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Authors

Dorr, Robert T
Soble, Michelle J
Meyskens, Frank L

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PULSED ELECTROMAGNETIC FIELDS DO NOT REDUCE

VESICANT SKIN ULCERS IN MICE

Robert T. Dorr and Michelle J. Soble
Departments of Medicine and Pharmacology/Toxicology
Colleges of Medicine and Pharmacy
University of Arizona Cancer Center
Tucson, Arizona 85724

Frank L. Meyskens, Jr.
University of Arizona Cancer Center
Tucson, Arizona 85724

ABSTRACT

A study was conducted in mice to test the efficacy of pulsed electromagnetic fields (PEMF) as a means of reducing acute and chronic skin ulceration from two model vesicant anticancer drugs. Adult female BALB/c mice were dehaired dorsally and given 0.5 mg intradermal injections of the DNA intercalators, doxorubicin and bisantrene. The mice were then housed in plexiglass cages and exposed to 2-Hz PEMF of repetitive pulse bursts of 250 μ sec or 20 μ sec duration. Skin lesions and survival were assessed daily. There was no significant skin toxicity reduction with either PEMF treatment for doxorubicin (acute ulceration) or bisantrene (chronic ulceration). The inclusion of topical DMSO treatments also did not reduce skin ulceration and appeared to increase doxorubicin lethality. The PEMF treatments appeared to slightly increase some of the vesicant ulcers and doxorubicin lethality.

INTRODUCTION

Recent studies have shown that pulsed, low energy electromagnetic fields (PEMF) can enhance the healing of nonunion fractures (1,2), facilitate nerve regeneration (3), and reduce persistent inflammation of joints (4). Wolcott et al. have also demonstrated accelerated clinical healing of ischemic skin ulcers using low intensity direct electric currents (5). The applied

electromagnetic energy was weaker than that required to depolarize cell membranes. Nevertheless, electrochemical gradients were produced in the extracellular fluid exposed to cell plasma membranes (6).

Severe skin ulcers are known to result from the extravasation of vesicant antineoplastic drugs (7). These lesions heal poorly and can require extensive excision and full thickness graft repair (8,9). Because of the severe nature of such vesicant-induced skin ulcers, a number of antidotal approaches have been systematically evaluated using experimental animal models (10-18). Most of these approaches have involved pharmacologic or topical temperature modifications to reduce rodent or pig skin ulcers. There are no studies which have attempted to evaluate the possible antidotal role for pulsatile electromagnetic fields in managing vesicant-induced skin ulcers. The following experiments were conducted to quantitatively assess this potential using a standardized mouse model. Two antineoplastic vesicants were selected for study: doxorubicin, which is an acute (less than 30 days) murine vesicant (19), and bisantrene, which causes chronic murine skin ulcers (greater than 75 days)(20).

METHODS

Two wave forms of PEMF were used in this study: repetitive pulse bursts of 250 μ sec at 2 Hz (Unit No. B50-2; Electro Biology Inc., Fairfield, New Jersey) or 20 μ sec at 2 Hz (Unit No. S20-2). Both wave forms were asymmetrical and had a peak value of less than 15 Gauss (Table 1). These fields were applied to adult female BALB/c mice (Jackson Laboratories, Bar Harbor, Maine) that were housed in specially fabricated plexiglass cages (10 x 30 x 30 cm, 1-cm thick plexiglass). An induction coil was placed along the upper outer perimeter of each cage.

