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The effects of doxorubicin and mitoxantrone on wound healing

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Summary. The goal of the present study was to determine whether mitoxantrone would impair wound healing to a similar degree as doxorubicin when given in equally cytotoxic doses. On day 0, male Fischer rats were wounded and treated with 5% dextrose (control), 6 mg/kg doxorubicin, or 1.2 or 2.4 mg/kg mitoxantrone. On day 5, WBCs for the doxorubicin group and the group that had been treated with 1.2 mg/kg mitoxantrone were 33% and 43% lower than control values, respectively. All rats that had been given 2.4 mg/kg mitoxantrone died within 1 week of being wounded due to drug toxicity. On day 21, wound-breaking strength (WBS) analysis was performed: two skin specimens were taken from each dorsal skin incision perpendicular to the scar axis and were subjected to wound disruption (grams of force) by uniaxial extension. The WBS analysis indicated significant differences between the doxorubicin treated group (1183 ± 96 g) and the control group (2422 ± 247 g). However, no significant difference was found between the group that had been given 1.2 mg/kg mitoxantrone (2140 ± 191 g) and control animals. Thus, mitoxantrone seems to exert myelosuppressive effects similar to those displayed by doxorubicin, but the former drug results in significantly less impairment of wound healing in the rat model.

Introduction

Mitoxantrone is a broad-spectrum chemotherapeutic agent that was recently approved for clinical use. Although its mechanism of action is not completely understood, it is thought that mitoxantrone intercalates into DNA base pairs (specifically, G-C base pairs), thereby inhibiting DNA synthesis. Since its development in 1979, clinical studies have revealed that the drug's efficacy and spectrum of activity are similar to that of doxorubicin, one of the most widely used antineoplastic agents [10]. In a study by Fujimoto and Ogawa [6], mitoxantrone was more effective than doxorubicin in treating certain leukemias and carcinomas in mice. Comparative studies of the two antitumor drugs were also done in cancer patients. Neidhart et al. [9] reported that mitoxantrone and doxorubicin produced similar responses as first-line chemotherapeutic agents in breast cancer patients.

Unfortunately, both mitoxantrone and doxorubicin cause adverse side effects such as myelosuppression, mucositis, nausea, vomiting, and cardiotoxicity. The degree of toxicity, however, differs greatly between the two drugs. For instance, doxorubicin causes skin necrosis after local extravasation, whereas mitoxantrone does not. In addition, doxorubicin causes major cardiotoxicity but mitoxantrone causes significantly less damage to the myocardium [5]. Overall, mitoxantrone is less systemically toxic than doxorubicin [9].

The lower toxicity of mitoxantrone may have important clinical implications. Many protocols for cancer therapy now implement a multimodality approach, with chemotherapy being given shortly before or after surgery. However, the use of these agents during the perioperative period is known to impair surgical wound healing [4, 7]. This is particularly for doxorubicin, the anthracycline antibiotic most widely used for the treatment of cancer patients. Since mitoxantrone apparently elicits less systemic toxicity than does doxorubicin, we postulated that it might also impede wound healing to a lesser degree. The purpose of the present study was to investigate the comparative effects of mitoxantrone and doxorubicin on wound healing in an established rat model.

Materials and methods

A total of 18 male Fischer F344 rats (330–422 g) were housed at 2/cage and were fed Purina rat chow and tap water ad libitum. On the day of wounding (day 0), all rats were anesthetized with ether and the fur on

