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RADIOPROTECTION FROM RADIATION-INDUCED LYMPHEDEMA WITHOUT TUMOR PROTECTION

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ABSTRACT

Lymphedema or tissue swelling from impaired lymph drainage commonly occurs after regional nodal dissection and/or radiation therapy for cancer control. Treatment options for this disabling and life-altering complication involve long-term labor-intensive commitments. Sentinel node biopsy can forestall removal of negative regional nodes, offering some protection against lymphedema, however, most preventive measures are elusive, ineffective, or unproven. Our goal was to determine whether the radioprotectant amifostine could prevent or retard the development of lymphedema in a rodent radiation therapy-dependent model yet not offer tumor protection from the therapeutic effects of radiation therapy. We pre-treated rats after unilateral radical groin dissection with the organic thiophosphate radioprotectant amifostine or placebo prior to single dose post-operative groin radiation therapy and monitored hindlimb volumes, wound scores, and tissue lymphostasis. In addition, we determined whether amifostine protected human MCF7 breast cancer cells exposed to a range of radiation therapy doses in an in vitro clonogenic assay and an in vivo MCF7 tumor xenograft model. Our findings indicate that amifostine markedly reduced the volume of limb lymphedema and dramatically improved wound healing and tissue lymphostasis in the rodent lymphedema model. The in vivo and in vitro studies further demonstrated that amifostine offered no MCF7 tumor protection from radiation therapy. These pre-clinical findings provide proof-of-principle to further delineate specific mechanisms underlying amifostine's beneficial effects, determine optimal amifostine-radiation therapy dosing regimens, and thereby expedite translation into clinical trials to reduce lymphedema incidence and severity in cancer patients at high lymphedema risk in whom radiation therapy is the recommended therapy.

Keywords: lymphedema, radioprotection, amifostine

Lymphedema is tissue swelling, usually confined to the limbs, due to congenital or acquired deficiencies in lymph transport. In the United States, the secondary/acquired form of lymphedema most commonly (estimates up to 50% over the patient's lifetime) (1-6) results from surgery (regional lymphadenectomy/lymphectomy) and/or radiation therapy to the regional nodal basin to eradicate or control the spread of breast, pelvic, melanoma and other cancers. Both modalities operate independently and synergistically as lymphedema risk factors (7-9). Whereas lymphedema risk-reducing approaches such as sentinel lymph node biopsy, more limited or directed anatomic

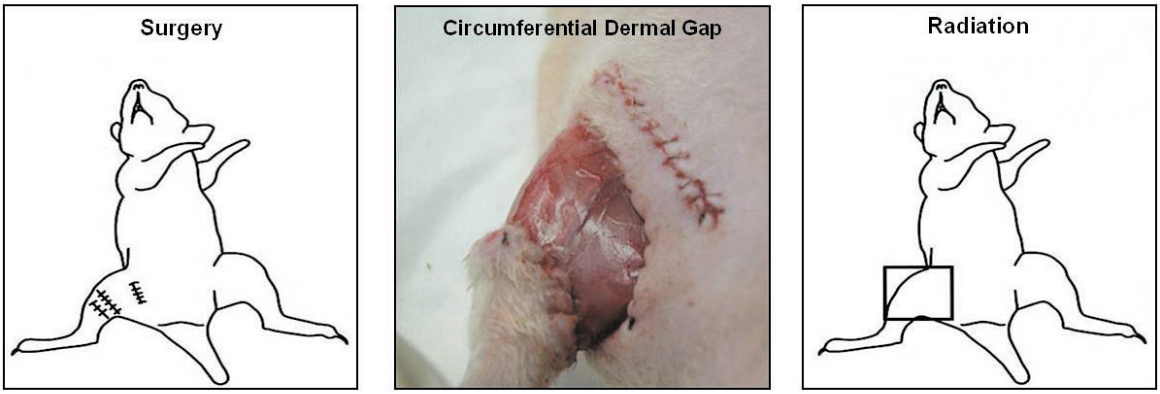

Fig. 1. Lymphedema model development. Rats underwent radical right lymphatic/nodal groin excision (A) followed by creation of a circumferential dermal gap (B) to impede skin lymphatics from establishing collateral lymph drainage. Two days following surgery, rats were administered either amifostine (200 mg/kg) or saline placebo administered subcutaneously 30 minutes prior to 45 Gy Cobalt-60 irradiation of the surgical site to further retard lymphatic regeneration (C).

dissections, or early lymphatic-venous anastomoses, may reduce lymphedema incidence and severity after surgery, no such options have been suggested to prevent or minimize radiation therapy-associated tissue damage underlying cancer treatment-related lymphedema. Indeed, more intense, higher dose, shorter duration radiation therapy regimens (hypofractionation radiation therapy) are currently being proposed to enhance long-term survival, specifically in node-positive patients with breast, pelvic, prostate and other cancers (10-14).

