

Low Dose, Alternating Electric Current Inhibits Growth of Prostate Cancer

Theodore D. Koreckij,¹ Charles Hill,² Larry Azure,² Holly Nguyen,¹
Lawrence L. Kunz,³ Andrew Azure,² Eva Corey,¹ Paul Lange,¹
and Robert L. Vessella^{1*}

¹Department of Urology, University of Washington, Seattle, Washington

²LaZure Scientific, Inc., Issaquah, Washington

³BioGenetics Research Laboratories, Inc. P.S., Greenbank, Washington

BACKGROUND. A number of minimally invasive technologies exist for the treatment of prostate cancer (CaP), each with their associated morbidities. We sought to test the efficacy of low dose alternating electric current (LDAEC) to inhibit CaP growth in a preclinical setting and determine its effect on normal tissue.

METHODS. In the first study, two power settings, 15 or 25 mA of current, and two treatment times, 15 or 60 min, were evaluated in C4-2B CaP xenografts. In the second study, power was regulated to maintain an intra-tumoral temperature of $\leq 45^{\circ}\text{C}$ in C4-2B and LuCaP 35 tumors. In both studies, tumor volume, serum PSA levels, survival and histology were analyzed. In a third study, LDAEC was applied to mice hamstrings with evaluation of gait and histology.

RESULTS. The most effective tumor volume reduction in the first study was seen with tumors treated with 25 mA for 15 min ($62 \pm 9.4\%$ decrease, $P = 0.001$). Longer treatment time did not enhance treatment effect. Using 45°C to govern delivery of LDAEC resulted in a near 100% reduction in tumor volume in 8/10 mice with C4-2B tumors ($P < 0.001$) with similar inhibition of LuCaP 35 tumors ($P = 0.01$). This treatment, although resulting in skeletal muscle necrosis, did not affect nerves, smooth muscle and blood vessels.

CONCLUSION. LDAEC demonstrates efficacy against C4-2B and LuCaP 35 CaP xenografts while causing no harm to nerves and blood vessels. These results warrant further investigations into the use of LDAEC as a treatment for CaP. *Prostate* © 2009 Wiley-Liss, Inc.

KEY WORDS: prostate cancer; alternating electric current; PCAP

INTRODUCTION

There is a constant search for better, less invasive methods for treating cancer. This is especially true in the treatment of prostate cancer (CaP) where the advent of prostate specific antigen (PSA) screening has revealed an incidence that far outweighs the mortality associated with this disease [1]. Although a number of nomograms exist which use various clinicopathologic parameters to aid in the prediction of aggressive and organ defined disease [2,3]; they are not exact and a great number of patients are treated who have potentially indolent disease. Although the utility of expectant management (active surveillance) is currently being investigated [4], the relatively poor

outcomes in patients who develop advanced disease, and the inaccuracies of current methods to predict those that will, or will not, develop advanced disease, has led to the current practice of treating localized CaP patients similarly, typically with surgery or radiation therapy [5].

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*Correspondence to: Robert L. Vessella, Department of Urology #356510, University of Washington, 1959 NE Pacific, Seattle, WA 98195. E-mail: vessella@u.washington.edu

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There are many modalities that have been developed in an effort to eradicate primary CaP while limiting the negative side effects associated with therapy. Of those considered to be minimally invasive, the most well known and often used is radiation therapy either through external beam or via brachytherapy [6]. Other minimally invasive therapeutic modalities include radiofrequency ablation (RFA) [7,8], cryosurgery [9], high frequency ultrasound (HIFU) [10,11], photodynamic therapy [12,13], and interstitial microwave therapy [14]. While all of these modalities have demonstrated efficacy against CaP, comparisons among treatment modalities is difficult (reviewed in Ref. [15]). However, common to all these therapies is the non-selective nature in the type of cell they can harm. This non-selective nature is of particular concern when treating CaP where collateral damage may lead to impotence, incontinence, and other side effects. As a result, development and evaluation of a minimally invasive technology that could selectively target tumor cells while sparing normal surrounding cells is still of great interest and would be of immense clinical significance.

The studies reported in this article involve the use of alternating electric current (AEC) to achieve inhibition of tumor growth. The cellular effects evoked by AEC can differ greatly depending on the dosage used and method of application. RFA consists of the application of an AEC delivered in very high doses (power of 300–500 W) via a probe that is inserted into the center of a tumor mass [16]. This results in localized tissue heating (i.e., 70 to >100°C) which causes immediate cell death of both tumor and normal cells. Radiofrequency ablation has been evaluated in the treatment of various solid tumors besides prostate, including hepatocellular and breast carcinomas [17].

Intermediate frequency, very low dose AEC (LDAEC) (<1 W) has also demonstrated the ability to inhibit proliferation of a wide range of tumor cell types *in vitro*, including CaP cells [18,19]. Whereas RFA uses extreme temperature elevation to exert its effects, efficacy using LDAEC was achieved through the inhibition of spindle formation and mitosis in the absence of an elevation in temperature. LDAEC has been successfully used to treat patients with glioblastoma multiforme [20], although patients were required to undergo daily, 16-hr treatments to achieve the anti-tumor effect. To date, no studies have used LDAEC to treat CaP *in vivo*.