The mice were injected intradermally (ID) with one of two vesicant antineoplastic drugs according to a standard model (9). Groups of five mice

(25-30 g each) were first prepared for injection by depilation of a 1 cm² dorsal area with Neet^R lotion (Whitehall Laboratories; containing thio-glycolic acid, mineral oil, steary and acetyl alcohols, and sodium hydroxide). Twenty-four hours after hair removal, the mice were injected ID with 0.5 mg/0.05 ml of either doxorubicin HCl (Adriamycin^R, Adria Laboratories, Columbus, Ohio) or 0.75 mg of bisantrene HCl (9-1, anthracenedicarboxaldehyde-bis-(4,5-dihydro-1-H-imidazole-2yl-hydrazone), American Cyanamid, Pearl River, New Jersey). The doses were selected to approximate human clinical equivalents and were known to produce standard skin ulcers in mice (19,20). Both drugs were diluted into unpreserved 0.89% sodium chloride immediately prior to use. In addition to these vesicants, some mice were treated topically with two 1-ml applications of 100% DMSO, chromatography grade, immediately after vesicant injection to determine if DMSO reduce vesicant ulcers in combination with PEMF.

Mice were housed five/plexiglass cage in a standard humidified vivarium with 12-hour light/dark cycles. Standard mouse food blocks (Ralston Purina) and tap water were provided ad libitum. Laminar air flow environments and slight acidification of water were used to reduce possible intercurrent infections. The generator/cages were placed on particle-board shelves to reduce possible metal interference with the induced fields. Each cage was separated from other cages and any other intervening structure by at least three feet in the horizontal direction and four feet in the vertical direction. Mice were unrestrained in the cages throughout the study.

For these studies, the widest perpendicular widths of skin lesions were measured with a micrometer (Mitutoyo, Japan). Toxicity parameters included: (1) induration (visible swelling with loss of fine skin architecture; (2) erythema; and (3) ulceration. Observations were recorded for each mouse and summary graphs were prepared from mean data. Ulceration was additionally analyzed by mathematical integration to provide the area under the ulceration-time curve (AUC or "total toxicity"). Statistical analyses of

TABLE 1

PEMF Generator Identification

<u>PEMF UNIT</u>	<u>BURST WIDTH</u>	<u>POSITIVE AMPLITUDE</u>	<u>DURATION</u>	<u>REPETITION CYCLE</u>
A-E	20 μ sec	4 mV	85 μ sec	2 Hz
F-J	250 μ sec	1.6 mV	50 msec	2 Hz
(E,J)	Controls (Device not activated)			

variance on the ulceration data included intergroup comparisons of: (1) the AUC ($\text{cm}^2 \times \text{days}$); (2) the peak lesion size (cm^2); (3) the total time of apparent ulceration (days); and (4) the number and time (days) of death of any mice dying in the course of the study. A multiple range test was used for all subsequent intergroup comparisons. For this analysis, the Student-Newman Keuls (SNK) test was performed at a 0.05 level.

Each study was repeated once and the data grouped for the statistical analyses. The PEMF units were allocated to the drug treatment groups such that each type of generator, B50-2 or S20-2, was tested against each drug regimen (Table 1) in an observer-blinded fashion. Vesicant treatments were also blinded until data analysis was complete to prevent bias in measuring skin lesions. The PEMF generators were identified only by alphabetical letter and were not tested by electromagnetic probe to determine the particular wave form produced by an individual generator prior to the end of the study. As an additional control, one generator of each wave form type (E and J) was not turned on throughout the course of the study. These served as controls for the effects, if any, of the special plexiglass cages on vesicant lesion healing. Table 2 outlines the allocation of individual PEMF generators to each vesicant evaluated in this study.

TABLE 2

Drug Treatments with PEMF Units

<u>PEMF GENERATOR</u>	<u>DRUG TREATMENTS</u>
A,F	Doxorubicin 0.5 mg/0.05 ml ID
B,G	Doxorubicin 0.5 mg/0.05 ml ID followed by daily DMSO applications
C,H	Bisantrene 0.5 mg/0.05 ml ID
D,I	Bisantrene 0.5 mg/0.05 ml ID followed by daily DMSO applications
E,J	Doxorubicin 0.5 mg/0.05 ml ID

RESULTS

Doxorubicin

Table 3 summarizes the mean ulceration and survival data for the doxorubicin-PEMF studies. Overall, the results did not demonstrate significant differences for either the 250 μ sec or 20 μ sec PEMF treatments compared to the controls (E and J). The lowest peak ulcer size was obtained with PEMF control unit J in which the device was not activated. There was also no significant doxorubicin toxicity reduction for the PEMF regimens combined with topical DMSO. This is consistent with our earlier observations (17). Indeed, the DMSO-containing regimens appeared to significantly increase doxorubicin lethality, which otherwise averaged 20% in this series.

