Original Article

Interstitial Wires Releasing Diffusing Alpha Emitters Combined With Chemotherapy Improved Local Tumor Control and Survival in Squamous Cell Carcinoma-bearing Mice

Tomer Cooks, MSc[1], Lior Arazi, MSc[2,3], Margalit Efrati, MSc[1], Michael Schmidt, MSc[2], Gideon Marshak, MD[1,4], Itzhak Kelson, PhD[2,3], and Yona Keisari, PhD[1]

BACKGROUND: The objective of this study was to examine the combined effect of diffusing alpha-emitter radiation therapy (DART) together with the chemotherapeutic agent cisplatin on tumor development. **METHODS:** BALB/c mice bearing squamous cell carcinoma tumors were treated with radium 224 (^{224}Ra-)–loaded stainless steel wires, releasing short-lived, alpha-emitting atoms from their surface. A concomitant regimen of cisplatin doses (5 mg/kg per dose) was given intravenously for the evaluation of the combined effect. Animals were monitored for tumor growth and survival. **RESULTS:** First, the authors observed that alpha particles and cisplatin inhibited SQ2 cell proliferation in vitro and promoted apoptosis. Treatment of tumor-bearing mice indicated that, when a regimen of 2 separate doses of cisplatin was given concomitantly with a single intratumoral ^{224}Ra-loaded wire, there was moderate tumor growth inhibition relative to what was observed from each treatment alone. When tumors were treated with 2 radioactive wires positioned near the tumor base and a similar drug administration, the growth arrest effect intensified, and there also was a significant increase in survival rates. The combined treatment reduced both local tumor growth and metastatic spread to the lungs. **CONCLUSIONS:** Antitumor activity and overall survival of metastatic tumor-bearing mice were improved significantly by the combined treatment. These results highlight the potential benefit of alpha radiation-based radiotherapy in combination with chemotherapeutic drugs for anticancer treatment. **Cancer 2009;115:1791–801. © 2009 American Cancer Society.**

KEY WORDS: alpha radiation, squamous cell carcinoma, chemoradiotherapy, cisplatin.

There is a growing interest in effective anticancer agents that also exhibit radiosensitizing ability.[1] The administration of systemic therapies allows modulation of radiation response to improve tumor control

Corresponding author: Yona Keisari, PhD, Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel; Fax: (011) 972-3-6406098; ykeisari@post.tau.ac.il

[1]Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; [2]School of Physics and Astronomy, Raymond and Beverly Sackler Faculty of Exact Sciences, Tel Aviv University, Tel Aviv, Israel; [3]Althera Medical, Tel Aviv, Israel; [4]Institute of Oncology, Davidoff Center, Rabin Medical Center, Petach Tikva, Israel

This work was performed in partial fulfillment of the requirements toward a PhD degree of Tomer Cooks, Sackler Faculty of Medicine, Tel-Aviv University. We thank Professor. Theodore S. Lawrence (Department of Radiation Oncology, University of Michigan Medical School, Ann Arbor, Mich) for his helpful comments and suggestions.

Received: July 9, 2008; **Revised:** September 8, 2008; **Accepted:** October 22, 2008

Published online: February 5, 2009 © 2009 American Cancer Society

DOI: 10.1002/cncr.24191, www.interscience.wiley.com

(radiosensitization) or to prevent normal tissue toxicity (radioprotection).[2] When used concurrently with radiation therapy, cisplatin (CP) acts as a radiosensitizer, and such treatment of advanced head and neck squamous cell carcinoma (HNSCC) achieved higher response rates, prolonged mean survival, and increased survival rates and was characterized by longer local recurrence-free survival rates and improved organ preservation.[3] The combination of low-linerar energy transfer (LET) (x-rays, gamma rays) radiation therapy (RT) and platinum derivatives is a common anticancer strategy[4-6] and achieves a better antitumor effect compared with each modality alone.[7,8] CP has been described as an apoptosis enhancer that cross-links cellular DNA, forming bifunctional adducts with the N7 of guanine bases,[9] and reportedly is remarkably effective when combined with RT in several different malignancies, including both small cell and nonsmall cell lung carcinoma, lymphoma, and head and neck carcinomas.[10-12]

Over 60 clinical trials that were conducted before 1993 indicated a 12% reduction in the risk of death from HNSCC when patients were treated with both definitive and postoperative chemoradiotherapy (CRT) involving CP.[7] Moreover, for patients who had carcinomas with a poor prognosis, adjuvant, postoperative, high-dose CP and irradiation given concomitantly resulted in better control of locoregional disease and disease-free survival than postoperative RT alone.[13]

In recent years, with the use of low-LET alongside traditional external RT, increasingly, more attention has been attracted toward the use of RT methods with high-LET.[14-16] This approach calls for studies on the biologic effects created by the combined use of DNA-affecting agents. such as CP and high-LET RT. Because CP sensitizes cells to ionizing radiation mainly by inhibition of the nonhomologous end-joining pathway (for DNA repair of double-strand breaks), and because a considerable fraction of double-strand breaks induced by high-LET particles are repairable, the combination of these 2 treatments potentially may achieve enhanced anticancer effects.[17,18]

