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ORIGINAL ARTICLE

Hideya Takeuchi · Gulshan Ara · Edward A. Sausville
Beverly Teicher

Jasplakinolide: interaction with radiation and hyperthermia in human prostate carcinoma and Lewis lung carcinoma

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Abstract *Purpose*: Jasplakinolide is a novel natural product anticancer agent which acts by inducing over-polymerization of actin. The aim of the current study was to explore the activity of jasplakinolide with hyperthermia and radiation. *Methods*: The response of human PC-3 and DU-145 prostate carcinoma cells and DU-145 xenografts and the response of the Lewis lung carcinoma to jasplakinolide were studied. *Results*: Jasplakinolide was cytotoxic toward human prostate carcinoma cells, DU-145, PC-3 and LNCaP in culture, killing 1 log of cells with 0.8, 0.3 and 0.07 μM of drug in 24 h, respectively. The combination of jasplakinolide and hyperthermia resulted in primarily additive cell killing by the two modalities in the three prostate carcinoma lines. In combination with radiation, jasplakinolide produced some diminution in the shoulder of the survival curve of normally oxygenated PC-3 cells and was a radiation sensitizer of hypoxic DU-145 cells and hypoxic PC-3 cells. In vivo, jasplakinolide was an active antitumor agent against the Lewis lung carcinoma and the DU-145 prostate carcinoma xenograft. Jasplakinolide was a radiation sensitizer in the Lewis lung carcinoma. Jasplakinolide was also effective against the systemic Lewis lung carcinoma, decreasing lung metastases. Lung metastases were further decreased when jasplakinolide was administered along with radiation to the subcutaneous primary tumor. In the DU-145 tumor, the effects of jasplakinolide and fractionated radiation for 1 or 2 weeks appeared to be primarily additive. *Conclusion*: Jasplakinolide is an interesting new anticancer agent for which further study both as an anticancer agent and in combined modality regimens is warranted.

Key words Jasplakinolide · Hyperthermia · Radiation sensitization · Prostate cancer

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H. Takeuchi · G. Ara · B. Teicher
Dana-Farber Cancer Institute and Joint Center for Radiation Therapy, 44 Binney St., Boston, MA 02115, USA

E.A. Sausville
Developmental Therapeutics Program,
Division of Cancer Treatment and Diagnosis,
National Cancer Institute, National Institutes of Health,
Bethesda, MD 20892, USA

B. Teicher (✉)
Lilly Research Laboratories,
Lilly Corporate Center, DC 0540,
Indianapolis, IN 46033, USA
Tel. +1-317-276-2739; Fax +1-317-277-6285;
E-mail TEICHER_BEVERLY_A@Lilly.com

Introduction

Jasplakinolide (NSC 613009), a cyclodepsipeptide isolated from *Jaspis johnstoni* Indo-Pacific sponge [16], has been shown to be a potent inhibitor of the proliferation of human breast carcinoma cells [16], human acute myeloid leukemia cells [7] and human prostate carcinoma cells [4]. Exposure of human PC-3 prostate carcinoma cells to jasplakinolide results in the formation of multinucleated cells and disruption of the actin cytoskeleton in the cells [2, 6, 10, 14]. In addition to inducing over-polymerization of actin, jasplakinolide inhibits bombesin-stimulated phosphorylation of focal adhesion kinase (FAK) blocking FAK signal transduction in human PC-3 prostate carcinoma cells [6].

Androgen-independent prostate cancer is a lethal disease against which conventional chemotherapy has been remarkably ineffective. It can be argued that prostate cancer patients are often elderly with other medical conditions requiring reduction in the doses of chemotherapy to less than effective levels [3, 4, 9]. However, even when drug doses are maintained at "therapeutic" levels, prostate cancer remains a chemotherapy-resistant disease. Thus, androgen-independent carcinoma is an important target for new drug discovery.