There was a large variation in the ulcer AUCs in the doxorubicin 0.5 mg series. This ranged from 28.5 ± 9.31 (50 msec PEMF) to 8.09 ± 5.12 (Unit J). This latter treatment, J in which the generator was turned off, was significantly different from the other regimens. Figure 1 shows the toxicity-time plots for three doxorubicin 0.5 mg treatments, control, 250 μ sec, and 20 μ sec PEMF. These semilogarithmic plots graphically show that

TABLE 3

Doxorubicin-PEMF Ulceration and Survival Data

DOX TREATMENT	PEMF REGIMEN		<u>MEAN SKIN ULCERATION (SD)</u>				
	Unit	Wave Form	AREA UNDER TOXICITY x TIME CURVE (cm ² x days)	PEAK LEVEL OF ULCERATION (cm ²)	TOTAL TIME OF ULCERATION (days)	SURVIVAL Median No. Day of Dying Death	
0.5 mg	A	250 μ sec	28.52 (9.31)	1.62 (0.55)	31.8 (3.1)	20%	6.0
0.5 mg + DMSO	B	250 μ sec	24.74 (19.04)	2.24 (0.73)	20.0 (17.3)	60%	9.0
0.5 mg	E	Off	25.75 (3.70)	2.12 (0.47)	25.0 (7.6)	20%	5.0
0.5 mg	F	20 μ sec	19.72 (6.03)	1.32 (0.38)	31.4 (2.5)	None	--
0.5 mg + DMSO	G	20 μ sec	10.31 (9.87)	1.05 (0.28)*	14.6 (15.4)	60%	4.0
0.5 mg	J	Off	8.09 (5.12)*	1.02 (0.26)*	15.6 (12.2)	20%	2.0

STATISTICAL COMPARISONS

<u>ANOVA</u>	<u>AUC</u>	<u>PEAK LEVEL</u>	<u>DURATION</u>	<u>SURVIVAL</u>
F-Ratio (F-Probability)	2.38 (0.02)	3.29 (0.01)	1.11 (0.38)	0.69 (0.74)

neither PEMF treatment reduced acute skin ulceration produced by this antineoplastic vesicant. The general pattern of skin toxicity evident in the PEMF groups was not different from our extensive prior work with doxorubicin in this murine skin model (17-19).

Bisantrene

Figure 2 and Table 4 display the summary results of the bisantrene trials. None of the mice in the bisantrene study died. This is consistent