Alpha particles are a form of high-LET radiation. Only a few hits of alpha particles to the nucleus usually are required to ensure cell death, including in hypoxic cell populations.[19,20] It is believed that the mechanisms by which cell death is initiated involve both apoptosis and necrosis, depending on numerous biologic and physical factors like the cell type, the microenvironment, and the characteristics of the DNA lesions formed by the particles.[21,22] Unlike photon- or electron-based RT, very little is known about the antitumor potential of the combination of CP and alpha-based RT, although there is an increasing number of studies dealing with the role of alpha particles in anticancer treatment modalities.[23,24]

In previous studies. we described a novel technique, termed diffusing alpha-emitter RT (DART), using the characteristics of the radium 224 (^{224}Ra) alpha decay chain for the treatment of solid tumors. It was reported that the intratumoral insertion of thin wires loaded with ^{224}Ra into SCC tumors promoted extensive local alpha radiation through decay products and subsequent tumor growth inhibition and improved animal survival rates.[25,26] The objective of the current work was to evaluate the possible synergism between the intratumoral alpha radiation treatment that we developed and the traditional, potent, radiosensitizing drug, CP,[27] against SCC-derived tumors.

MATERIALS AND METHODS

Tumors

The SQ2 cell line[28] was kindly provided by Dr. Gad Lavie from the Sheba Medical Center, Tel-Hashomer, Israel. This is a murine anaplastic cell line and was generated from an SCC tumor that developed spontaneously in a male BALB/c mouse. The tumor was characterized and determined as SCC at the Pathology Institute of Sheba Medical Center. The cells were grown in Dulbecco Modified Eagle Medium supplemented with 10% fetal calf serum (Biological Industries, Beit Haemek, Israel), L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (100 μg/mL).

Radioactive Microplates

An experimental setup was developed to implant ^{224}Ra on the bottom of 96-well microplates (Corning, Corning, NY). The implantation was performed inside a vacuum chamber using an 8-headed 'stamp' that fit a single column of the microplate, and each head carried a small amount of thorium 228 (^{228}Th) activity. The ^{224}Ra activity implanted in each well was controlled by the time of exposure to the ^{228}Th stamps.

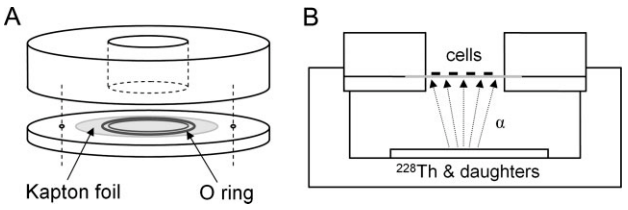

FIGURE 1. (A,B) The kapton well apparatus.

Cell Proliferation Assay

The antiproliferative effects of alpha particles and CP, alone and in combination, were determined using a 3-bis (2 methoxy-4 nitro-5 sulfenyl)-(2H)-tetrazolium-5-carboxanilide (XTT) assay (Cell Proliferation Kit; Biological Industries). Cells (10^4 per well) were seeded in 96-well microplates that were implanted with escalating levels of ^{224}Ra activity (radioactive microplates). Cells were allowed to grow for the required period; then, the activated XTT mixture was added to a final concentration of 0.33 mg/mL according to the manufacturer's instructions. After 2 hours of incubation, absorbance was analyzed using an automated spectrophotometer (VersaMax; Molecular Devices, Sunnyvale, Calif) at 475 nm wavelength.

Kapton Wells Setup

Cells seeded on a thin (7.5 μm) kapton (polyimide) foil were irradiated by alpha particles traversing the foil from below. The setup (see scheme in Fig. 1) was comprised of 2 stainless-steel rings that were identical in diameter (35 mm) with a center hole of 9 mm. One of the rings was 3 mm high, and the second ring was 12 mm high. The kapton foil (Dupont, Luxembourg) was placed between the 2 rings (with the 12-mm ring on top) covering the hole; then, the rings were screwed tightly with a rubber O-ring to ensure impermeability (Fig. 1A). After ultraviolet light sterilization of the wells for at least 1 hour, cells were seeded on the foil at a density of $5 \cdot 10^4$ cells per well and were exposed to the alpha particle flux 24 hours later. Exposure was performed by positioning the cells seeded on the foil 9.8 mm above a silicon wafer coated with a thin layer of 4.0 μCi (148 kBq) ^{228}Th in secular equilibrium with its daughters in air (Fig. 1B). The total alpha particle flux across the kapton foil was measured by an EG&G solid-state alpha particle detector (EG&:G ORTEC, Oak Ridge, Tenn). Exposure times were 0 minutes, 1 minute, and 3 minutes, and the average flux was 2.8×10^4 alpha particles/mm^2 · miniutes across the exposed area. The calculated average dose rate, which was based on a Monte-Carlo calculation (not shown) and was performed using the SRIM-2003 code,[29] was 0.75 ± 0.05 grays (Gy) per minute. The variations of the average dose rate across the irradiated area were on the order of 10% to 15%.

For detection of the fraction of apoptotic, cells we used the annexin-V/phosphatidyl inositol (PI) assay (MBL, Naka-ku Nagoya, Japan). SQ2 cells were seeded in kapton wells, as described above, and treated either with CP, or alpha particle flux, or a combination of both modalities. Four hours after any treatment, cells were collected using trypsin and washed once with phosphate-buffered saline (PBS) followed by another wash with binding buffer. The cells were incubated with 10 μL annexin-V–fluorescein isothiocyanate and 5 μL PI in the dark for 15 minutes and analyzed in a flow cytometer (Facsort, Becton Dickinson, NJ).