This study was undertaken to determine whether amifostine (Ethyol, WR2721), an organic thiophosphate radioprotectant developed by the United States Army to minimize tissue damage from nuclear fallout and now FDA-approved to reduce acute radiation therapy toxicity in head and neck cancer patients and to reduce cumulative renal toxicity of cisplatin chemotherapy in patients with ovarian cancer (15-18), might also prevent or minimize longer term complications such as lymphedema and delayed wound healing. Using our refined rodent model of stable cancer treatment-related lymphedema, requiring both extirpative surgery as well as post-operative radiation therapy (19), we followed the course of development of limb swelling, surgical wound healing, and associated tissue lymphostasis changes with and without amifostine treatment just prior to radiation therapy. In addition to assessing effectiveness of amifostine in this setting, we also further examined a key aspect of its safety – specifically whether amifostine did or did not protect MCF7 breast cancer cells from radiation therapy in an in vitro clonogenic assay and in an in vivo tumor xenograft model.

MATERIALS AND METHODS

Animal Model

Adult male Wistar-fuzzy rats (~250g), bred in-house at the University of Arizona's University Animal Care facility were used for these studies. Animal protocols were approved by the Institutional Animal Care and Use Committee and were in accord with institution guidelines. The fuzzy mutation causes hypotrichosis, or a deficiency of hair, allowing for easier and more accurate limb manipulation, measurement and examination. Model development required a surgery-plus-radiation sequence to produce sustained, stable hindlimb lymphedema. Surgery or radiation alone produces only minimal and

transient lymphedema and both were required for model development (*Fig. 1*) (19). Rats were induced with isoflurane and remained under ketamine/ace- promazine/ xylazine (0.1 ml/100g) anesthesia during the operation, which was performed under a dissecting microscope (Weck Surgical Systems, Evergreen, CO, 10X). Immediately prior, 0.1 ml intradermal Evans blue dye was injected into the dorsal skin of the right foot to highlight lymphatic vessels and nodes. A vertical incision was then made in the mid-groin and lymphatic vessels and popliteal node excised by cautery. A circumferential dermal incision was made in the right thigh and the separated skin edges sutured to the underlying muscle layer to produce a circumferential integumentary gap. Two days following surgery, rats were randomized into two groups, anesthetized, and administered either 200 mg/kg amifostine (MedImmune, Gaithersburg, MD) or an equivalent amount of isotonic saline (placebo controls) subcutaneously between the scapulae approximately 30 minutes prior to 45 Gy irradiation delivered to the surgical site using a Cobalt-60 gamma machine (Theratron 80 model) (*Fig. 1*).

Monitoring of Limb (Lymphedema) Volume and Wound Healing

Body weight, limb volumes, and wound quality were monitored weekly for 12 weeks and at termination. Absolute lymphedema volumes of both limbs were calculated from serial circumferential measurements taken by the same observer with a modified tape measure using the truncated cone formula: $(h)(C^2+Cc+c^2)/12\pi$, where C=circumference at bottom (foot), c=circumference at top (ankle), and h=height or distance between bottom and top (distance between foot and ankle). Lymphedema volume in the experimental limb was determined by subtracting the control contralateral limb volume and also expressed as percent increase in experimental limb volume compared to contralateral limb volume. This serial circumference/ truncated cone method to calculate limb/ lymphedema volume is also the standard used in evaluating and treating lymphedema patients and agrees closely with the water displacement method. During the weekly examinations, the operative wounds were inspected, photographed, and assigned a blinded wound healing quality score ranging from 1-5, with 1 representing a completely healed wound exhibiting a small, faint pink scar and 5 representing a poorly healed wound which usually included open draining areas with exposed bone. Comparisons with baseline values were summarized at 4 weeks after initial surgery (early), 8 weeks (midpoint) and 12 weeks (endpoint).

Histology and Immunostaining

At the study's termination, at 12 weeks, rats were sacrificed and tissue sections taken from both experimental limb and contralateral limb for comparison. Four small pieces of skin (approximately 5 mm^2 diameter or less) with underlying muscle tissue were removed from the top of the foot, ankle, wound area, and thigh from the experimental leg. Three small pieces of tissue from the foot, ankle, and thigh of the contralateral leg were also taken. Tissue samples were fixed in formalin, washed in 70% ethanol, processed, embedded in paraffin, and 5µm sections cut using a microtome. Following deparaffinization and tissue rehydration, hematoxylin and eosin (H&E) staining was performed to provide a general impression of edema accumulation, acanthosis and fibrosis, inflammatory cell infiltration, presence of mast cells, and relative density of both blood and lymphatic vessels. Immunohistochemistry was also performed on randomly selected deparaffinized, rehydrated tissues to further identify lymphatic channels. Antigen retrieval was accomplished using a 1X solution of a pretreatment reagent decloaking solution in a decloaking chamber (071207, Biocare Medical, Concord, CA). Slides were