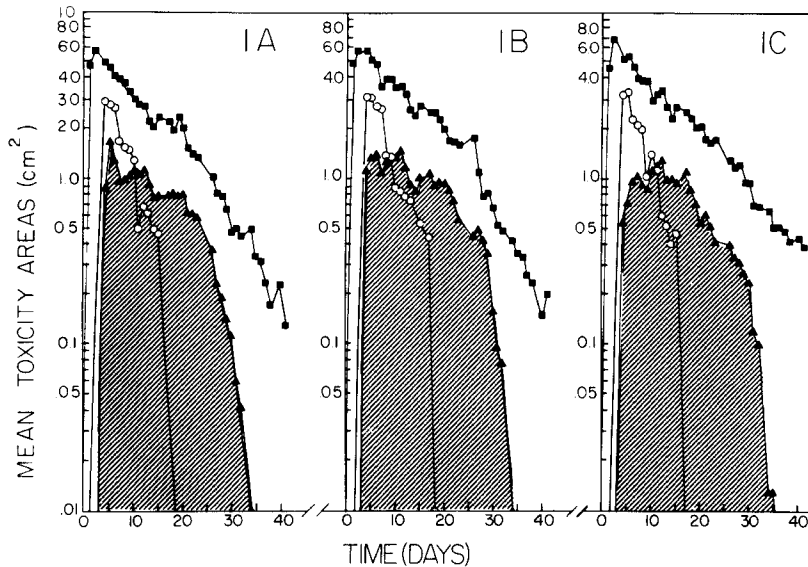


FIGURE 1

The semilogarithmic plots of skin toxicity areas in cm^2 (y) versus time in days (x-axis) for mice receiving 0.5 mg doxorubicin ID and no PEMF treatment (a), bursts of 250 μsec -2 Hz PEMF continuously (b) or single pulse 20 μsec -2 Hz PEMF (c). Each point represents the mean of five mice. Toxicity areas were calculated from the widest perpendicular diameters of induration (lines bounded with squares), erythema (lines bounded with circles), and ulceration (lines bounded with triangles, shaded area).

with earlier studies with this drug dose (20). Figure 2 shows the characteristic prolonged, chronic ulcers created by this drug. The average duration of ulceration with the PEMF regimens ranged from 55-72 days which was not different from the controls. While a large variation in ulcer AUCs was apparent, the largest ulcer AUC was again found with the 250 μsec , 2 Hz, PEMF treatment.

It should be stressed that an effective bisantrene antidote, sodium bicarbonate, has been identified for this chronic ulcerant (20). In the current study, there was no evidence that PEMF treatments reduced bisantrene murine skin toxicities.

TABLE 4

Bisantrene-PEMF Ulceration Data

BIS TREATMENT	PEMF REGIMEN		AREA UNDER TOXICITY x TIME CURVE cm ² x days	PEAK LEVEL OF ULCERATION (cm ²)	TOTAL TIME OF ULCERATION (Days)
	Unit	Wave Form			
0.5 mg	C	250 μ sec	15.41 (10.50)	0.86 (0.54)	72.2 (37.8)
0.5 mg + DMSO	D	250 μ sec	5.23 (6.46)	0.38 (0.39)	55.0 (43.4)
0.5 mg	H	20 μ sec	5.61 (6.93)	0.48 (0.55)	56.0 (51.1)
0.5 mg + DMSO	I	20 μ sec	5.28 (4.22)	0.53 (0.39)	69.2 (44.1)

STATISTICAL COMPARISONS

<u>ANOVA</u>	<u>AUC</u>	<u>PEAK LEVEL</u>	<u>DURATION</u>
F-Ratio (F-Probability)	0.98 (0.43)	1.15 (0.36)	0.29 (0.84)

DISCUSSION

The results of the current studies do not demonstrate a significant benefit for PEMF as an antidote to vesicant-induced skin ulceration in an established mouse skin toxicity model. Neither of the repetitive pulse bursts (20 μ sec nor 250 μ sec) PEMF treatment was effective in this study. It is possible that the unrestrained mobility of the mice did not produce an optimal perpendicular orientation of the electromagnetic field to the dorsal skin lesions. A restrained animal model might overcome this problem but would be impractical for prolonged PEMF treatments.