Western Immunoblotting

Cells were washed with PBS and lysed with lysis buffer (50 mmol/L Tris-HCl, pH 7.6; 20 mmol/L $MgCl_2$; 200 mmol/LNaCl; 0.5% NP40; 1 mmol/L dithiothreitol; and 1 mmol/L antiproteases). Lysates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by immunoblotting with 1:1000 anticleaved caspase-3 antibody. Then, the immunoblots were exposed to 1:5000 peroxidase/goat-antirabbit immunoglobulin G, and protein bands were observed by enhanced chemiluminescence (ECL) and quantified by densitometry with an Image Master VDS-CL (Amersham Pharmacia Biotech, Arlington Heights, Ill) using TINA 2.0 software (Ray Tests). The ECL kit was obtained from Amersham.

Animals

Male BALB/c mice (8-12 weeks old) were obtained from the breeding colony of Tel-Aviv University, Israel. Animal care and experimentation was carried out in accordance with Tel-Aviv University guidelines. All surgical and invasive procedures were conducted under anesthesia by intraperitoneal inoculation of Imalgen (100 mg/kg;; Fort

Dodge Laboratories, Germantown Hills, Ill) and xylazine hydrochloride (10 mg/kg; VMD, Arendonk, Belgium) solution in 0.25 mL of PBS.

Tumor Cell Inoculation

Animals were inoculated intracutaneously with 5×10^5 SQ2 cells in 0.2 mL Hank Balanced Salt Solution (Biological Industries) into the low lateral side of the back. Local tumor growth was determined by measuring 3 mutually orthogonal tumor dimensions with a digital caliper (Mitutoyo, Onomy, Japan). The volume of tumor was calculated using the formula: $V = (\pi/6) \cdot D_1,D_2,D_3$, where D_1, D_2, and D_3 indicate the measured dimensions.

^{224}Ra-Loaded Wire (Diffusing Alpha-Emitter Radiation Therapy Wire) Preparation

^{224}Ra-loaded wires were prepared using a ^{228}Th generator (as described in detail by Arazi et al[25]). In this setup, positive ^{224}Ra ions that were emitted by recoil from a surface layer containing ^{228}Th were collected electrostatically near the tip of a thin stainless-steel wire (0.3 mm in diameter; Golden Needle, Suzhou, China). Then, the wires were heat-treated to induce radium diffusion away from the surface to a typical depth of 10 nm. The ^{224}Ra-impregnated wires then were characterized by an alpha particle detector to account for their ^{224}Ra activity and the release rate of radon 220 (^{220}Rn). The wires that were used in the in vivo experiments had ^{224}Ra activity in the range of 11.5 to 29.7 kBq with ^{220}Rn desorption probabilities of 22% to 36% (ie, for each ^{224}Ra decay on the wire, the probability of ^{220}Rn release into the tumor was 22%-36%).

Wire Insertion

Wires, either loaded with ^{224}Ra or inert, that were cut to a length of 5-6 mm were placed near the tip of a 23-gauge needle attached to a 2.5 mL syringe (Picindolor, Rome, Italy) and inserted into the tumor by a plunger placed internally along the syringe axis.

Histology

Histologic analysis was performed on BALB/c mice lungs, both treated and untreated. Immediately after their removal, the lungs were fixed in a 4% formaldehyde solution (Sigma, Rehovot, Israel) for at least 24 hours. The preserved specimens were embedded in paraffin, and sections (5-10 μm) were stained with hematoxylin and eosin (Surgipath, Richmond, Va) and inspected for metastases. Metastatic burden quantification was done by summing the gray values of all pixels in the region of interest divided by the number of pixels using Image J free software (available at: http://rsb.info.nih.gov/ij/ accessed January 25, 2009).

Statistical Analysis

The statistical significance (*P* value) of the differences between tumor volumes in the various groups was assessed by applying using a 2-sided Student *t* test and a repeated-measures analysis of variance. Survival analysis (Mantel-Cox test) was carried out using StatSoft Statistica 7.

RESULTS

Combined Alpha Particles and Cisplatin Enhanced Cell Death and Arrested Proliferation in Culture

SQ2 cells were plated in 96-well plates implanted with ^{224}Ra atoms (0, 0.02, 0.063, 0.2, 0.63, and 2 Bq/mm^2 radioactive microplates). For each radioactive dose, 3 concentrations of CP were added to the microplate (0.3, 3, and 30 μM) to determine whether cells that received both treatments would be affected more compared with cells that received each treatment alone. Cell numbers were assessed by the XTT assay after 24, 48, and 72 hours of incubation and are expressed as the percentage of nontreated control cells. Substantial proliferation arrest caused by alpha irradiation alone was detected after 48 hours, and the effect intensified after 72 hours (Fig. 2). A dose-dependent inhibition for cell growth effect was observed and ranged from 18% in wells that were exposed to 0.63 Bq/mm^2 up to 52% inhibition in 2 Bq/mm^2 wells that were incubated for 72 hours. A similar but stronger antiproliferative effect was observed for cells that were incubated with 0.3 μM CP and radioactivity. A higher proliferation inhibition, as illustrated in Figure 2, was evident after 48 hours and after 72 hours. Cells that were exposed to 0.2 Bq/mm^2 for 72 hours demonstrated 18% inhibition, and 0.3 μM of CP caused 21% inhibition.