then washed in phosphate buffered saline (PBS) for three minutes and blocked using TNB blocking reagent (FP1020, Perkin Elmer, Waltham, MA) containing Triton X-100 for one hour at room temperature in a humidified chamber. After one hour, Lyve-1 antibody, Lymphatic Vessel Endothelial Receptor 1 (07-538, Millipore, Billerica, MA 07-538), a lymphatic vessel endothelial cell marker, was diluted 1:100 in TNB, pipetted onto tissue sections, and left for one hour at room temperature in a humidified chamber. Tissue sections were washed and the secondary antibody Alexa Fluor 555, goat anti-rabbit (A21428, Invitrogen, Carlsbad, CA), was diluted 1:250 in TNB, pipetted onto tissue sections, and left to incubate for one hour in a humidified chamber. Slides were then washed in PBS and the fluorescent DNA stain DAPI was added onto tissue sections prior to coverslip mounting using Citifluor (19470, Ted Pella, Inc., Redding, CA). D2-40 (Cell Marque, Rocklin, CA), also a lymphatic endothelial marker, was used to further examine lymphatic density, growth, and histopathologic changes in randomly selected samples. Tissues were stained using a Ventana Medical Systems BenchMark XT IHC/ISH staining module (Ventana Medical Systems, Inc., Tucson, AZ). A "lymphostasis index" profiling the presence and severity of changes at 12 weeks in skin and subcutaneous tissue on the dorsum of the left and right foot (distant from the operative and radiation site) was also assigned in a blinded fashion by the pathologist (EB) based on the following six criteria: stratum corneum thickening, collagenosis, lymphatic density (number/dilatation), chronic inflammation, mast cell accumulation, and edema. Scores for each of the six criteria ranged from 0 (absent) to 3 (severe) with a theoretical maximum of 18 for the lymphostasis index.

In Vitro Clonogenic Assay

Human MCF7 breast cancer cells (ATCC # HTB-22), courtesy of the Experimental Mouse Shared Service facility at the University of Arizona Cancer Center cell repository ranging in number from 2,000 to 80,000 cells were grown in Cellgro RPMI-1640 (1X) media (Mediatech, Herndon, VA) supplemented with 10% fetal bovine serum (Omega Scientific, Tarzana, CA) were plated in 60mm collagen-coated culture dishes (BD Biosciences, San Jose, CA) following irradiation and treatment with either 5µM amifostine or isotonic saline placebo control thirty minutes prior to Cobalt-60 irradiation, ranging from 0-10 Gy. Culture dishes were then placed in a 5% CO_2/95% air humidified atmosphere at 37° for 12-14 days to allow sufficient colony formation. Colonies were then fixed for approximately 15 minutes with 3:1 methanol:acetic acid plus 0.5% crystal violet (Sigma, St. Louis, MO) and counted using an automated "Colcount" colony counter (Optronix, Milton Port, Oxford UK).

Tumor Xenograft Model

Athymic nude mice, approximately 5-6 weeks old, weighing 20-25 grams and housed in the University Animal Care facility were used for in vivo studies. The Experimental Mouse Shared Service (EMSS) facility at the Arizona Comprehensive Cancer Center assisted with performing the in vivo experiments and monitored the mice during the experimental period. Two days post 17-ß estradiol supplementation (Innovative Research of America, Sarasota, FL), $6x10^6$ MCF7 cells were injected into the right flank. After approximately two weeks to allow for tumor growth to achieve ~100 mm^3, mice were randomized into two groups, one group receiving subcutaneous amifostine (200 mg/kg), and the other group receiving isotonic 0.9% NaCl (placebo control) thirty minutes prior to a single 20 Gy radiation dose delivered to the tumor using a Cobalt-60 gamma unit. Mice were monitored and weighed twice weekly, and antitumor effect was monitored through direct measurement of tumor size (tumor-growth delay) using

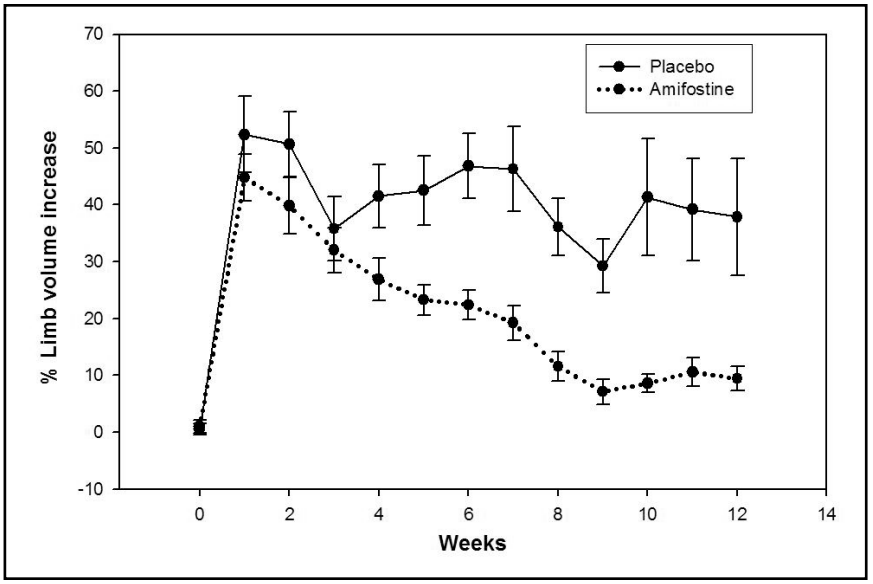

Fig. 2. Effect of amifostine on lymphedema volume. Graph shows time course leading to severe sustained lymphedema in placebo controls (solid line) compared to mild lymphedema in the amifostine-treated group (dotted line) (X ± SE).

calipers to plot growth curves and subsequent calculation of growth delay. Once tumors reached ~1,000 mm^3 (approximately 80 days after tumor cell injection), mice were sacrificed and tumors measured and collected for histological analysis.