Marine organisms have been a rich source of compounds with pharmacologic activity and pharmaceutical potential [1, 12, 13]. The cytotoxic cyclodepsipeptide jasplakinolide was isolated from a sponge of the genus *Jaspis* [5, 17, 22]. Jasplakinolide emerged from the NCI cell line screen as a chemical structure with potent activity against the growth of human prostate cancer cell lines [4]. In independent studies jasplakinolide has been found to be cytotoxic toward human breast cancer cell lines [16] and human acute myeloid leukemia cells [7]. Jasplakinolide stabilizes F-actin microfilaments by altering the dynamic process of polymerization and depolymerization [6, 10, 14]. Jasplakinolide may be the first potential antineoplastic agent with this mechanism of action. Jasplakinolide's action to promote F-actin microfilament polymerization recalls the action of agents such as paclitaxel and crytophycin whose mechanism of cytotoxicity involves stabilization of tubulin microfilaments in cells.

The current studies were undertaken as an initial exploration of the potential application of jasplakinolide as a chemotherapeutic agent in vitro alone and in combined modality regimens against human prostate carcinoma using cells, and in vivo using the murine Lewis lung carcinoma or human DU-145 prostate carcinoma xenograft.

Materials and methods

Drugs

Jasplakinolide was obtained as a gift from Dr. David J. Newman, Natural Products Branch, National Cancer Institute, Fredrick, Md.

Cell lines

The human DU-145 prostate carcinoma line was developed from a lesion in the brain of a patient with widespread metastatic carcinoma of the prostate. The DU-145 line is not hormone-sensitive and is weakly positive for acid phosphatase. The line is near triploid with a mode of 64 chromososomes. The DU-145 cell line has been maintained in cell culture since 1976 and is grown in Eagle's minimum essential medium supplemented with 10% fetal bovine serum. For experiments, DU-145 cells (5×10^6) were implanted as stock tumors then a tumor brei was prepared. Cells (5×10^6) from the stock tumors were implanted subcutaneously into a hind leg of male nude mice (Harlan-Sprague-Dawley, Indianapolis, Ind.). The DU-145 tumors grew to 150 mm^3 in 12.3 $\pm$ 1.4 days [20, 21].

The human LNCaP prostate carcinoma line was developed from a needle aspiration biopsy of the left supraclavicular lymph node of a patient with metastatic prostate carcinoma in 1977. LNCaP cells produce human prostatic acid phosphatase and prostate-specific antigen and have androgen receptors. The androgen receptor status and response to androgens and antiandrogens for LNCaP cells has been characterized [8, 12, 14]. The line is hypotetraploid with a mode chromosome number of 84. The LNCaP cell line was maintained in Waymouth's medium supplemented with 15% fetal bovine serum.

The human PC-3 prostate carcinoma line was developed from a grade IV prostatic adenocarcinoma in 1978. The PC-3 line is androgen-independent, exhibits low acid phosphatase and testosterone 5α-reductase activity. The cells are near triploid with a modal chromosome number of 62. The PC-3 cell line was maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum.

All three prostate tumor lines were purchased from the American Type Culture Collection, Rockville, Md.

Cell survival experiments

Human DU-145, LNCaP or PC-3 cells in exponential growth in monolayer culture were exposed to various concentrations of jasplakinolide from 0.001 to 5 μM for 1, 3, 6, 18, 24 or 48 h at 37 °C in a humidified atmosphere of air with 5% carbon dioxide. After the drug exposure the cells were washed with phosphate-buffered 0.9% saline, then suspended by treatment with trypsin, and known numbers of cells were plated in duplicate dishes for colony formation. The colonies were allowed to grow for 2 weeks and then were visualized by staining with crystal violet. The colonies were counted manually and the surviving fractions compared with untreated control cells were calculated. The results are expressed as the means of three experiments $\pm$ SEM.