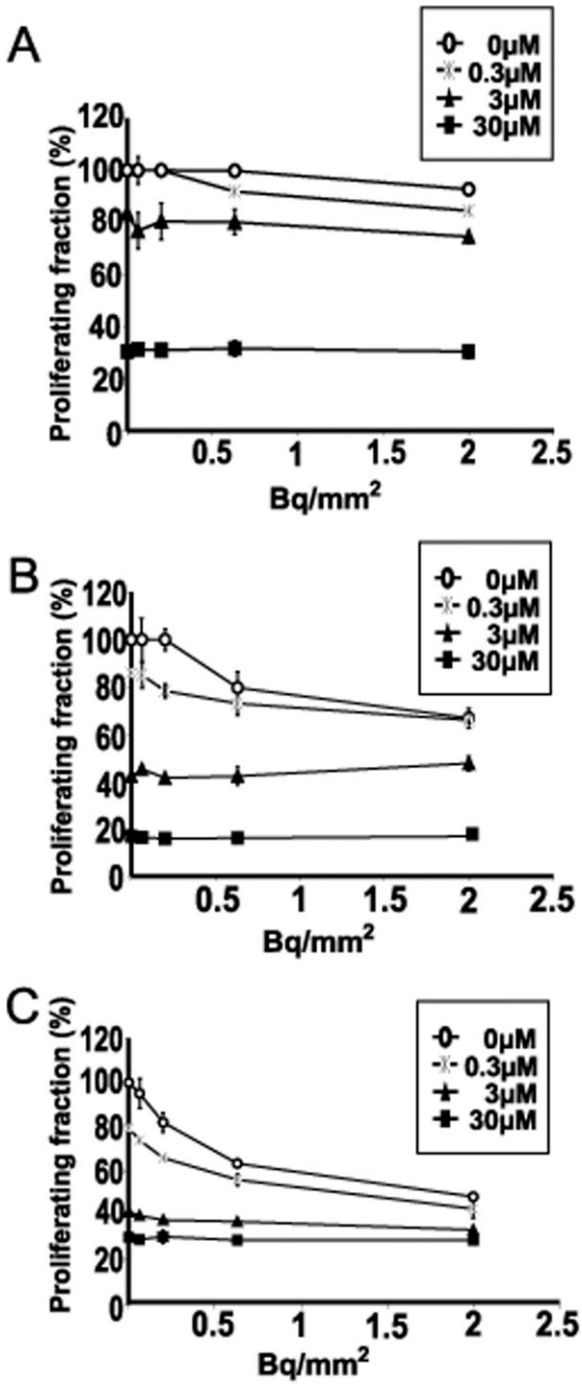

FIGURE 2. Inhibitory effect of alpha particles and cisplatin on SQ2 cell proliferation. Cells were irradiated by implanted radium 224 (^{224}Ra) atoms in escalating intensities, with or without the addition of different cisplatin concentrations. Three different irradiation durations were tested: 24 hours (A), 48 hours (B) and 72 hours (C). Bars indicate the standard error.

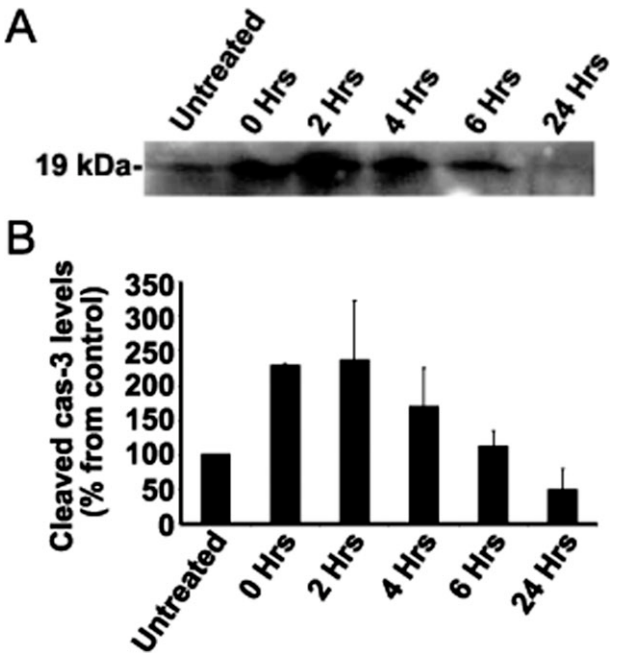

FIGURE 3. Alpha irradiation initiates apoptotic cell death in SQ2 cells. Cells that were exposed to 1.5 grays of alpha particles were examined for cleaved caspase-3 (cas-3) levels at 5 different time points postirradiation. (A) Typical immunoblot of cell lysates that were subjected to Western blot analysis. (B) The ratios between the optical densities obtained from blotting were normalized to the untreated control lysates. Bars indicate the standard deviation.

However, the combined treatment gave rise to 34% proliferation arrest. At higher levels of the drug (3 and 30 μM), a strong antiproliferative effect (>60%) was induced by the drug alone and obscured any additive effects with alpha radiation.