Herein, we present our results on the efficacy of a novel treatment system, the Preferential Cancer Ablation Procedure (PCAPTM; LaZure Scientific, Inc., Issaquah, WA), which destroys tumor cells through the application of intermediate frequency LDAEC. Our results demonstrate its effectiveness in two different

CaP xenograft models within a treatment time span of 15 min. Importantly, these same therapeutic parameters, when applied to normal tissues, did not cause damage to nerves and vasculature.

MATERIALS AND METHODS

Cell Lines

The C4-2B CaP cell line was originated from a bone metastasis of C4-2 cells, a subline of LNCaP. It is castration-resistant and produces PSA [21]. This line is maintained under standard tissue culture conditions. The C4-2B and C4-2 cell lines were kind gifts from Leland Chung, Ph.D. formerly of Emory University (Atlanta) and now at Cedars Sinai (Los Angeles).

LuCaP 35 is an androgen-dependent, PSA-producing xenograft line developed from the lymph node of a patient with metastatic CaP. LuCaP 35 is maintained via serial passage in intact SCID male mice [22].

Delivery Device

The PCAPTM ablation device (LaZure Scientific, Inc.) is an electrosurgical instrument system that delivers a low-intensity, intermediate frequency alternating electric field to the desired area via the use of a therapeutic transducer (Fig. 1). The System Control Module (SCM) generates, delivers, monitors and controls the characteristics of the therapeutic field within the specified

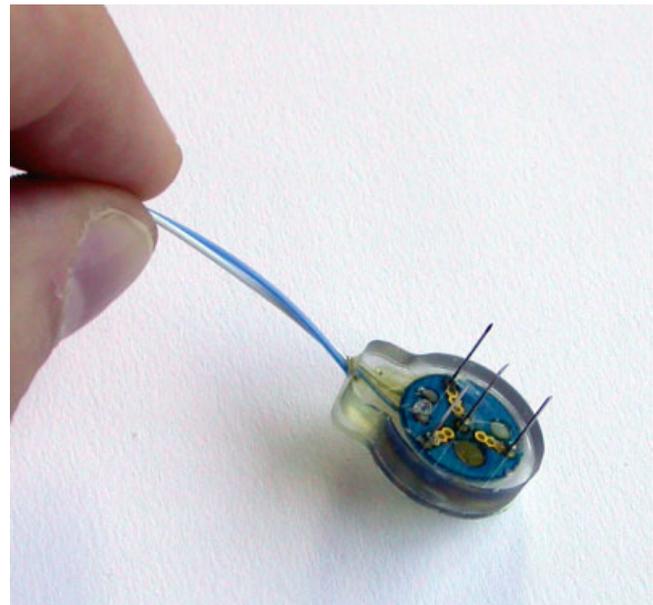


Fig. 1. Design of probe. The picture above represents the electrode configuration used in the studies. The center electrode represents the sole anode surrounded by peripheral cathodes set in a triangle.

treatment parameters. The SCM consists of an integrated direct current (DC) battery power source, an alternating current (AC) inverter, a signal generator, a signal amplifier, an oscilloscope, an operator interface monitor, and a central processing unit (CPU). An AC current is derived from the integrated power inverter. An intermediate frequency (100 kHz) AEC sinusoidal wave is produced from the signal generator. The signal can be amplified to a current range of 5–60 mA and voltage of up to 20 Vrms. Field characteristics including waveform, frequency, current and voltage are monitored by an integrated oscilloscope. An integrated CPU monitors overall system power consumption and availability, and controls the output of the signal generator and amplifier based on input of the treatment parameters.

The therapeutic transducer (Fig. 1) or “probe” is the minimally invasive component that is inserted percutaneously into the target tumor tissue for the purpose of delivering the energy field. It consists of a central needle anode surrounded by three needle cathodes set in a triangular fashion. The therapeutic field is contained within the volume of the area encompassed by the peripheral needle electrodes. The distance from anode to cathode can vary as described below.

In Vivo Study Monitoring

All animal procedures were performed in compliance with the University of Washington Institutional Animal Care and Use Committee and NIH guidelines. In the subsequent animal studies, tumor volumes were measured twice weekly and calculated using the equation: length \times width \times height \times 0.5236. Blood samples were collected weekly for determinations of serum PSA levels (IMx Total PSA Assay, Abbott Laboratories, Abbott Park, IL). In Study #1, intra-tumoral temperature measurements were made using a hypodermic thermocouple needle type T (Omega Technologies, Stamford, CT) manually inserted into the center of the tumor. In Study #2, the probe was altered to include a

built-in thermocouple (type T, Omega Technologies) capable of automatically monitoring changes in intra-tumoral temperatures at 15 sec intervals.

Study #1: Effects of LDAEC dose adjustments in treatment of C4-2B xenografts. Sixty 6-week-old intact male CB-17 SCID mice (Charles River Laboratories Inc., Wilmington, MA) were injected subcutaneously on the right flank with 2×10^6 C4-2B cells with Matrigel™ (1:1, BD Biosciences, San Jose, CA). Animals were randomized to treatment as shown in Table I when tumor volumes reached 200 mm³. Table I also provides details on the study design and the number of mice treated per group. Effects on tumor volume were monitored as described.

