stratum corneum to facilitate diffusion does not appear to be necessary, as those sites injected more than once did not have any additional routes of egress created (as was done with the initial injection), and the subatmospheric pressure–treated sites still did not develop any ulcers.

This technique has the potential to prevent the formation of many of these very difficult-to-treat ulcers, greatly improving the quality of life for these patients compared to patients who do develop ulcers. In addition, in this time of increasing concern over cost of care, this preventative modality is more economical than treatment of established ulcers.

ACKNOWLEDGMENTS

The V.A.C.™ dressings and pumps were the generous gift of Kinetic Concepts (San Antonio, TX). The statistical analysis was performed by Greg Russell (Section on Biostatistics, Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC).

REFERENCES

1. Rudolph R, Stein RS, Pattillo RA: Skin ulcers due to adriamycin. Cancer 1976;38:1087–1094.
2. Rudolph R, Larson DL: Etiology and treatment of chemotherapeutic agent extravasation injuries: A review. J Clin Oncol 1987; 5:1116–1126.
3. Bowers DG Lynch JB: Adriamycin extravasation. Plast Reconstr Surg 1978;61:86–92.
4. Rudolph R, Suzuki M, Luce JK: Experimental skin necrosis produced by adriamycin. Cancer Treat Rep 1979;63:529–537.
5. Gianni L, Corden BJ, Myers CE: The biochemical basis of anthracycline toxicity and antitumor action. Rev Biochem Toxicol 1983;5:1–82.
6. Arcamone F: Doxorubicin anticancer antibiotics. In "Medicinal Chemistry," Vol. 17. New York: Academic Press, 1981.
7. Moore HW: Bioactivation as a model for drug design bioreductive alkylation. Science 1977;197:527–532.
8. Bachur NR, Gee MV, Friedman RD: Nuclear catalyzed antibiotic free radical formation. Cancer Res 1982;42:1078-81.
9. Tewey KM, Rowe TC, Yang L, Halligan BD, Liu LF: Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. Science 1984;226:466–468.
10. Ling YH, Priebe W, Perez-Soler R: Apoptosis induced by anthracycline antibiotics in P388 parent and multidrug-resistant cells. Cancer Res 1993;53:1845–1852.
11. Richardson DS, Johnson SA: Anthracyclines in haematology: preclinical studies, toxicity and delivery systems. Blood Rev 1997; 11:201–223.
12. Desai MH Teres D: Prevention of doxorubicin-induced skin ulcers in the rat and pig with dimethyl sulfoxide (DMSO). Cancer Treat Rep 1982;66:1371–1374.
13. Okano T, Ohnuma T, Efremidis A, et al.: Doxorubicin-induced skin ulcer in the piglet. Canc Treat Rep 1983;67:1075–1078.
14. Harwood KV Bachur N: Evaluation of dimethyl sulfoxide and

local cooling as antidotes for doxorubicin extravasation in a pig model. Oncol Nurs Forum 1987;14:39–44.
15. Lebredo L, Barrie R, Woltering EA: DMSO protects against adriamycin-induced tissue necrosis. J Surg Res 1992;53:62–65.
16. Averbuch SD, Boldt M, Gaudiano G, et al: Experimental chemotherapy-induced skin necrosis in swine. Mechanistic studies of anthracycline antibiotic toxicity and protection with a radical dimer compound. J Clin Invest 1988;81:142–148.
17. Monstrey SJ, Mullick P, Narayanan K, et al: Hyperbaric oxygen therapy and free radical production: An experimental study in doxorubicin (Adriamycin) extravasation injuries. Ann Plast Surg 1997;38:163–168.
18. Disa JJ, Chang RR, Mucci SJ, et al: Prevention of adriamycin-induced full-thickness skin loss using hyaluronidase infiltration. Plast Reconstr Surg 1998;101:370–374.
19. Bayati S, Zook N, Brown RE, et al: Improving outcome in adriamycin extravasation injury using oral dapsone therapy. Presented at the 77th annual meeting, American Association Plastic Surgery, Montreal, Quebec, Canada, May 17–20, 1998.
20. Potmesil M, Isreal M, Silber R: Two mechanisms of Adriamycin-DNA interaction in L1210 cells. Biochem Pharmacol 1984;33: 3137–3142.
21. Dorr RT: Antidotes to vesicant chemotherapy extravasations. Blood Rev 1990;4:41–60.
22. Meyer W, Schwartz R, Neurand K: The skin of domestic mammals as a model for the human skin with special reference to the domestic pig. Curr Prob Dermatol 1979;7:39–52.
23. Bartek MJ, LaBudde JA, Maibach HI. Skin permeability in vivo: comparison in rat, rabbit, pig and man. J Invest Dermatol 1972; 58:114-23.
24. Morykwas MJ, Argenta LC, Shelton-Brown EI, et al: Vacuum-assisted closure: a new method for wound control and treatment: animal studies and basic foundation. Ann Plast Surg 1997;38:553–562.
25. Argenta LC, Morykwas MJ: Vacuum-assisted closure: a new method for wound control and treatment: clinical experience. Ann Plast Surg 1997;38:563–576.