Combined Alpha Particles and Cisplatin-Induced Apoptosis

A time course measurement of the expression levels of cleaved caspase-3 was conducted in SQ2 cells that were exposed to 1.5 Gy of alpha irradiation to assure that these cells die through apoptosis. Figure 3 shows that apoptosis mechanisms are initiated immediately postirradiation in a process that remains intensified 2 to 4 hours after the treatment. A similar examination was dedicated to determine the optimal apoptosis-enhancing concentration of CP, which was set at 30 μM (data not shown). Apoptotic cell death was monitored by the annexin-V dye-binding assay. Cells were costained with propidium iodide, which permeates into dead cells, to distinguish apoptotic cells

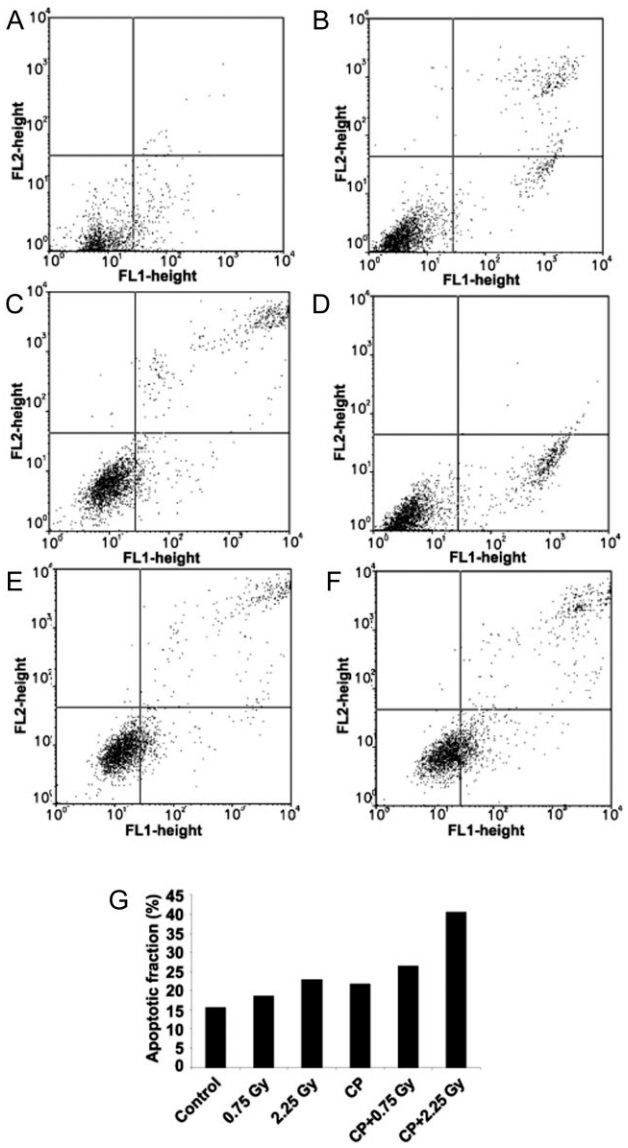

FIGURE 4. Apoptosis induction in SQ2 cells by alpha particles and cisplatin (CP)-treated cell cultures stained with annexin-V and phosphatidyl inositol revealed untreated cells (control) (A), cells that were exposed to 0.75 grays (Gy) of alpha particles (B), cells that were exposed to 2.25 Gy of alpha particles (C), cells that were treated with 30 μM CP for 4 hours (D), cells that were treated with both 0.75 Gy of alpha particles and 30 μM CP for 4 hours (E), cells that were treated with both 2.25 Gy of alpha particles and 30 μM CP for 4 hours (F), and the percentage of apoptotic cells analyzed by flow cytometry (G).

from necrotic cells. Cells that were seeded in the kapton wells were exposed to 2 doses of alpha irradiation (0.75 Gy and 2.25 Gy) without or with CP (30 μM) and were compared with treatment by CP only or nontreated cells. Figure 4 shows the percentage of apoptotic cells in all treated cultures. Less than 16% of untreated cells stained positive with annexin-V, and only a moderate increase was detected for cells that were irradiated with 0.75 Gy (19%). When cells were exposed to 2.25 Gy or to CP alone, more apoptosis occurred (22%-23% of cells stained positive with annexin-V). Furthermore, when chemotherapy and alpha-radiation were applied together, the apoptotic fraction increased for both radioactivity dose levels (27% for CP and 0.75 Gy; 41% CP and 2.25 Gy).

A Single ^{224}Ra-Loaded Wire Insertion Combined With 2 Cisplatin Treatments Moderately Retarded Tumor Growth

SQ2 tumors in BALB/c mice were implanted with a ^{224}Ra-loaded wire in combination with CP given intravenously. The ^{224}Ra-loaded wire treatment was performed as tumors reached the average size of 6 to7 mm in greatest dimension. The chemotherapeutic agent was injected in 2 separate doses of 5 mg/kg per animal—the first dose was administrated 1 day before ^{224}Ra-loaded wire treatment, and the second dose was given 5 days later. Inert (nonradioactive) wires that were identical in shape to the radioactive wires were used as controls. The outcome of this line of experiments (Fig. 5) suggests that both α radiation and chemotherapy (the ^{224}Ra wire and CP groups) contribute to tumor growth retardation as stand-alone treatments. Average tumor volumes 30 days after tumor transplantation were very similar for both treatment groups (48%-51% for the inert control group). The average tumor volumes in mice that received the combined treatment (^{224}Ra wire + CP), were smaller relative to the volumes in mice that received each treatment alone (40% for the inert control group on Day 30), but the differences were not statistically significant (*P* values between the combination group and the CP or ^{224}Ra wire groups were .054 and .105, respectively).