In this study, all tumors were treated once with a probe that measured 3 mm from the central anode to the peripheral cathodes. All needle electrodes measured 8 mm in length. Upon initiation of treatment, intra-tumoral temperatures were monitored every 5 min. Animals received 15 or 25 mA treatments for 15 or 60 min. Control tumors received probe placement without application of current. Animals were sacrificed 7 and 14 days after the treatment unless otherwise compromised. After sacrifice, tumor tissue was collected for histological analysis.

Study #2: Temperature-controlled delivery of LDAEC. Based on observations from the first study, Study #2 was designed to allow for adjustment of LDAEC in order to maintain an intra-tumoral temperature of 45°C, which was associated with the greatest anti-tumor response in Study #1. The probe was modified as described to allow for monitoring of intra-tumoral temperatures and subsequent adjustment of the power being delivered by the SCM. Two different tumor types were utilized to ensure that efficacy with LDAEC was not specific to one cell line. Forty 6-week-old intact male CB-17 SCID mice were injected subcutaneously on the right flank with 2×10^6 C4-2B cells as described above. An additional 40 mice were implanted subcutaneously on the right flank with

TABLE I. Animal Allocation and Treatment Conditions in the First Study*

Group	Animals (n)	Treatment duration (min)	Current (mA)	Number of animals sacrificed (n)	
				Day 7	Day 14
1	10	60	0	4	6
2	12	15	15	5	7
3	9	15	25	4	5
4	10	60	15	4	6
5	10	60	25	5	5

*Animals were treated at a frequency of 100 kHz.

TABLE II. Animal Allocation and Treatment Conditions in the Second Study*

Group	Animal number	Tumor xenograft	Treatment time (min)	Sacrifice time points (number)	
				Day 3	Followed for recurrence
1	16	C4-2B	15	4	12
2	14	C4-2B	15	4	10
3	18	LuCaP 35	15	4	14
4	18	LuCaP 35	15	4	14

*Animals were treated at a frequency of 100 kHz; power was adjusted so as to maintain an intra-tumoral temperature of 45°C.

~20 mg tissue pieces of LuCaP 35 CaP xenografts as we described previously [22]. Animals were randomized to treatment groups once tumor volumes reached 200 mm³ (~3–4 weeks) as summarized in Table II. Tumor growth was monitored as described.

All tumors in this study were treated once with a probe that measures 5 mm from the central anode to the peripheral cathodes. This probe was larger than the 3 mm probe in the first study in an attempt to improve tumor coverage. Needle electrodes remained at 8 mm in length. Control tumors received probe placement without application of current.

Four animals from each group in both the C4-2B and LuCaP 35 xenograft studies were sacrificed 3 days after treatment for histologic examination. The remaining animals were followed until tumor volumes reached 1,000 mm³ or for 90 days after treatment.

Study #3: Effects of LDAEC on normal tissue. Ten 6-week-old intact male CB-17 SCID male mice without tumors were used to evaluate the effects of LDAEC on normal tissue. The therapy was applied to the hind leg, with subsequent evaluation of histology.

Animals were allocated to treatment as summarized in Table III. Five animals received treatment with the application of LDAEC governed by a max temperature of 45°C to the right hamstring. A probe that measured 4 mm from anode to cathode was used in this study as the 5 mm probe was too large to be completely encompassed within the hamstring. However, using 45°C to govern delivery of LDAEC to normal tissue

TABLE III. Animal Allocation and Treatment Conditions in the Third Study*

Group	Animal number	Treatment Variable	Duration (min)
1	5	Fixed, 45°C	15
2	5	Fixed Power	15

*Animals were treated at a frequency of 100 kHz.

resulted in the need to deliver much higher overall power to reach the same temperature as in the previous tumor studies. Thus, an additional five animals received treatment with LDAEC set at a constant power (mW). This constant power setting was selected according to the average power delivered to tumors in the second study (1.65 mW/mm³ of tissue treated, see results below).

Animals were monitored closely for any signs related to a decrease in function of the affected leg and sacrificed 4 days after treatment to allow for assessment of cell death, but limit infiltration of inflammatory cells into treated areas in order to restrict histological analysis to the effects of the treatment modality itself.

Histology

All tumored and non-tumored tissues were prepared for histological evaluation in a similar fashion. After sacrifice, tissues were fixed in 10% buffered formalin overnight. Tissues were cut into 2–3 mm serial blocks, embedded in paraffin, sectioned at 5 µm, placed on glass slides and stained with either hematoxylin and eosin or Masson's Trichome. The Masson's Trichome is a special stain that stains nuclei black, cytoplasm, keratin, muscle fibers, and intercellular fibers red, and collagen blue. All histopathology and morphometric analysis was reviewed and performed by an ACVP board certified veterinary pathologist, LK (listed author). For measurements of the tumor morphology, the microscopic slides were viewed on a Nikon Labophot-2 microscope (Nikon Inc., Melville, NY), with high-resolution digital images captured by a Nikon D1 digital microscopic camera (Nikon Inc.), and high-resolution, low magnification digital images captured by a Nikon Super CoolScan 5000 microscopic slide scanner (Nikon Inc.). The digitized images were morphometrically analyzed using Image-Pro Plus software version 4.5.1.22 for Windows XP (Media Cybernetics, Silver Spring, MD). The images were calibrated for the Image-Pro Plus software using

Schaedler Precision Rules that is calibrated in half-millimeter increments (Schaedler Quinzel, Parsippany, NJ). Measurements of the treatment area were captured on the digital microscopic images as a screen capture from Image-Pro Plus.