Statistical Analysis

Limb (lymphedema) volumes, wound scores and tissue lymphostasis indices were expressed as mean ± standard error (SE) of the mean. Plating efficiency and survival fractions for the clonogenic assays were calculated and data expressed as mean ± standard deviation (SD). Regression analysis was also performed on the survival fractions. Tumor xenograft sizes were expressed as mean ± standard deviation. Student's t-test (two-tailed with unequal variance) was used to test for significance. Statistical calculations were performed using SigmaStat, version 2.03 (SPSS, Inc., Chicago, IL).

RESULTS

Amifostine Pre-Radiation Therapy Reduced Lymphedema Volume

Absolute lymphedema volume and percentage increase in limb volume (experimental limb compared to contralateral control limb) for amifostine-treated rats was significantly and progressively lower at all three time points measured – 4 weeks (initial), 8 weeks (midpoint), and 12 weeks (endpoint). The mean ± SE percent increase in experimental limb volume vs. the contralateral limb volume for amifostine-treated and placebo control rats over the course of 12 weeks is illustrated in *Fig. 2*. After acute swelling subsided one to two weeks following surgery + radiation, the placebo control and amifostine-treated groups' lymphedema volumes began to diverge, with the controls slowly increasing to a stable plateau and the amifostine-treated rats gradually losing excess limb (lymphedema) volume reaching significance at week 4 ($p < 0.05$) with this trend accelerating and gaining increased significance by twelve

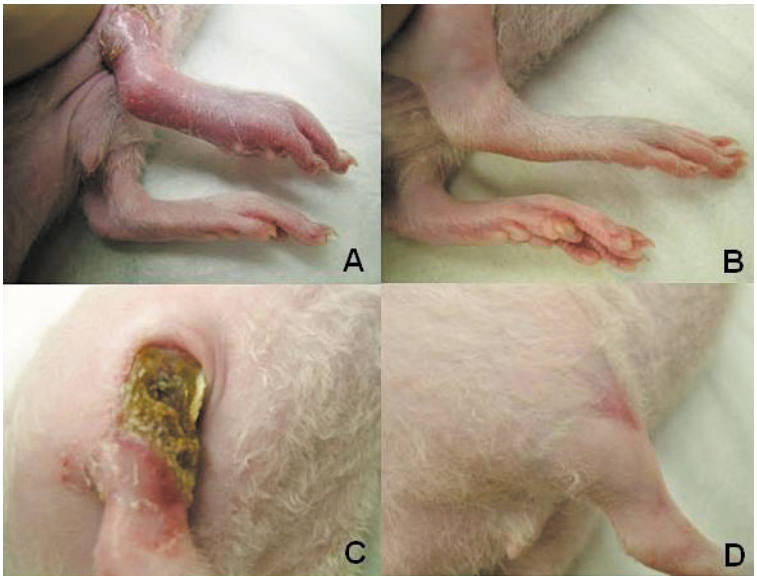

Fig. 3. Lymphedema volume and wound healing illustrations. Comparison of placebo control rats (left) with amifostine-treated rats (right). At 12 weeks, typical severe right hindlimb lymphedema (A) and impaired wound healing (C) in placebo controls (left panels) compared to mild lymphedema and nearly healed wounds in amifostine-treated rats (right panels, B, D).

TABLE 1
Effect of Pre-RT Amifostine on Limb Volume, Wound Score, and Tissue Lymphostasis Index

Outcome	Group	Week 4	Week 8	Week 12
% Limb Volume Increase	Placebo (15)	41.5±5.5	36.1±5.0	37.9±10.3
	Amifostine (21)	26.9±3.7*	11.6±2.5***	9.4±2.2**
Wound Score	Placebo (10)	—	4.4±0.3	4.5±0.2
	Amifostine (14)	—	2.7±0.3***	2.3±0.3***
Tissue Lymphostasis Index	Placebo (4)	—	—	12.3±0.5
	Amifostine (5)	—	—	7.0±0.9**

Wound score scale =1 (best, healed) – 5 (poorest, open gap). Tissue lymphostasis index scores =0 (no lymphostasis) – 18 (severe all features present). (X±SE); n=( ); *p<0.05, **p<0.01, ***p<0.001. See text for further explanation.

weeks (37.9 ± 10.3% c.f. 9.4 ± 2.2% at 12 weeks p<0.01) (*Fig. 3A,B*) (*Table 1*).