Hyperthermia

Human DU-145, LNCaP or PC-3 cells in exponential growth in monolayer culture were exposed to various concentrations of jasplakinolide from 0.001 to 5 μM for 24 or 48 h with hyperthermia treatment (42 °C or 43 °C) during the final hour of drug exposure. Hyperthermia was accomplished by submerging the sealed flasks in a circulating waterbath set to temperature. After the hyperthermia treatment the cells were washed, suspended by treatment with trypsin and plated for colony formation as described above.

Radiation

Human DU-145, LNCaP or PC-3 cells in exponential growth in monolayer culture were exposed to jasplakinolide (0.1 μM and 1 μM) for 24 h. Normally oxygenated cells were exposed to jasplakinolide for 24 h at 37 °C prior to and during radiation delivery. Hypoxic cells were exposed to jasplakinolide for 24 h at 37 °C, with the final 4 h under hypoxic conditions (under a circulating 95% nitrogen/5% carbon dioxide atmosphere) prior to and during radiation delivery. Radiation was delivered using a Gamma Cell 40 (Atomic Energy of Canada, Ottawa, Canada; ^{137}Cs, 0.88 Gy per min). After radiation delivery the cells were washed and then suspended by treatment with trypsin and plated for colony formation as described above.

Tumor growth delay experiments

The doses of jasplakinolide used were the maximally tolerated doses that produced no deaths in these tumor/host systems.

Lewis lung tumor

The Lewis lung tumor [9, 10, 11] was carried in male C57BL mice (Taconic Laboratories, Germantown, N.Y.). For the experiments, 2×10^6 tumor cells prepared from a brei of several stock tumors were implanted subcutaneously into a hind leg of male mice aged 8–10 weeks. Tumors reach a volume of 100 mm^3 by day 7 after tumor cell implantation.

For experiments, animals bearing subcutaneously implanted Lewis lung carcinoma were treated with: (1) jasplakinolide (1.5 mg/kg) by intraperitoneal injection twice per day on days 5 through 11 after tumor implantation, (2) jasplakinolide (10.5 mg/kg) administered subcutaneously by continuous infusion using a 7-day Alzet pump (Alza Corporation, Palo Alto, Calif.) on days 5 through 11 after tumor implantation or (3) jasplakinolide (21 mg/kg) administered subcutaneously by continous infusion using a 14-day Alzet pump on days 5 through 18 alone or along with fractionated radiation (2, 3 or 4 Gy) delivered locally to the tumor-bearing limb on days 7 through 11 using a Gamma Cell 40 ^{137}Cs irradiator (0.88

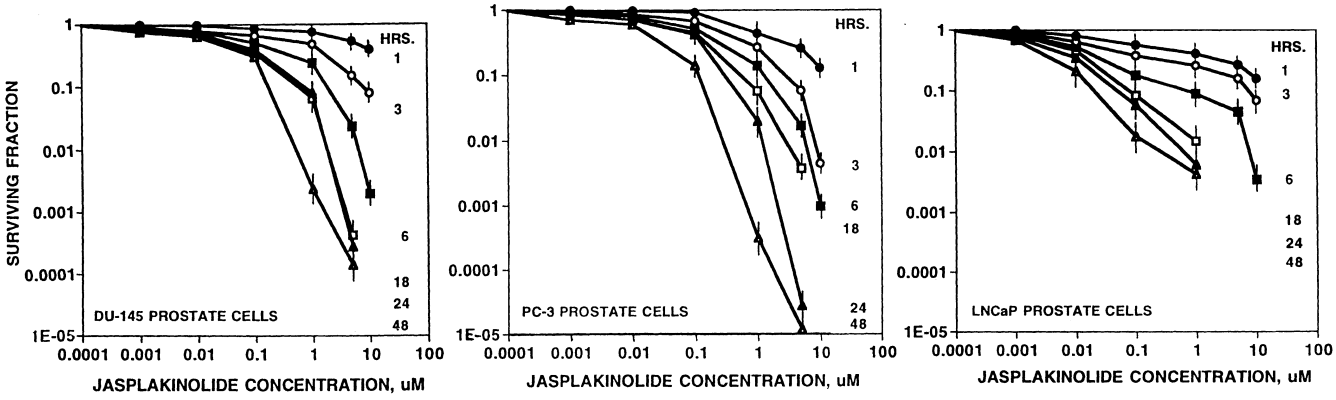

Fig. 1 Survival of human prostate carcinoma cells, DU-145, PC-3 and LNCaP, exposed to various concentrations of jasplakinolide for 1 h (●), 3 h (○), 6 h (■), 18 h (□), 24 h (▲) or 48 h (△). Points are the means of three independent experiments; *bars* are the SEM