Statistical Analysis

Statistical analyses were performed using unpaired student t-tests and Mantel-Cox tests for survival curve analyses (Prism Graphpad, Graphpad Software, San Diego, CA). Due to large variations in serum PSA levels, levels were normalized to their enrollment values for comparison. Results were considered significant when $P \leq 0.05$.

RESULTS

Study #1: Effects of LDAEC Dose Adjustments in Treatment of C4-2B Xenografts

We first sought to determine the dose of LDAEC that would produce the greatest tumoricidal effect. Treatment with 25 mA resulted in a $62 \pm 9.4\%$ (mean \pm SEM, Fig. 2) decrease in tumor volume at the lowest nadir following enrollment. Seven out of the 20 animals treated with 25 mA had complete ablation of the tumor as measured by calipers, and on average, the maximum reduction in tumor volume was reached approximately 6 days after therapy. The volume reduction in 25 mA treated tumors was significantly greater than those reductions observed in both the 15 mA treated animals ($17 \pm 4.7\%$, $P < 0.001$) and the control group ($9.8 \pm 6.9\%$, $P = 0.001$). This slight reduction in the volume of control tumors is attributed to the loss of blood from within the tumors that occurred upon placement of the probe. Treatment with 15 mA did not result in

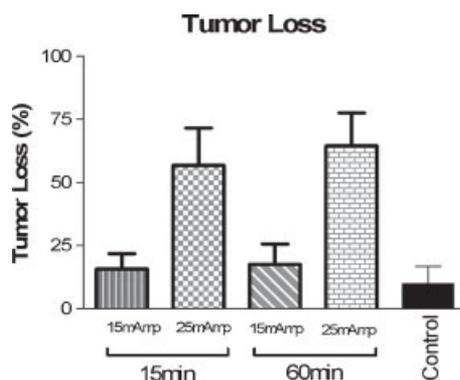


Fig. 2. Results of dose adjustments of LDAEC on C4-2B xenografts. Tumor volume reduction at lowest nadir expressed as a percent of enrollment tumor volume. Maximum tumor volume reduction was seen 6 days after therapy. Tumors treated with 25 mA demonstrated the greatest reduction with no added benefit observed with longer treatment duration.

significant reductions in tumor volumes compared to those seen in the control group ($P = 0.436$), and the application of LDAEC for longer treatment durations did not result in further tumor volume reductions beyond those observed with the shorter treatment duration at either current setting.

The normalized PSA levels in the 25 mA treated animals at 14 days was 0.67 ± 0.3 which represents a significant reduction versus the 15 mA treated animals (4.4 ± 1.1 ; $P = 0.005$) and control animals (2.2 ± 0.66 ; $P = 0.024$). No significant reductions were seen in normalized PSA levels in the 15 mA treated animals versus control at 14 days (2.2 ± 0.66 , $P = 0.27$). Similar to the above tumor volumes, serum PSA levels measured between groups receiving the same current but at different time intervals were not significantly different.

Intra-tumoral temperatures were measured immediately prior to and during each treatment. The maximum temperature in the 25 mA treatment groups rose to $44 \pm 0.6^\circ\text{C}$ which represents a $15 \pm 0.6^\circ\text{C}$ increase in temperature over baseline ($29 \pm 0.83^\circ\text{C}$). There were no further increases in intra-tumoral temperatures with the longer treatment period. The temperature elevation in 25 mA treated tumors was a significantly higher elevation than those observed in 15 mA treated tumors ($P < 0.001$). In the 15 mA treatment groups, the temperature rose to a maximum of $36 \pm 0.6^\circ\text{C}$; a $6.5 \pm 1.1^\circ\text{C}$ increase above baseline temperature ($29 \pm 0.15^\circ\text{C}$).

Histologically, the time points of seven and 14 days proved to be too far removed from the actual treatment. The tumors treated with 25 mA had become necrotic and fallen off by day 7, yielding little histological data regarding the actual effect of LDAEC on treated tissue. The remaining tissue most likely represented tumor outside the treatment array (data not shown). Due to the limitations of histological analysis in Study 1, Study 2 was designed so that histological analyses could be performed.