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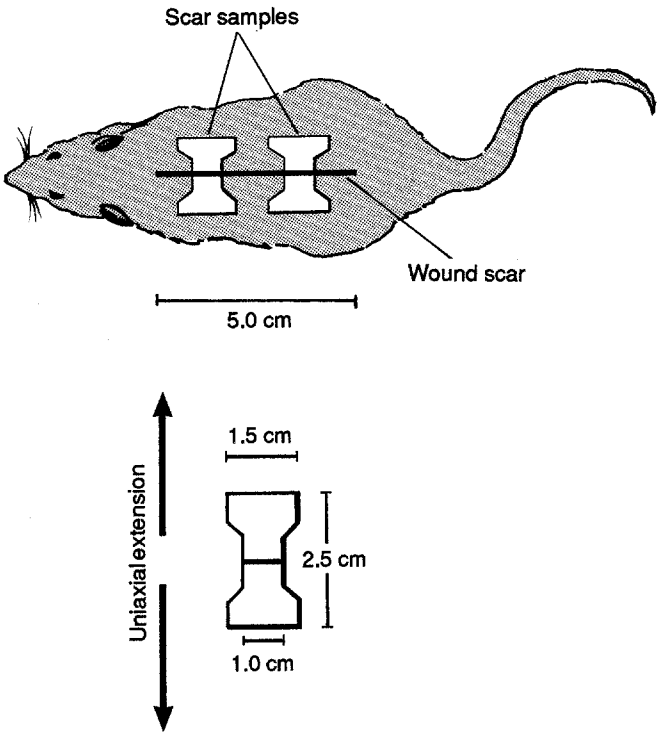

Fig. 1. All rats were killed by CO_2 asphyxiation (one at a time). Immediately thereafter the dorsal skin was harvested, scar samples were taken using a preformed cutter, and the skin specimens were subjected to uniaxial extension perpendicular to the scar axis

their backs was clipped. A 5-cm-long, midline, dorsal, full-thickness incision was made with a number 10 blade by one investigator (D. F. D.). The wounds were closed with stainless steel skin clips. The skin clips were removed on the 7th postoperative day without the use of anesthesia.

Following wounding, the rats were randomly divided into four experimental groups. All animals were treated by tail-vein injection on day 0. Group 1 (n = 3) received 5% dextrose in water and served as the control group. Group 2 (n = 6) received 6 mg/kg doxorubicin, the dose lethal for 10% of the group (LD_{10}); it has previously been shown that this concentration significantly impairs wound healing [1, 2, 4, 7]. Group 3 (n = 6) was given 1.2 mg/kg mitoxantrone. (In human cancer patients, mitoxantrone is given at 1/5 the dose of doxorubicin; extrapolation of this proportion to the rat model resulted in a dose of 1.2 mg/kg.) Group 4 (n = 3) received 2.4 mg/kg mitoxantrone. We included this group for purposes of comparison to determine whether there was a dose-related effect.

On day 5, WBCs were performed using a Coulter counter (model ZF, Coulter Electronics, Hialeah, Florida). Two rats were randomly selected from each group and two capillary tubes of blood were collected from the tail vein of each rat. The WBC analysis was carried out on day 5 because it has previously been shown that doxorubicin-treated rats manifest the lowest WBC at 5 days after treatment.

On day 21, all rats were killed by CO_2 asphyxiation (one at a time). Immediately thereafter, the dorsal skin was harvested, scar samples were taken using a preformed cutter, and the skin specimens were subjected to uniaxial extension perpendicular to the scar axis (Fig. 1). The wound-breaking strength (WBS), or the force required to disrupt the wound, was graphically measured using the Instron Tensiometer (model 4201, Instron Corp., Canton, Mass.). A constant cross-speed of 20 cm/min was used throughout the experiment [1, 4]. Student's t-test was used to compare the WBS values for each group.