Gy/min, Atomic Energy of Canada). The animals were placed in lead jigs so that their bodies received less than 2% of the delivered dose.

DU-145 prostate carcinoma

For experiments, male nude mice (Harlan-Sprague-Dawley) bearing subcutaneously implanted DU-145 human prostate carcinoma xenografts were treated with: (1) jasplakinolide (10 mg/kg) subcutaneously by continuous infusion using a 7-day Alzet pump on days 9 through 15 alone or along with radiation (2, 3 or 4 Gy) delivered locally to the tumor-bearing limb on days 12 through 16 or (2) jasplakinolide (20 mg/kg) subcutaneously by continuous infusion using a 14-day Alzet pump on days 9 through 22 alone or along with radiation (2, 3 or 4 Gy) delivered locally to the tumor-bearing limb on days 12 through 16 and 19 through 23. Radiation therapy was delivered as described above.

Tumor growth delay

The progress of each tumor was measured thrice weekly by caliper measurements in two dimensions until it had reached a volume of 1500 mm^3. Tumor growth delay was calculated as the number of days required for each tumor to reach a volume of 500 mm^3 as compared with untreated control tumors. Each treatment group comprised five mice and the experiment was repeated three times. Tumor growth delay was taken as the mean value ± SE calculated for the treatment group as compared with the control group [18, 19].

Results

Human DU-145, PC-3 and LNCaP prostate carcinoma cells in exponential growth were exposed to various concentrations of jasplakinolide (0.001–10 μ*M*) for various periods of time from 1 to 48 h (Fig. 1). There was a marked concentration and time dependence for jasplakinolide cytotoxicity in each of the prostate tumor cell lines. The DU-145 cells were least sensitive to jasplakinolide and the LNCaP cells were most sensitive. After 24 h exposure to the drug, 1 log of DU-145 cells had been killed by 0.8 μ*M* of the drug, 1 log of PC-3 cells had been killed by 0.3 μ*M* of the drug and 1 log of LNCaP cells had been killed by 0.07 μ*M* of the drug.