Study #2: Treatment-Controlled Delivery of LDAEC

Temperature-controlled delivery of LDAEC in treatment of C4-2B xenografts. The ability to govern LDAEC therapy by monitoring temperature would increase usability for treatment delivery and thus an intra-tumoral temperature of 45°C was used to govern delivery of LDAEC to these animals. This temperature setting was ascertained from Study #1 where intra-tumoral temperatures in this range resulted in the greatest reductions in tumor volumes. The current, voltage, frequency and temperature being applied were measured every 15 sec throughout the 15 min treatment (Table IV). In this study, the average power

TABLE IV. Treatment Delivered in Study #2

Xenograft	Voltage	Current (mA)	Frequency (kHz)	Temperature (°C)	Power (mW)	Power per volume tissue (mW/mm ³)
C4-2B						
Mean ± SD	11.41 ± 0.7	36.56 ± 4.36	100 ± 0.01	43.98 ± 0.46	429.2 ± 52.52	1.65
LuCaP 35						
Mean ± SD	11.95 ± 1.38	29.41 ± 6.51	100 ± 0.01	42.86 ± 1.19	368.3 ± 117.9	1.42
>50% tumor reduction	12.87 ± 1.64	34.12 ± 7.31	100 ± 0.01	42.43 ± 0.48	460.9 ± 137.2	1.77

applied that resulted in the eventual elevation of intratumoral temperatures to 45°C was 1.65 mW/mm³ of tissue treated (current × voltage/volume with probe array; see Table IV).

Overall, LDAEC therapy resulted in an 89 ± 7.9% reduction in tumor volumes at lowest nadir from baseline. This reduction in tumor volume was seen at approximately 4 days (2 animals did not have their lowest reduction until days 14 and 18) after treatment. The decrease in tumor volumes of LDAEC treated tumors was significantly greater than that seen with sham-treated controls (3.3 ± 1.9%, $P < 0.001$) (Fig. 3A). Treatment resulted in the complete ablation of the tumor as measured by calipers in eight out of 10 animals. Three out of those 10 animals failed to show any signs of disease recurrence (non-detectable PSA or tumor volume) 90 days after therapy; leading to a significant improvement in survival for treated animals ($P < 0.001$, Fig. 3B). Recall that this study used a larger size probe to more effectively encompass the tumor.

Significant reductions in normalized PSA levels were seen in the treatment group as early as 1 week after treatment versus control group (0.07 ± 0.06 vs.

2.15 ± 0.48, $P < 0.001$). These significant reductions were maintained up to 4 weeks after the treatment, after which, statistical analysis was no longer possible due to a limited number of animals remaining in the control group.

Based on the failure to obtain meaningful histological data at 7 days or longer post-treatment in Study #1, tumors in this study were excised at day 3. Histological analysis showed complete necrosis of the tumor contained within the probe array (Fig. 4A). Histo-morphometry revealed a significantly higher percentage of non-viable areas in treated tumors compared to control (87 ± 3.9% vs. 35.97 ± 7.2%, $P < 0.001$). The areas of cell death noted in control tumors are attributed to the ischemic necrosis that occurs with rapid tumor growth. A representative picture is shown demonstrating histomorphometric analyses as well as a photomicrograph showing a treated tumor with foci of viable tumor that appear at the edges of the tumor boundaries which likely accounts for tumor recurrence in a few animals where probe coverage was inadequate (Fig. 4B). There were no viable tumor cells detected within the treatment field induced areas of necrosis.

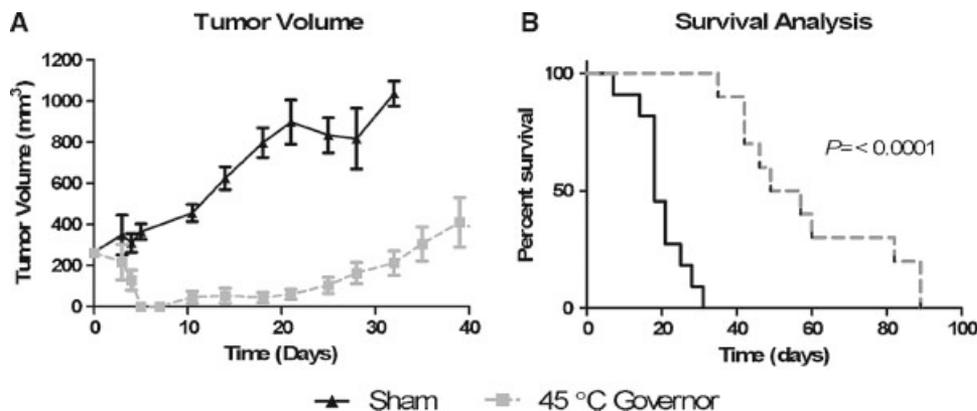


Fig. 3. Results of temperature-controlled delivery of LDAEC on C4-2B xenografts. **A:** Rapid reduction in tumor volumes immediately following therapy. Time along X-axis is shortened to highlight rapid reduction in tumor volumes. **B:** Significant improvements in survival were observed in treated animals. Three animals showed no evidence of disease recurrence (no tumor volume or detectable serum PSA levels) at the end of study period (90 days).