Insertion of 2 ^{224}Ra-Loaded Wires Combined With 2 Cisplatin Doses Significantly Retarded Tumor Growth and Prolonged Survival

In light of the findings described above and previous studies conducted in our laboratory,[26] we examined the effect

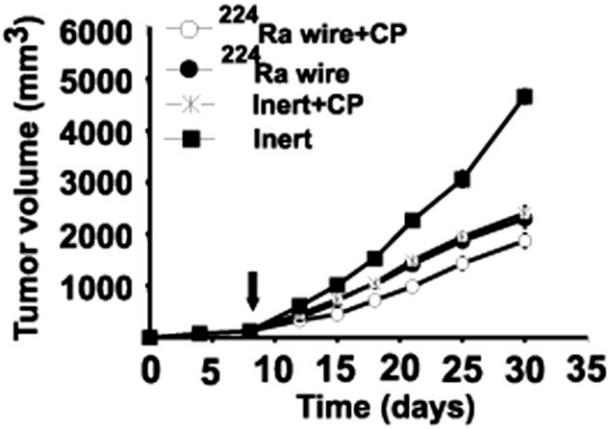

FIGURE 5. Tumor growth inhibition by cisplatin (CP) and a single alpha-radiation treatment. BALB/c mice bearing SQ2 tumors were treated with a single, radium 224 (^{224}Ra)-loaded, stainless-steel wire (^{224}Ra wire), or with 2 separate doses of CP (5 mg/kg each), or with both and were monitored for tumor growth (bars represent standard errors). Inert indicates tumor-bearing mice that were treated with inert wires (n = 15); Inert + CP, tumor-bearing mice that were treated with inert wires and CP (n = 15); ^{224}Ra wire, tumor-bearing mice that were treated with radioactive wires loaded with ^{224}Ra atoms (n = 14); ^{224}Ra wire + CP, tumor-bearing mice that were treated with radioactive wires loaded with ^{224}Ra atoms and CP (n = 15).

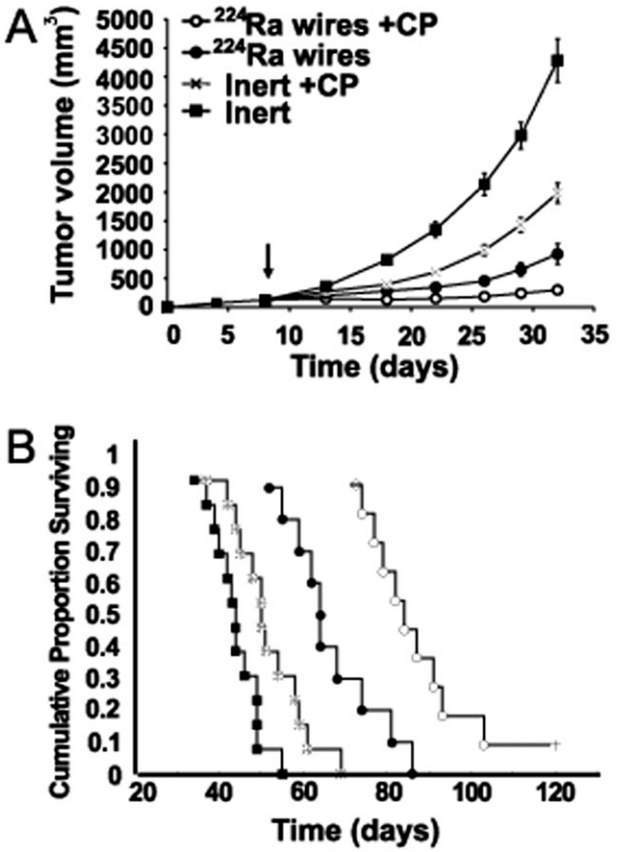

FIGURE 6. Tumor growth inhibition and prolonged survival after treatment with cisplatin (CP) combined with 2 radium 224 (^{224}Ra)-loaded, stainless-steel wires (^{224}Ra wires). BALB/c mice bearing SQ2 tumors were treated either with 2 ^{224}Ra wires, or with 2 separate doses of CP (5 mg/kg each), or with both and were monitored for tumor growth and survival (bars represent standard errors). Inert indicates tumor-bearing mice that were treated with inert wires (n = 15); Inert + CP, tumor-bearing mice that were treated with inert wires and CP (n = 15); ^{224}Ra wire, tumor-bearing mice that were treated with radioactive wires loaded with ^{224}Ra atoms (n = 14); ^{224}Ra wire + CP, tumor-bearing mice that were treated with radioactive wires loaded with ^{224}Ra atoms and CP (n = 15). (A) Tumor development. (B) Survival curve.