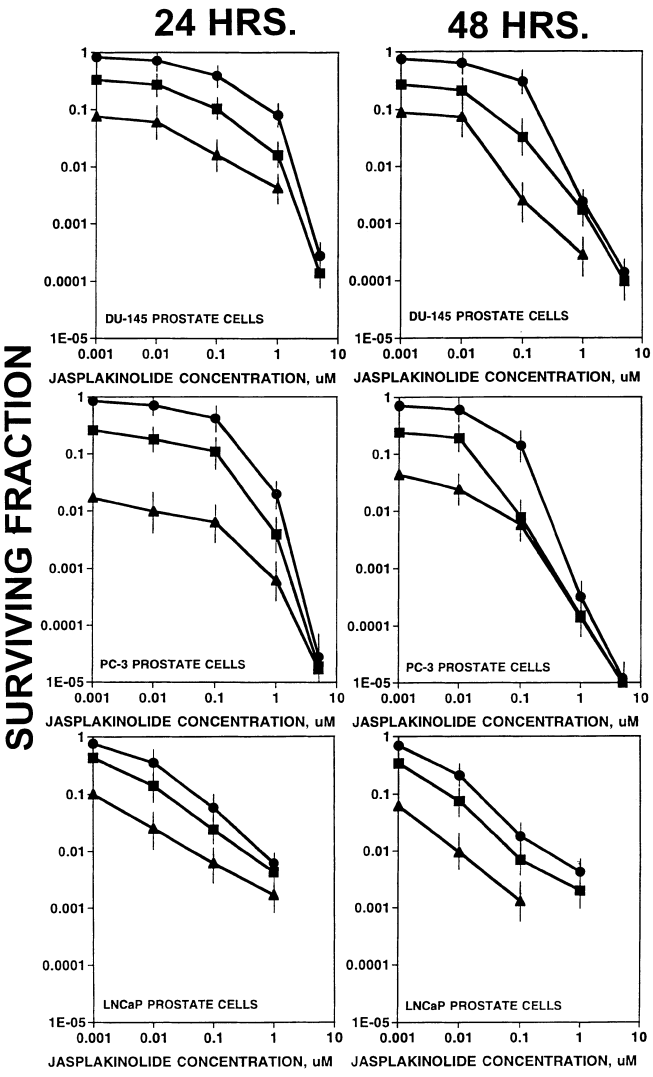

Fig. 2 Survival of human prostate carcinoma cells exposed to various concentrations of jasplakinolide for 24 h or 48 h and hyperthermia during the last hour of drug exposure. The hyperthermia treatments were: 37 °C, control (●), 42 °C, 1 h (■) and 43 °C, 1 h (▲). Points are the means of three independent experiments; *bars* are the SEM

The combination of jasplakinolide exposure for 24 or 48 h and clinically relevant levels of hyperthermia (42 °C or 43 °C) during the last hour of drug exposure was

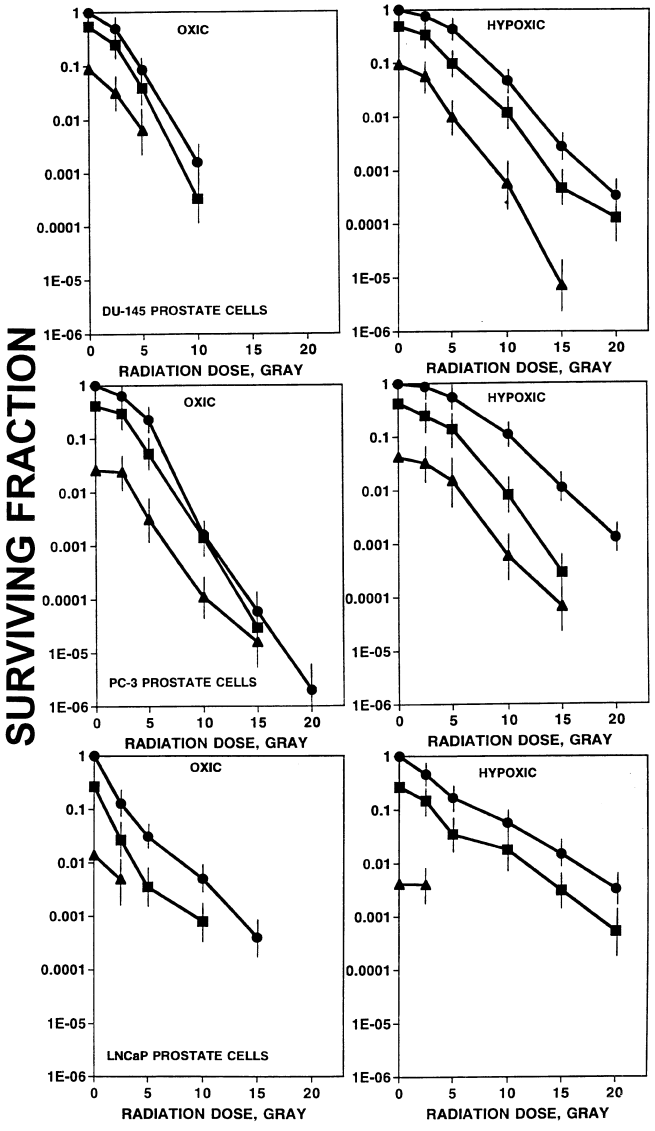

Fig. 3 Survival of human prostate carcinoma cells exposed to various concentrations of radiation alone (●) or along with 0.1 μ*M* jasplakinolide for 24 h (■) or along with 1 μ*M* jasplakinolide for 24 h (▲) under oxygenated or hypoxic conditions. Radiation was delivered at the end of the drug exposure. Points are the means of three independent experiments; *bars* are the SEM