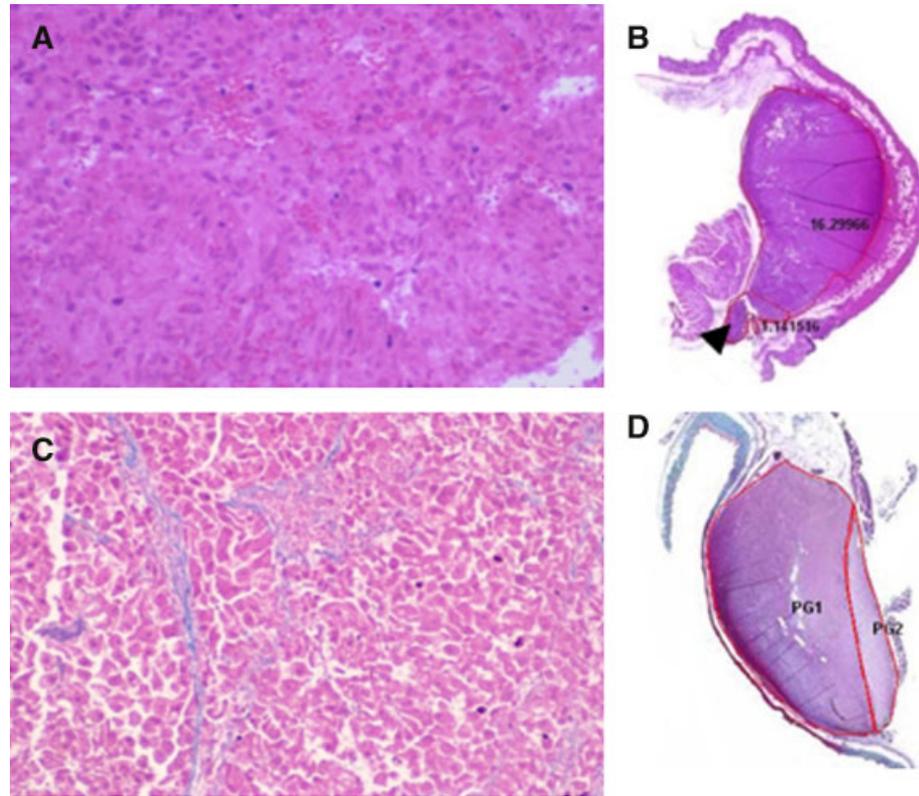


Fig. 4. Representative sample of the histologic appearance of tumor cells after treatment with LDAEC (A) C4-2B tumors treated 3 days after therapy (200 \times magnification). B: Depiction of histomorphometric analysis of C4-2B tumors. C: LuCaP 35 tumors treated 3 days after therapy (200 \times magnification). D: Depiction of histomorphometric analysis of LuCaP 35 tumors.

Temperature-controlled delivery of LDAEC in treatment of LuCaP 35 xenografts. LuCaP 35 tumors were treated to investigate the ability of LDAEC to inhibit growth of an additional CaP xenograft line and ensure that its effects were not limited to C4-2B cells. With the LuCaP 35 xenografts, the average power applied that resulted in the elevation of intra-tumoral temperatures to 45 $^{\circ}$ C was 1.42 mW/mm 3 . The average treatment parameters were recorded as described and are listed in Table IV.

LDAEC applied to LuCaP 35 tumors resulted in a significant inhibition of tumor growth after treatment (Fig. 5A). Inhibition of growth was detected 1.5 weeks after treatment and was maintained up to 4.5 weeks post-treatment until a lack of remaining animals in the control group limited the statistical power necessary to detect a difference. As such, treatment resulted in significant improvement in survival compared to control animals ($P = 0.010$, Fig. 4B). One treated animal had no measurable tumor or detectable serum PSA level 90 days after treatment. However, treatment did not always result in the exuviation of these tumors as was seen with C4-2B tumors. Interestingly, a subset analysis of treated LuCaP 35 tumors that did display >50% reduction in tumor volume ($n = 4$) revealed that

the power during those treatments averaged 1.77 mW/mm 3 of tissue treated (Table IV). These values are higher than the overall mean for all LuCaP 35 treated tumors and more similar to those witnessed in the C4-2B tumors.

PSA levels corresponded with the tumor volume reductions and were normalized for statistic analysis. Treatment resulted in a drop in serum PSA levels at 1 week following treatment with LDAEC compared to control (0.24 ± 0.08 vs. 1.05 ± 0.22 , $P < 0.001$). This reduction in serum PSA levels remained significantly reduced for 3 weeks after treatment until too few animals remained in the control groups for analysis.