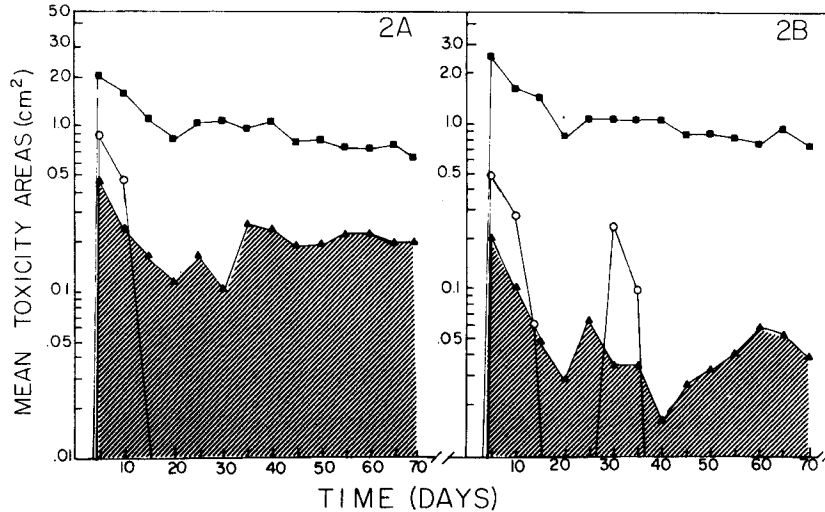


FIGURE 2

This semilogarithmic plot of skin toxicity areas in cm^2 (y-axis) versus time in days (x-axis) for mice receiving 0.75 mg bisantrene HCl and a continuous 2 Hz PEMF treatment of either 20 μsec single pulse (a) or 250 μsec bursts (b). Each point represents the mean of five mice. Toxicity areas were calculated as in the methods for induration (lines bounded by squares), erythema (lines bounded by circles), and ulceration (lines bounded by triangles, shaded area).

Previous pharmacologic studies with this mouse skin model have demonstrated a dose-dependency for vesicant antineoplastics injected ID (17-19). Topical cooling and low dose corticosteroids have been shown to reduce doxorubicin lesions (12). For chronic bisantrene ulcers, sodium bicarbonate has proved to be significantly antidotal (20). Thus, skin lesions in these model vesicant drugs can be reduced with effective local antagonists. This was not seen in the current trials using PEMF to enhance healing.

With doxorubicin and PEMF, the smallest ulcers were produced in the control group (device J) in which the PEMF unit was not activated. This was

statistically significant by the SNK analysis. There was no effect of adding DMSO to the PEMF treatments. This is consistent with an earlier assessment of DMSO alone as a local doxorubicin antidote (17). The range of doxorubicin ulcers produced in the PEMF studies are also similar to earlier trials of doxorubicin alone (12,17-19).

The lesions produced by bisantrene in this study were characteristically prolonged over 70 days. This is consistent with earlier experiments with this vesicant (20). There was no reduction in bisantrene-induced ulcers using any of the PEMF treatments. Likewise, the addition of topical DMSO to the bisantrene-PEMF experiments did not reduce chronic ulceration.

The plasma membrane is believed to be the likely foci for PEMF-cell interactions. Following an ionic cell surface change mediated by a PEMF-type field, a variety of biologic stimulatory effects have been observed in different species. These changes include enhanced DNA synthesis in isolated rat calvaria bone cells, dendritic neural regeneration in embryonic chick ganglia, increased limb regeneration in the salamander, and enhanced healing of radial osteotomy repair in rats (21). It is believed that the electrochemical transformations at the cell membrane probably involve Ca^{++} and/or metal ion fluxes (22). These ionic and metal ion changes may then facilitate the unfolding of chromatin-DNA complexes to produce altered cell activity (23,24). In each of these basic studies, PEMF-type currents appear to modulate the rate of normal cellular processes in both a wave form and frequency-dependent fashion. Thus, the PEMF treatments chosen for the current study were specifically selected to optimize electromagnetic interactions for soft tissues, such as skin. We further believed that the increased DNA synthesis seen in cartilage cells stimulated by PEMF-type currents (25) might facilitate augmented skin tissue healing after vesicant-induced damage had occurred.

In contrast, there was no reduction in murine skin ulceration following continuous PEMF exposure in our study. Both vesicant drugs produced

characteristic skin ulcers and the PEMF treatments appeared to slightly increase some of these ulcers. However, this enhancement did not reach statistical significance. Further studies could be aimed at defining the potential for PEMF enhancement of anticancer drug cytotoxicity and antitumor efficacy with treatments similar to those used in the present studies.

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