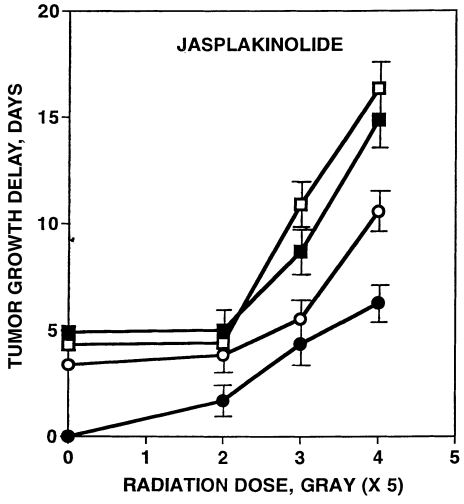

Fig. 4 Tumor growth delay of subcutaneously growing Lewis lung carcinoma after treatment locally to the tumor-bearing limb with fractionated radiation daily for 5 days on days 7–11 (●), with 1.5 mg/kg. i.p. jasplakinolide twice per day on days 5–11 and fractionated radiation on days 7–11 (○), with 10.5 mg/kg jasplakinolide by continuous infusion on days 5–11 and fractionated radiation on days 7–11 (■) or with 21 mg/kg jasplakinolide by continuous infusion on days 5–18 and fractionated radiation on days 7–11 (□). The tumors were 150–200 mm^3 in volume at the initiation of radiation therapy. Points are the means of three independent experiments; *bars* are the SEM

Table 1 Growth delay and number and percent large (> 3 mm) lung metastases on day 20 in animals bearing subcutaneously implanted Lewis lung carcinoma after treatment with fractionated radiation with or without jasplakinolide (*JAS*). Tumor growth delay is the difference in days for treated vs. control tumors to reach 500 mm^3. Control tumors reached 500 mm^3 in 11.4 ± 0.7 days after subcutaneous implantation of 2×10^6 Lewis lung carcinoma cells

Treatment group	Tumor growth delay (days)	No. of lung metastases (% large)
Controls		30 (57)
5 × 2 Gy	1.7 ± 0.3	18 (48)
5 × 3 Gy	4.4 ± 0.3	15 (48)
5 × 4 Gy	6.3 ± 0.4	13 (40)
JAS (1.5 mg/kg)		
Twice daily, days 5–11	3.4 ± 0.3	10 (42)
+ 5 × 2 Gy	3.9 ± 0.3	11 (47)
+ 5 × 3 Gy	5.6 ± 0.5	8 (47)
+ 5 × 4 Gy	10.6 ± 0.6	2.5 (20)
JAS (10.5 mg/kg)		
7-day pump, days 5–11	4.9 ± 0.4	6 (38)
+ 5 × 2 Gy	5.0 ± 0.5	5 (36)
+ 5 × 3 Gy	8.7 ± 0.9	4.5 (25)
+ 5 × 4 Gy	14.9 ± 1.3	4 (10)
JAS (21 mg/kg)		
14-day pump, days 5–18	4.4 ± 0.3	9 (33)
+ 5 × 2 Gy	4.4 ± 0.3	8 (30)
+ 5 × 3 Gy	10.9 ± 1.1	5 (24)
+ 5 × 4 Gy	16.4 ± 1.6	3.5 (33)