of 2 ^{224}Ra-loaded wires inserted horizontally into the base of each tumor in combination with 2 doses of chemotherapy. The double ^{224}Ra-loaded wire insertion had a prominent effect on tumor development, as shown in Figure 6A. A pronounced difference between tumor volumes of the irradiated group (^{224}Ra wires) and the CP-treated animals was evolving 10 days after radioactive treatment. This difference became more evident with time; and, 32 days after tumor cell inoculation, the average tumor volume in the CP-treated group was 2.14-fold greater than the average tumor volume in the ^{224}Ra-treated group. Moreover, major growth inhibition of tumors was achieved when both modalities, CP and ^{224}Ra wires, were administrated concomitantly. Greater than 50% of the animals in the combination group had tumor retardation at some point of the monitoring, with complete tumor eradication in 1 animal. Twenty-four days after ^{224}Ra treatment, the average tumor volume in the combined treatment group was 14-fold smaller compared with the average tumor volume in the inert control group (300 mm^3 and 4290 mm^3, respectively) and 3-fold smaller compared with the best effect achieved by the radioactive wires alone (920 mm^3). A survival follow-up was done on all 4 tested groups to examine the differences in effects on life expectancy between treatments. The findings presented in Figure 6B indicate that all 3 treatments prolonged lifespan significantly. Mice that were treated with CP alone survived longer than the control group (mean survival, 51.4 days and 43.9 days, respectively; P = .0093), and the group that received ^{224}Ra-loaded wires survived even longer (mean survival, 66.5 days; P = .00001). Moreover, the integration of both CP and intratumoral radioactive wires yielded a pronounced and significantly larger effect on life expectancy and almost

doubled the average lifespan (range, 87.3 days-98% compared with the inert group) of the tumor-bearing mice.

Insertion of 2 ^{224}Ra-Loaded Wires Combined With 2 Cisplatin Doses Significantly Reduced Metastatic Load in the Lungs

Tumor-bearing mice were treated with 2 radioactive wires, or with 2 CP injections, or with both. Twenty-six days after tumor transplantation, as the average tumor size of the inert control group exceeded 2 cm (greatest dimension), the mice were killed, and histologic assessment of lung metastases in tissue sections[26] was conducted. Each of the 4 groups (inert, ^{224}Ra wires, CP, and CP + ^{224}Ra wires) contained 3 animals, and normal lung tissues were taken from healthy BALB/c mice. Figure 7 describes the inhibition of lung metastatic load in mice that were treated with both intratumoral alpha radiation and CP compared with the lungs from mice that were treated with inert wires. Both treatments given alone (CP or ^{224}Ra-loaded wires) also decreased metastatic burden although to a smaller extent than the combined treatment.

DISCUSSION

We investigated the combined anticancer effects of a commonly used chemotherapeutic agent with a novel technique based on the release of alpha-emitting daughters of ^{224}Ra. Both in vitro and in vivo examinations were done to assess the collaboration between CP and intratumoral dispersed radioactive atoms against SCC-derived tumors.

In vitro experiments indicated that treatment of SCC cells with CP for 4 hours initiated apoptotic cell death mechanisms and retarded cell proliferation. A similar observation was made when cells were exposed to doses greater than 0.75 Gy of alpha particle fluxes. When both treatments were applied concomitantly, enhanced apoptosis and reduced proliferation were detected.

To examine the collaboration between alpha radiation and CP in vivo, tumor-bearing mice were treated with each treatment modality alone and in combination. A single ^{224}Ra wire inserted into the center of each tumor, given together with 2 equal and separate intravenous CP injections, produced a moderate gain compared with the effects of chemotherapy or RT administered alone.