Histological analysis of treated tumors excised at day 3 revealed complete necrosis of areas contained within the probe array. Similar to the C4-2B tumors, there were the areas of viable tumors bounding the edge of the tumor, likely accounting for tumor recurrence. Histomorphometry revealed that $67 \pm 9.6\%$ of LuCaP 35 treated tumors were non-viable; a significantly higher percentage compared to that observed in control tumors ($12 \pm 11.2\%$, $P = 0.01$). Representative photomicrographs of treated areas and histomorphometry are shown (Fig. 4C,D). No

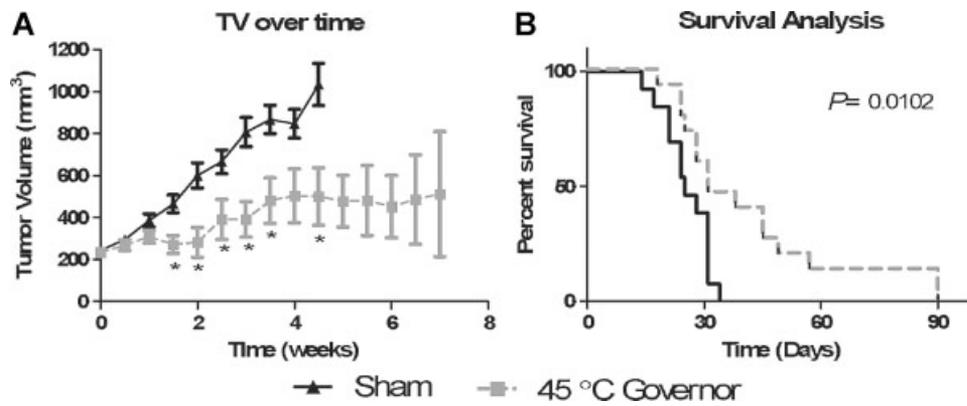


Fig. 5. Results of temperature-controlled delivery of LDAEC on LuCaP 35 xenografts. **A:** Inhibition of tumor growth in response to treatment. * $P < 0.05$. **B:** Significant improvements in survival in those animals receiving treatment with one animal showing no evidence of disease recurrence at 90 days post-treatment.

viable tumor cells were detected within the treatment field induced areas of necrosis.

Study #3: Effects on Normal Tissue

In order to determine the preferential nature of LDAEC two separate studies were performed with normal, non-tumored tissue. In the first study with non-tumored tissue, we used 45°C to govern delivery of LDAEC to mice hamstrings. However, the power required in these studies averaged 3.20 mW/mm³ of tissue treated in order to reach the temperature threshold of 45°C. This was more than double the power needed to treat the tumors in Study #2. With this treatment, animals displayed significant malfunction of the treated limb and signs indicative of compromise, and thus were sacrificed within a few hours after therapy and the hind legs embedded for histology.

The elevated power levels observed when using 45°C to govern delivery of LDAEC to non-tumored tissue study led to a second study on normal tissue using a constant power setting. We used the power setting from our experiments with the C4-2B xenografts (1.65 mW/mm³), as this was the higher of the power settings necessary to produce the desired effect in the two different tumor types treated in Study #2. This potentially represents the upper threshold that would need to be applied to a prostate gland in order to treat the primary cancer and thus important to determine the effects of this amount of power on normal tissue. However, at this power setting there still appeared to be a very mild impairment of limb function but not necessitating euthanasia. Interestingly, at this power setting, the temperature within the treatment area only reached 39.2°C. Animals were sacrificed at day 3 for histological analysis.

In these non-tumored tissue studies, cell death of skeletal muscle is seen in both the temperature

controlled and power controlled studies. However, within areas of skeletal muscle necrosis, the nerves were histologically normal (Fig. 6A). Large blood vessels were also histologically normal, including the surrounding smooth muscle.

DISCUSSION

Given the large number of patients diagnosed with CaP and the inability to accurately predict those cancers that will behave aggressively, most patients will elect to have treatment of their primary disease. Thus, a number of minimal invasive modalities have been developed, with more currently in development, for the treatment of primary CaP. Herein, we report on the significant tumoricidal capabilities of short-term LDAEC in two preclinical models of CaP.

In the first study, intra-tumoral temperatures elevated to ~45°C, and the use of 45°C to regulate delivery of LDAEC in the second study resulted in significant inhibition in tumor growth of both C4-2B and LuCaP 35 tumors. Hyperthermia (42–45°C) itself is a very old form of cancer therapy and has had renewed interest with the recent therapeutic success of Lance Armstrong against metastatic testicular cancer (a thermosensitive cell type) or what is being described as “the Lance Armstrong Effect” [23]. The mechanisms behind hyperthermic cell death include protein unfolding, DNA and mitochondrial damage and generation of reactive oxygen species (reviewed in Ref. [24]). Hyperthermia may also stimulate natural immunity as is observed by the body’s response to infection with fever, and it may also increase antigenicity of tumor cells upon clearance [23]. Exploration of the mechanistic role that the immune response may play in the tumoricidal effects seen with LDAEC could not be carried out in the immune-compromised model system used herein but is certainly an intriguing possibility with perhaps