Amifostine Pre-Radiation Therapy Improved Surgical Wound Healing

A marked improvement in healing of the irradiated operative wound was also noted in the amifostine-treated group. Most of these wounds were nearly completely healed by 12 weeks with just a minimal scar remaining (*Fig. 3D*). In contrast, placebo-control rats typically displayed open, draining, non-healing wounds with exposed or broken bone fragments by the study's end (*Fig. 3C*). By week 8, wounds in amifostine-treated rats were healing as evidenced by skin closure, normal skin color, and scabbing. Placebo-

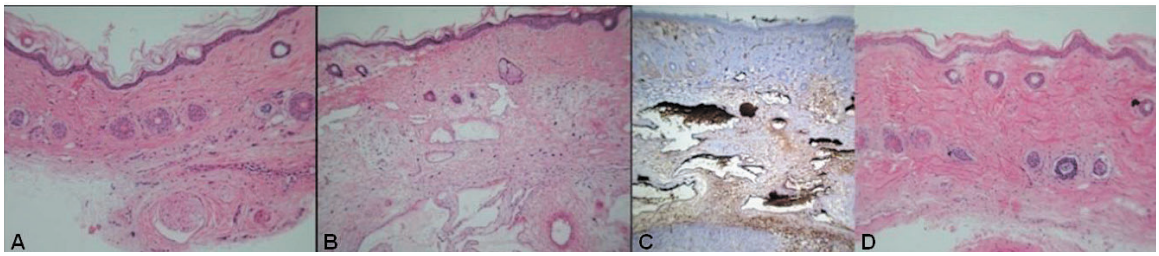

Fig. 4. Histology and Immunostaining. Comparison of skin histology from normal control left foot (A), severely lymphedematous right foot in placebo rat demonstrating enlarged lymphatic spaces and extensive mast cell infiltration (B), same section stained with D2-40 lymphatic marker (C), and (D) minimal lymphatic dilation and mild edema in right foot of amifostine-treated rat (H&E, original magnification 10X).

control wounds continued to worsen with secondary infection, serous discharge, bone exposure, and necrosis. Wound scores reflected this drastic difference between the two groups (4.5 ± 0.2 in placebo control c.f. 2.3 ± 0.3 in amifostine-treated rats at 12 weeks p<0.001) (*Table 1*).

Amifostine Pre-Radiation Therapy Reduced Histologic Features of Tissue Lymphostasis

Placebo control rats showed typical findings of severe lymphostasis, proximally associated with stratum corneum thickening, increased collagen deposition, and chronic inflammatory cell infiltrates (monocytes and lymphocytes) characterized by increased mast cells, and edema compared to normal control foot (*Fig. 4A*). Increased numbers of dilated lymphatics were noted deep in the subcutaneous tissue (*Fig. 4B,4C*). In contrast, amifostine-treated rats showed minimal or absent edema, mild or absent stratum corneum thickening, and sparse scattered dilated lymphatics. Inflammation was minimal to absent, and mast cells were seldom seen (*Fig. 4D*). At 12 weeks, the mean tissue lymphostasis index (derived from histopathologic changes on the dorsum of the right foot distant from the operative/radiation site in the groin) (*Table 1*) was substantially reduced to 7.0 in amifostine-treated compared to placebo control rats (12.3) (p=0.002) although still significantly higher than the contralateral control left foot (3.4) (p=0.012).

Amifostine Did Not Protect Cultured MCF7 Cells from Radiation Therapy

MCF7 cell survival curves with amifostine pre-radiation therapy were indistinguishable from placebo controls as evidenced by an r^2 value of 0.954. No significant difference was observed between the survival curves and survival fractions of amifostine-treated and placebo-treated cells with increasing radiation doses from 0-10 Gy as illustrated in *Fig. 5*.

Amifostine Did Not Protect Implanted MCF7 Tumors from Radiation Therapy

Figure 6, graphically displays the mean MCF7 tumor burden in placebo control and amifostine-treated mice after receiving 20 Gy radiation treatment. There was no significant difference in tumor size between the two groups at any time point during the study. Overlying skin in the amifostine-treated mice did, however, show less radiation damage (*Fig. 7B*) compared to placebo controls (*Fig. 7A*).

DISCUSSION

With today's improved, including more aggressive, approaches to cancer therapy, patients are living longer. It is therefore critical that the long-term adverse effects from these treatments be documented and avoided to preserve the quality of life of