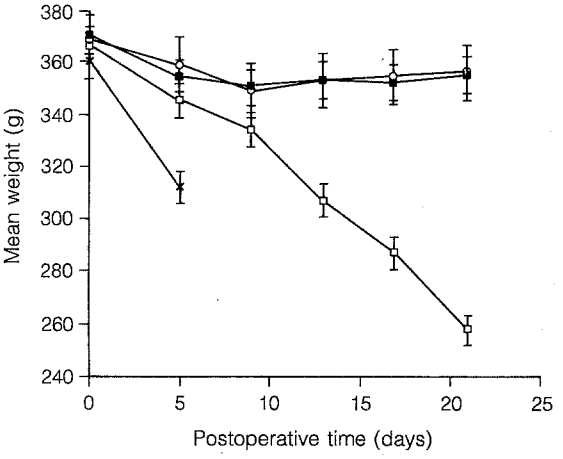

Fig. 2. Postoperative time in days versus mean body weight in grams is shown for each treatment group: control (○), doxorubicin (□), 1.2 mg/kg mitoxantrone (■), 2.4 mg/kg mitoxantrone (×). On day 17, Student's t-test revealed significant differences in weight loss between doxorubicin-treated and control animals and between and doxorubicin-treated and mitoxantrone-treated rats (1.2 mg/kg). However, no significant difference was found between mitoxantrone-treated (1.2 mg/kg) and control animals

Results

No surgical wound complications such as infections or hematomas occurred in any of the rats.

Weight loss

All groups initially lost weight, but rats in group 1 (control) and group 3 (1.2 mg/kg mitoxantrone) regained the weight such that by day 21, they had lost only 4% of their original weight (Fig. 2). Animals in group 2 (6 mg/kg doxorubicin) continued to lose weight throughout the 21-day period such that by the end of this time, they had lost 30% of their original weight. Rats in group 4 (2.4 mg/kg mitoxantrone) rapidly lost weight, and all animals died within the 1st postoperative week. Student's t-test was used to compare the weight loss between the groups on day 17; a value of $P < 0.05$ was considered to be significant. There were significant differences between control and doxorubicin-treated rats ($P \leq 0.005$) and between doxorubicin-treated and mitoxantrone-treated animals (1.2 mg/kg; $P \leq 0.005$). However, no significant difference was found between the control group and mitoxantrone-treated rats (1.2 mg/kg; ($P > 0.38$).

WBC analysis

All chemotherapy-treated groups showed a statistically significant decrease in WBC as compared with control values but were not statistically distinguishable from each other (Fig. 3). The WBC obtained for the doxorubicin-treated group was 33% of the control value. In group 3 (1.2 mg/kg mitoxantrone) and 4 (2.4 mg/kg mitoxantrone), the counts were 43% and 21% of the control value, respectively.

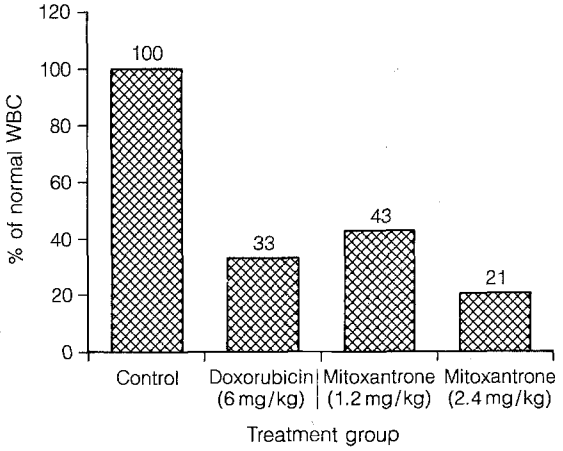

Fig. 3. Relative WBCs of chemotherapy-treated groups as compared with control values. WBCs were carried out on day 5 as described in Materials and methods

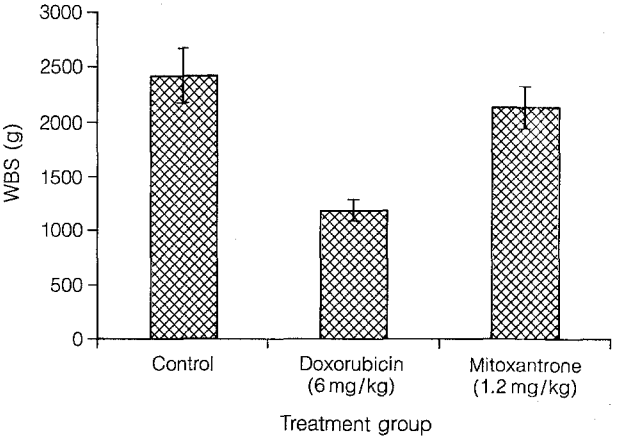

Fig. 4. The mean values ±SEM obtained for WBS is shown for control, doxorubicin-treated, and mitoxantrone-treated groups. Student's *t*-test revealed significant differences in WBS between doxorubicin-treated and control animals and between doxorubicin-treated and mitoxantrone-treated rats. However, no significant difference was found between mitoxantrone-treated and control animals. Student's *t*-test: $P = 0.00002$ for control vs doxorubicin-treated rats, $P = 0.38$ for control vs mitoxantrone-treated animals, and $P = 0.00011$ for doxorubicin-treated vs mitoxantrone-treated groups