examined over a jasplakinolide concentration range in each of the three prostate carcinoma cell lines (Fig. 2). The hyperthermia treatments alone produced surviving fractions of 0.45 and 0.88 in DU-145 cells, 0.38 and 0.016 in PC-3 cells and 0.63 and 0.10 in LNCaP cells at 42 °C for 1 h and 43 °C for 1 h, respectively. In general, concentrations of jasplakinolide up to about 0.1 μ*M* exhibited additive cytotoxicity with the hyperthermia treatments. At concentrations of jasplakinolide of 1 μ*M* and greater, the additivitiy with hyperthermia was lost and the cytotoxicity of jasplakinolide became dominant. The shoulders on the survival curves for the LNCaP cells treated with hyperthermia and jasplakinolide was less prominent than the shoulders on the survival curves of the DU-145 cells and the PC-3 cells. Therefore 1 log of cell killing was obtained with 42 °C for 1 h along with 0.13, 0.10 and 0.02 μ*M* jasplakinolide for 24 h in the

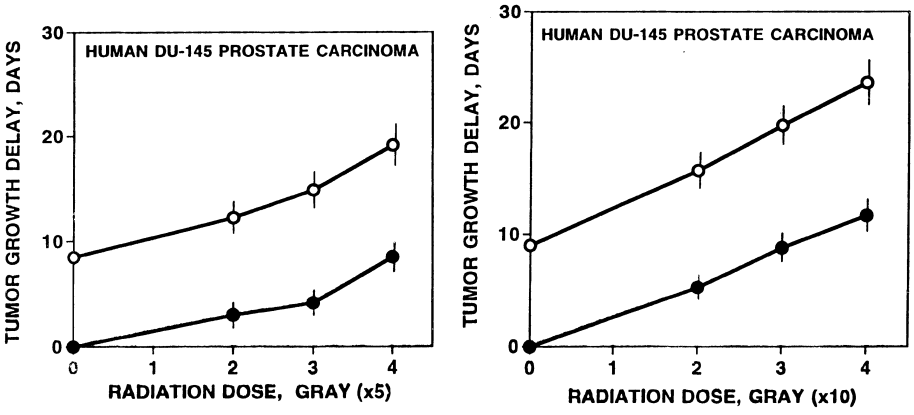

Fig. 5 Tumor growth delay of subcutaneously growing human DU-145 prostate carcinoma xenografts after treatment locally to the tumor-bearing limb with fractionated radiation daily for 5 days on 9 days 12–16 (●) or for 10 days on days 12–16 and 19–23 (●) alone or along with 10 mg/kg jasplakinolide by continuous infusion on days 9–15 (○) or along with 20 mg/kg jasplakinolide by continuous infusion on days 9–22 (○). Points are the means of three independent experiments; *bars* are the SEM

DU-145, PC-3 and LNCaP cells, respectively. Two logs of cell killing was obtained with 42 °C for 1 h along with 1.5, 0.7 and 0.7 μ*M* jasplakinolide for 24 h in the DU-145, PC-3 and LNCaP cells, respectively. The pattern of cell killing with 48 h of jasplakinolide exposure was similar.

Two concentrations of jasplakinolide (0.1 μ*M* and 1 μ*M*) were selected for cell culture studies with jasplakinolide in combination with radiation under normally oxygenated and hypoxic conditions in the three human prostate carcinoma cell lines (Fig. 3). Jasplakinolide and radiation produced primarily additive cytotoxicity in normally oxygenated prostate carcinoma cells. Exposure to jasplakinolide for 24 h prior to radiation delivery produced some diminution of the shoulder of the radiation survival curve in the PC-3 cells by a factor of 2. In hypoxic cells radiation sensitization was observed in the DU-145 cells and PC-3 cells as evidenced by an increased slope of the radiation survival curves. The radiation dose-modifying factor observed with 1 μ*M* jasplakinolide and radiation in hypoxic DU-145 cells and in hypoxic PC-3 cells was 1.5.