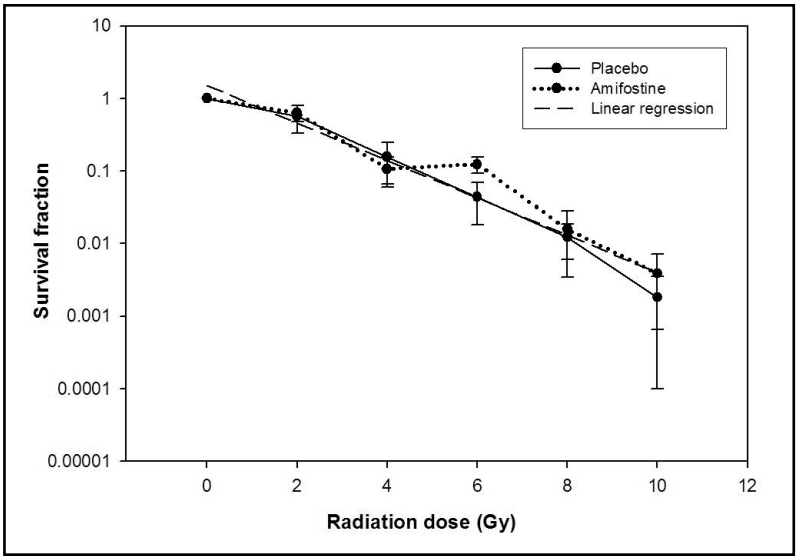

Fig. 5. In vitro clonogenic assay. MCF7 human breast cancer cell line survival curves from in vitro clonogenic assay as a logarithmic function of radiation dose (Gy). Dashed line represents linear regression of placebo control and amifostine-treated survival cell curves (r^2 = 0.954) indicating no significant protection by amifostine (X ± SD).

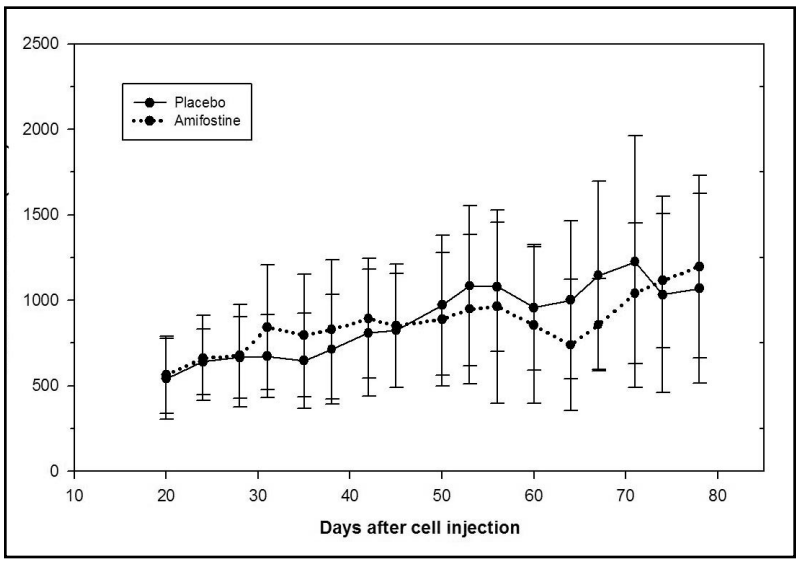

Fig. 6. Irradiated MCF7 tumor implants with and without amifostine. MCF7 tumor burden graph (X ± SD) showing equivalent tumor size (i.e., no tumor protection) in amifostine-treated compared to control mice following irradiation.

surviving patients. Lymphedema frequently occurs after treatments for cancer that include lymph node dissections and/or removal coupled with radiation to the nodal regions of the head, neck, axilla, groin, pelvic, or retroperitoneal regions. Lymphatic obstruction that results causes lymphatic dilatation, collateral formation, and pooling of lymph, which gradually give way to massive ectasia, valvular destruction, retrograde flow, and lymph coagulation (20-22). As high protein lymphedema persists,

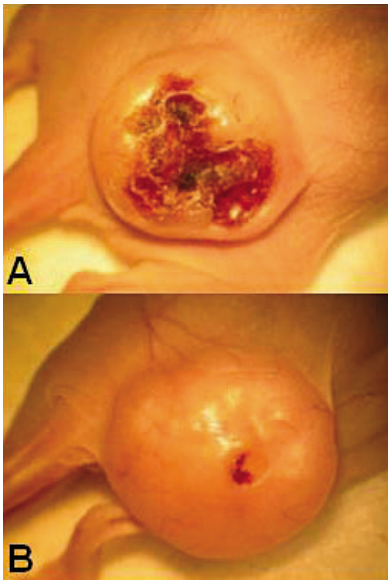

Fig. 7. Tumor xenograft model. MCF7 tumor implants in athymic nude mice. Compared to placebo-treated tumors in the implant model (A), radiation damage was markedly reduced in the overlying skin of the amifostine-treated mice (B).

"skin failure" ensues with stratum corneum thickening, chronic inflammation particularly attracting mast cells, intense lymphangiogenesis/hemangiogenesis, progressive fibrosis and adipose deposition, and interference with immune cell trafficking and particulate clearance, associated with increased susceptibility to recurrent opportunistic infections, lymphorrhea, and occasionally rapidly fatal lymphangiosarcoma (20-22). Current modes of physical, pharmacological, and operative lymphedema treatment (such as debulking, liposuction, lymphatic transplantation, and lymphatic-venous derivative shunts) (23) offer symptomatic relief, generally to a limited degree, and require lifelong labor-intensive and costly compliance.