WBS analysis

The mean WBS for group 1 (control) was 2422±247 g, that for group 2 (doxorubicin) was 1183±96 g, and that for group 3 (1.2 mg/kg mitoxantrone) was 2140±191 g (Fig. 4). The group that received high-dose mitoxantrone was not tested because all rats had died prior to the performance of WBS analysis. Student's *t*-test was used to compare the WBS data; a value of $P < 0.05$ was considered to indicate significance. There were significant differences between group 1 (control) and group 2 (doxorubicin; $P = 0.00002$) and between group 2 (doxorubicin) and group 3 (1.2 mg/kg mitoxantrone; $P = 0.00011$). However, no significant difference was found between group 1 (control) and group 3 (1.2 mg/kg mitoxantrone $P = 0.38$).

Discussion

The reduction in WBS produced by treatment with doxorubicin has previously been demonstrated in this laboratory [1, 2, 4]. Devereux et al. [2] noted that doxorubicin exerted a significant effect on WBS when the drug was given at prior to or after wounding and that this effect was even greater effect when the compound was given on the day of wounding (day 0). Further evaluation revealed that the impairment of wound healing was attributable to a reduction in collagen accumulation in the scars as well as to a reduction in collagen-fiber diameter [4].

For the surgical oncologist, the inhibitory effect of doxorubicin and other chemotherapeutic agents on wound healing is an important consideration in their use in cancer treatment. Cancer patients often manifest reduced wound healing due to factors such as poor nutrition. However, Devereux et al. [2] demonstrated that nutritional factors alone were not sufficient to result in a significant impairment of wound healing. It is the use of doxorubicin or similar drugs in cancer patients that dramatically impairs wound healing. Thus, the use of a drug that exhibits chemotherapeutic efficacy equal to that of doxorubicin but produces less wound healing impairment may be advantageous.

In the present experiment, the WBC indicated that doxorubicin and mitoxantrone had displayed similar myelosuppressive activity by day 5. In addition, WBS analysis demonstrated that mitoxantrone had caused less impairment of wound healing than doxorubicin by day 21. In fact, there was no significant difference in WBS between the mitoxantrone-treated (1.2 mg/kg) group and control animals.

However, Mullen et al. [8] found no demonstrable difference between doxorubicin and mitoxantrone on wound healing in rats that had undergone chemotherapy immediately prior to wounding. The reasons for the difference between the results reported by these authors and those obtained in the present study are not clearly evident. However, variations in methodology may have contributed to the disparate findings. For example, Mullen et al. used number 10 blades to "free cut" each scar specimen by hand. In the present experiment, a preformed cutter that obtains scar specimens of identical size and shape was used. Also, Mullen et al. took only one scar specimen from each dissected skin sample. In our experiment, two scar specimens were dissected from each skin sample and tested, with care being taken to avoid the edges of the wound scar; this enabled us to test the WBS of the entire wound scar. Finally, Mullen et al. [8] reported that at 2 weeks after wounding, the WBS values found for rats that had been treated with mitoxantrone or doxorubicin immediately prior to wounding were *greater* than the control value. This result is troublesome and contradicts the results reported by other investigators as well as our own [1–4, 7].

Because mitoxantrone seems to manifest cytotoxicity similar to that of doxorubicin and yet causes less impair-

ment of wound healing, we believe that mitoxantrone should be considered a reasonable chemotherapeutic alternative to doxorubicin if such treatment is needed in the immediate perioperative period.

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