For initial in vivo studies, jasplakinolide was administered to animals bearing the Lewis lung carcinoma in three dosage regimens alone or along with fractionated radiation delivered locally to the tumor-bearing limb (Fig. 4). Jasplakinolide was an active antitumor agent in the Lewis lung carcinoma producing tumor growth delays between 3 and 5 days (Table 1). Jasplakinolide was also a radiation sensitizer of the Lewis lung carcinoma. With the jasplakinolide regimen of 1.5 mg/kg injected intraperitoneally twice per day on days 5 through 11 along with fractionated radiation delivery on days 7 through 11, the radiation dose-modifying factor was 1.4 ± 0.1. When jasplakinolide (10.5 mg/kg) was administered subcutaneously by continuous infusion over days 5 through 11 along with fractionated radiation on days 7 through 11, the radiation dose-modifying factor was 2.0 ± 0.2. Finally, when jasplakinolide (21/mg/kg) was administered subcutaneously by continuous infusion over days 5 through 18 along with fractionated radiation on days 7 through 11, the radiation dose-modifying factor was 2.4 ± 0.2.

The Lewis lung carcinoma metastasizes avidly to the lungs from the subcutaneously implanted primary tumor. Untreated control animals bearing the Lewis lung carcinoma die between days 21 and 25 after tumor cell implantation. Therefore, lungs were collected on day 20 to determine the effect of the treatments on systemic disease (Table 1). Fractionated radiation delivered locally to the tumor-bearing limb decreased the number of lung metastases on day 20 to 45–60% of the control number. Jasplakinolide administered by each of the three regimens decreased the number of lung metastases to 20–33% of the control numbers. The effect of jasplakinolide/radiation combination regimens on the number of lung metastases increased at higher radiation doses, reaching a maximum at 4 Gy of radiation giving lung metastases levels 8–13% of control numbers.

The human DU-145 prostate carcinoma grown as a xenograft in nude mice was also responsive to the antitumor effects of jasplakinolide which resulted in tumor growth delays of 8 to 9 days when the drug was administered subcutaneously by continuous infusion (Fig. 5). The effects of jasplakinolide and fractionated radiation in the DU-145 tumor appeared to be additive as evidenced by the parallel rise in the tumor growth delay curves. With the 1-week treatment regimen, adding jasplakinolide to the fractionated radiation increased the tumor growth delay 5-fold, and with the 2-week regimen adding jasplakinolide to the fractionated radiation increased the tumor growth delay 12-fold.

Discussion

Jasplakinolide was a potent cytotoxic agent in each of the three human prostate cancer cell lines. The level of cell killing by jasplakinolide increased over 24 h of exposure to the drug indicating that the drug is stable in cells for some time. The cytotoxicity of hyperthermia and of jasplakinolide appeared to be independent when cells were exposed to both treatments. The non-interactive nature of this combined modality treatment indicates that the intracellular targets of jasplakinolide are available and are not altered by hyperthermia treatment.

There is evidence for interaction between the cytotoxic mechanism evoked by jasplakinolide and ionizing radiation. In normally oxygenated cells, DNA repair was inhibited, and in hypoxic cells there was evidence of radiation sensitization. In vivo, when administered prior to each radiation fraction, jasplakinolide and ionizing radiation appeared to exert independent antitumor activity. Only when jasplakinolide was administered intermittently with daily ionizing radiation did radiation sensitization appear to occur in vivo. Thus, there is a basis for postulating that combining jasplakinolide and radiation may lead to much greater therapeutic response than expected from either agent alone. The value of systemic jasplakinolide administration was also evidenced in the response of metastatic disease to the drug in animals bearing the Lewis lung carcinoma.

The interaction of jasplakinolide with appropriately sequenced radiation doses is of interest, as the drug is known to cause cytokinetic block to cell division [14]. Other workers have reported that cells in cytokinetic block induced by, for example, cytochalasin are more susceptible to the effects of a variety of therapeutic measures including antimetabolites and radiation [11, 15]. Our results may indicate that such sensitization may occur in vivo, and encourage efforts to develop jasplakinolide as a radiation sensitizer or enhancer.

These results indicate that jasplakinolide is an active antitumor agent in vitro and in vivo and support further study of this interesting new antitumor agent.

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