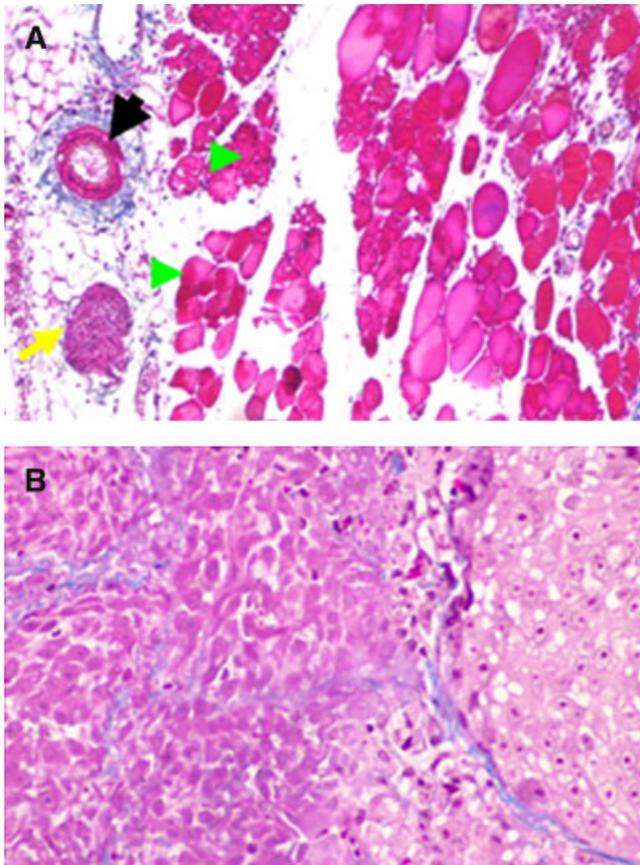


Fig. 6. Histologic effects of LDAEC. **A:** Effects on skeletal muscle, nerves, and blood vessels. Masson's trichrome staining demonstrating normal appearing blood vessels (black arrow) and nerve (white arrow) within an area of skeletal muscle necrosis (arrowheads) 3 days after treatment using 45°C to govern overall power delivery. **B:** Masson's trichrome stain of the zone of demarcation between viable (right) and non-viable (left) tumor cells in a LuCaP 35 tumor treated 3 days previously with LDAEC. The treated area of acute coagulation necrosis with karyolysis and cytolysis, was sharply delineated from the viable tumor outside the treatment array area (100× magnification).

considerable complementary therapeutic potential. At temperatures ranging between 42 and 45°C, time to cell death decreases exponentially with elevations in temperature [25]. Although hyperthermia at 45°C will result in cell death, typically the treatment time needs to be greater than 15 min; a time shown to be effective in this study with LDAEC [16,26,27]. In addition, the average temperature delivered over the full treatment period averaged less than 45°C (Table IV) as it took several minutes for the intra-tumoral temperatures to reach this point. Electric current has also been shown to be effective in tumor cell killing by causing a variety of changes within the tumor including changes in pH, membrane depolarization, and electrolysis [28,29]. Our data suggest that hyperthermia and LDAEC, acting in

combination, will cause death of CaP *in vivo* in a relatively short treatment course. The high rate of success observed with the current therapy warrants further studies to better understand the mechanisms behind the cell death elicited by the combination of LDAEC and hyperthermia.

The results of the first study demonstrated that there exists a threshold (i.e., 25 mA) in the power applied that will ultimately result in a tumoricidal effect. Interestingly, there was no statistical difference between groups receiving the same current at the different time intervals with the maximal effect seen as early as 15 min. As a result, it may be possible to further reduce the treatment duration with equal effectiveness but further investigations are necessary to support this premise.

When temperature was used to control delivery of LDAEC to normal tissue, higher amounts of power were required than those needed in tumored tissue to reach the same temperature which is why an additional non-tumored tissue study was performed using constant power. Studies have shown that CaP tissue has a higher impedance [30] which may explain why more power delivery was required in non-tumored tissue to elevate temperatures. Nonetheless, LDAEC had a cytotoxic effect on skeletal muscle cells but nerves and the smooth muscle surrounding blood vessels were unharmed. In the treatment of CaP, the potential to spare nerves is a very attractive feature of this modality; potentially helping to reduce the rates of impotence associated with current therapies. These results also imply that the major constituent of the stromal compartment of the prostate, smooth muscle, will be left intact after treatment.

Histology shows that LDAEC therapy results in a very precise area of demarcation; (Fig. 6B) only a few cell layers thick of a mixed response between viable and non-viable cells as well as no islands of viable tumor found within areas of the treatment field. Other modalities such as RFA and photodynamic therapy can result in a large gradation and heterogeneity in treatment effect [31,32]. Ultimately, a treatment probe array will be created that allows for the treatment of an entire prostate using a template similar to those used for brachytherapy. This ability to control the actual treatment area will aid in avoiding the urinary sphincter (i.e., skeletal muscle) which may be susceptible to therapy based on our non-tumored tissue studies. In addition to sparing normal tissue by controlling the treatment field, a cooling catheter can be placed to limit damage to the prostatic urethra that may be caused by the mild temperature elevation during treatment. However, the effect of LDAEC on normal prostatic tissue has yet to be tested and those studies are currently underway utilizing a canine

model. The trademark for LDAEC therapy as provided herein is now PCAPTM.

CONCLUSIONS

The application of LDAEC had a significant tumoricidal effect on two different human CaP xenografts. This same therapy leaves nerves and vasculature unaffected. Although these results are encouraging it is still early in development of this novel therapeutic approach. Further investigations regarding the mechanism of action, the best method for delivering and monitoring treatment effects as well as the overall preferential nature of is necessary and ongoing. Nonetheless, these studies warrant the continued investigation of LDAEC in the treatment of CaP.

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