Our study examined a novel approach to prevent the development of lymphedema by selectively protecting lymphatic tissue from the damaging effects of radiation. The results show that administering the radioprotectant amifostine pre-radiation therapy can sharply minimize development of lymphedema while also improving skin wound healing. Amifostine probably exerted its beneficial effect both by reducing damage to existing lymphatic and blood vessels at the borders of the irradiated circumferential skin gap as well as by accelerating lymphangiogenesis/regeneration producing bridging collaterals through the better healing wound so that lymphedema volume, inflammation, and tissue changes of lymphostasis were all markedly reduced.

Amifostine was selected as the radioprotectant for this study because of its FDA-approved status and safety in mitigating mucositis and xerostomia during radiation therapy in head and neck cancer patients and its wider use outside the United States in reducing acute radiation therapy toxicity in a wide variety of other cancers. Other potential longer term beneficial effects on delayed radiation therapy complications have received little attention and study. Amifostine is an organic thiophosphate which acts as a free radical scavenger enhancing oxygen removal and induction of hypoxia produced by thiol oxidation reactions. It is rapidly converted from its inactive form WR2721 to the active WR1065 by alkaline phosphatase present in circulating blood (and serum-containing culture media) and on the endothelial cell surface. The striking differential cytoprotective effect of normal in contrast to tumor tissue is multifactorial and thought to be due to lower concentration of alkaline phosphatase, acidic pH, passive rather than active absorption of the drug, and poor vascularization in tumor tissue (24,25). Variation in amifostine's protective effect has been exhibited among normal tissues that have been studied. Bone marrow and skin show the highest levels of protection against radiation therapy-toxicity with radiation dose-modifying factors of 2.7 ± 0.1 and 2.4 ± 0.1, respectively (25). The rapidly proliferating cells of the skin make it very susceptible to radiation damage (26), and the marked improvement observed in wound

healing in our study, although not previously reported in clinical trials, is consistent with amifostine's selective protective effect on the skin. To some extent, this action may have also contributed to the reduction in lymphedema volume and tissue evidence of lymphostasis. However, the important issue of radiation damage to the lymphatic and blood vasculatures and dose-modifying angioprotection by amifostine has not been directly investigated.

In this study, we added further evidence of amifostine's safety from any tumor protection by demonstrating specifically that MCF7 human breast cancer cells exposed to amifostine do not exhibit any survival advantage from radiation therapy damage either in vitro or in vivo. There is strong experimental and clinical involving many different tumor types (breast cancers were not previously included) demonstrating a lack of radio- and chemoprotection by amifostine. A recent meta-analysis (27) illustrates the safety and efficacy of amifostine for the protection of normal tissues during radiation therapy and the lack of tumor protection during radiation therapy. Further, the meta-analysis provides strong evidence for proceeding with radiation therapy approaches and treatment schedules using higher doses, hypofrequency, and greater chances of cancer control.

Finally, radiation therapy is used to treat approximately 60% of all cancers in the United States, and the dosage prescribed is based on the tolerance of the patient and the surrounding normal tissue and is usually less than the dose optimal for cure (26,28). If given prior to radiation therapy in optimal dosing regimens, amifostine would allow radiation dose escalation, improving chances of more complete cancer control while reducing damage to normal tissue and improving quality of life. The current study suggests that pre-radiation therapy amifostine may prevent or minimize one of the most important and frequent detractors from this quality of life in breast, pelvic, and melanoma cancer survivors: the development of lymphedema and in many such patients, poorly healing draining wounds and fistulae. The results thereby provide proof-of-principle for expeditiously translating these experimental findings into Phase II clinical trials in this at-risk patient population and also incorporating lymphedema and wound healing outcome measures into ongoing amifostine trials.

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REFERENCES

1. Petrek, JA, RT Senie, M Peters, et al: Lymphedema in a cohort of breast carcinoma survivors 20 years after diagnosis. Cancer 92 (2001), 1368-1377.
2. Kissin, MW, G Querci della Rovere, D Easton, et al: Risk of lymphoedema following the treatment of breast cancer. Br. J. Surg. 73 (1986), 580-584.
3. American Cancer Society. Lymphedema: Results from a workshop on breast cancer treatment-related lymphedema and lymphedema resource guide. Cancer. 83 (1998), 2775-2890.
4. Petrek, JA, MC Heelan: Incidence of breast carcinoma-related lymphedema. Petrek J, PI Pressman, RA Smith (Eds). Cancer 83 (1998), 2776-2781.
5. Agrawal, S, JM Kane, BA Guadagnolo, et al: The benefits of adjuvant radiation therapy after therapeutic lymphadenectomy for clinically advanced, high-risk, lymph node-metastatic melanoma. Cancer 115 (2009), 5836-5844.
6. Lawenda, BD, TE Mondry, PA Johnstone: Lymphedema: A primer on the identification and management of a chronic condition in oncologic treatment. CA Cancer J. Clin. 59 (2009), 8-24.