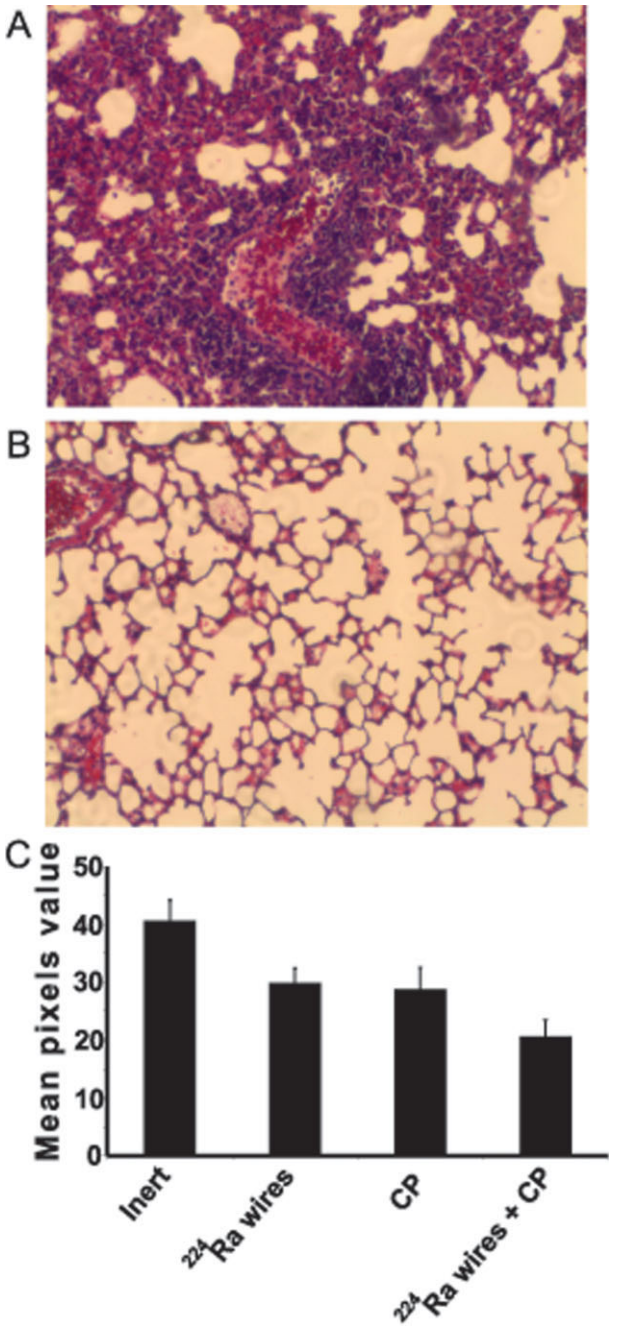

FIGURE 7. Metastatic lung burden. Hematoxylin and eosin-stained lung cross-sections were analyzed by using Image J software to assess for the presence of metastases. (A) Lung section from a mouse that was treated with inert wires (Inert). (B) Lung section from a mouse that was treated with both cisplatin (CP) and radioactive wires loaded with radium 224 (^{224}Ra) atoms (^{224}Ra wires + CP). (C) The ratio between lungs from inert wire-treated mice compared with mice that received CP either alone or combined with ^{224}Ra wires compared with normal healthy lungs from mice without tumors (original magnification, ×10 in A and B).

In a previous study, it was observed that 2 ^{224}Ra-loaded wires positioned at the tumor base significantly arrested the growth of primary SCC tumors and reduced the metastatic process.[26] Thus, we combined this efficient treatment protocol with chemotherapy. The findings revealed that combined treatment with 2 ^{224}Ra-loaded wires and 2 doses of CP caused extensive tumor retardation in almost all treated mice. The combined treatment also resulted in substantial prolongation in average life expectancy for this group of mice relative to all other treatments. The enhanced tumor destruction of 2 wires implanted into the tumor base may have been caused by the higher radioactive dose delivered to the most aerated domains of the tissue. This may result in increased distribution of radioactive atoms throughout the tumor on 1 hand and destruction of blood vessels, which irrigate the periphery of the tumor, on the other hand.

Because BALB/c mice bearing SQ2 tumors die primarily from lung metastases,[28] the major increase in life expectancy induced by the combination of ^{224}Ra-loaded wires and CP indicated that inhibition of the metastatic process may be involved. Thus, we examined the metastatic burden in the lungs from untreated and treated mice and observed that the ^{224}Ra wires + CP treatment group attained a significant reduction of 51% in the lung metastatic load compared with the group of inert-treated animals.

The extensive experimental and clinical knowledge on the effects of external RT combined with CP can be deployed to only a limited extent in understanding the effects of radionuclide therapy, even if it is comprised of beta particles.[30] Furthermore, therapy based on alpha particle dispersion is complicated, because factors like diffusion inside the tissue and the blood vessel network may play a vital role alongside the macroscopic dose concept.

Although alpha radiation is the most lethal form of radiation and has the highest LET, its short range in tissue limited the use of alpha particles in the treatment of cancer to single cells, small cell clusters, or micrometastases and still is in early clinical trials. To date, alpha irradiation has been considered unsuitable for external-beam RT of solid tumors, and brachytherapy was not feasible because of the lack of a practical treatment modality to effectively cover the tumor volume with these short-range particles.

DART, for the first time, may provide an efficient and secure method for prolonged treatment of the entire volume of solid tumors with a therapeutic dose of alpha particles. The first clinical indication considered for the use of DART is the treatment of recurrent HNSCC, which is a highly prevalent disease with high mortality. HNSCC tumors are fast growing, hypoxic, and, in many cases, inoperable when considering the preservation of organ function and quality of life. Conventional RT therapy (either external beam or brachytherapy) for HNSCC tumors usually is complicated by the proximity of normal tissues and organs, such as the spinal cord, brainstem, parotid glands, and optic pathway structures, which inevitably are exposed to high radiation doses and manifest side effects that include chronic radiation toxicities like mucosal fibrosis, bone necrosis, and atrophy. Not only can these effects compromise optimal treatment delivery, they also can lead to a lifetime risk with profound effects on patient quality of life.

The results described in this study suggest that radionuclides emitting alpha particles could cause more damage to primary tumors as well as to metastases when targeted into the tumors and strengthened by an apoptosis-enhancing agent. Because of the finding that CP is not the only agent that can be used for CRT, the concurrent treatment of SCC patients with RT and other radiosensitizing agents should be considered. Gemcitabine,[31] paclitaxel,[32] or concurrent docetaxel and CP with hyperfractionated irradiation[33] produced significant antitumor activity and encouraging preliminary response and survival data. Concomitant CRT is evidently a preferential treatment for patients who have unresectable SCC.[34-37]

Alpha radiation in the form of ^{224}Ra-loaded wires may add a clinical tool to the arsenal of radiotherapy for SCC and calls for additional research on its combination with other drugs in addition to CP. The results presented here indicate that DART coupled with a platinum derivative is an effective treatment against SCC tumors in mice. The mechanisms by which an apoptosis-triggering agent like CP can intensify the killing effect that alpha particles exerts on malignant tissues will be studied further.

Conflict of Interest Disclosures

Supported in part by the Hamer Medical Research Fund and the Alexander Germanis Fund.

Tomer Cooks, Lior Arazi, Michael Schmidt, Itzhak Kelson, and Yona Keisari received financial support from Althera Medical Ltd.

The authors received financial support from Althera Medical. A patent was filed by the Tel Aviv University on this treatment modality. Several the authors are shareholders in the company (T.C., L.A., M.S., I.K., Y.K.) and have served as Althera employees (I.K., L.A.), or consultants (Y.K.).

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