7. Tsai, RJ, LK Dennis, CF Lynch, et al: The risk of developing arm lymphedema among breast cancer survivors: A meta-analysis of treatment factors. Ann. Surg. Oncol. 16 (2009), 1959-1972.
8. Ozaslan, C, B Kuru: Lymphedema after treatment of breast cancer. Am. J. Surg. 187 (2004), 69-72.
9. Pierce, LJ: The use of radiotherapy after mastectomy: a review of the literature. J. Clin. Oncol. 23 (2005), 1706-1717.
10. Koukourakis, MI, PG Tsoutsou, IM Abatzoglou, et al: Hypofractionated and accelerated radiotherapy with subcutaneous amifostine cytoprotection as short adjuvant regimen after breast-conserving surgery: Interim report. Int. J. Radiat. Oncol. Biol. Phys. 74 (2009), 1173-1180.
11. Manavis, J, J Ambatzoglou, K Sismanidou, et al: Computed tomography (CT) scan evaluation of late toxicity following hypofractionated/accelerated radiotherapy with cytoprotection (HypoARC) in breast cancer patients treated with conservative surgery. Am. J. Clin. Oncol. 29 (2006), 479-483.
12. Koukourakis, MI, PG Tsoutsou, I Abatzoglou: Computed tomography assessment of lung density in patients with lung cancer treated with accelerated hypofractionated radiochemotherapy supported with amifostine. Am. J. Clin. Oncol. 32 (2009), 258-261.
13. Koukourakis, MI, C Simopoulos, M Pitiakoudis, et al: Hypofractionated accelerated radiotherapy, cytoprotection and capecitabine in the treatment of rectal cancer: A feasibility study. Anticancer Res. 28 (2008), 3035-3040.
14. Koukourakis, MI, S Touloupidis, J Manavis, et al: Conformal hypofractionated and accelerated radiotherapy with cytoprotection (HypoARC) for high risk prostatic carcinoma: Rationale, technique and early experience. Anticancer Res. 24 (2004), 3239-3243.
15. Koukourakis, MI, PG Tsoutsou, I Abatzoglou, et al: Postoperative accelerated radiotherapy with cytoprotection followed by three-dimensional conformal boost in patients with early endometrial/cervical cancer. Tumori. 95 (2009), 455-460.
16. Santini, V: Amifostine. Chemotherapeutic and radiotherapeutic protective effects. Expert Opin. Pharmocother. 2 (2001), 479-489.
17. Waselenko, JK, TJ MacVittie, WF Blakely, et al: Medical management of the acute radiation syndrome: Recommendations of the Strategic National Stockpile Radiation Working Group. Ann. Intern. Med. 140 (2004), 1037-1051.
18. Hensley, ML, KL Hagerty, T Kewalramani, et al: American Society of Clinical Oncology 2008 clinical practice guideline update: Use of chemotherapy and radiation therapy protectants. J. Clin. Oncol. 27 (2009), 127-145.
19. Lee-Donaldson, L, MH Witte, M Bernas, et al: Refinement of a rodent model of peripheral lymphedema. Lymphology 32 (1999), 111-117.
20. Witte, CL, MH Witte: Lymph circulatory dynamics, lymphangiogenesis, and pathophysiology of the lymphvascular system. In: *Vascular Surgery*. Rutherford, RB (Ed.), 6th ed., Philadelphia, PA: W.B. Saunders Company, pp. 2379-2396, 2005
21. Witte, MH, DL Way, CL Witte, et al: Lymphangiogenesis: Mechanisms, significance and clinical implications. In: *Regulation of Angiogenesis*. Goldberg, ID, EM Rosen (Eds.), 1st ed. Basel, Switzerland: Birkhäuser Verlag, pp. 65-112, 1997.
22. Witte, CL, MH Witte: Physiology and imaging of the lymphatic vascular system. In: *Vascular Surgery: A Comprehensive Review*. Moore, WS (Ed.), 6th ed., Philadelphia, PA: W.B. Saunders Company, pp. 837-859, 2002.
23. The diagnosis and treatment of peripheral lymphedema. 2009 Consensus document of the International Society of Lymphology. Lymphology 42 (2009), 42, 51-60.
24. Dorr, RT, K Lagel, S McLean: Cardioprotection of rat heart myocytes with amifostine (Ethyol) and its free thiol, WR-1065, in vitro. Eur. J. Cancer 32A(suppl 4) (1996), S21-S25.
25. Yuhas, JM: Active versus passive absorption kinetics as the basis for selective protection of normal tissues by s-2-(3-aminopropylamino)-ethylphosphorothioic acid. Cancer Res. 40 (1980), 1519-1524.
26. Stone, HB, CN Coleman, MS Anscher, et al: Effects of radiation on normal tissue: consequences and mechanisms. Lancet Oncol. 4 (2003), 529-536.
27. Sasse, AD, LG Clark, EC Sasse, et al: Amifostine reduces side effects and improves complete response rate during radiotherapy: Results of a meta-analysis. Int. J. Radiat. Oncol. Biol. Phys. 64 (2006), 784-791.
28. Wasserman, TH: Radiotherapeutic studies with amifostine (Ethyol). Semin. Oncol. 21(suppl 11), (1994), S21-S25

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