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Electrochemical red-ox therapy of prostate cancer in nude mice

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ABSTRACT

Minimally invasive therapies are increasingly in demand for organ-confined prostate tumors. Electrochemical therapy (EChT) is attractive, as it relies on locally-induced reduction–oxidation reactions to kill tumor cells. Its efficacy for prostate cancer was assessed in human PC-3 and LNCaP tumor xenografts growing subcutaneously in nude mice ($n = 80$) by applying 2 Stainless Steel vs. 4 Platinum–Iridium (Pt–Ir) electrodes to deliver current densities of 10 to 35 mA/cm^2 for 30 or 60 min. The procedure was uneventful in 90% of mice. No difference in tumor vs. body temperature was observed. Changes at electrode-tumor junctions were immediate, with dryness and acidity (pH 2–3) at the anode and oedema and alkalinity (pH 10–12) at the cathode. This was accompanied by cellular alterations, found more pronounced at the cathode. Such acidic and alkaline conditions were cytotoxic in vitro and dissolved cells at pH > 10. In mice, tumor destruction was extensive by 24 h with almost undetectable blood prostate specific antigen (LNCaP model) and covered the whole tumor surface by 4 days. EChT was most efficient at 25–30 mA/cm^2 for 60 min, yielding the longest recurrence-free survival and higher cure rates, especially with 4 Pt–Ir electrodes. EChT is a promising option to optimize for organ-confined prostate tumors.

1. Introduction

Prostate cancer (PCa) is the most common solid primary tumor in the adult male. It is potentially curable using surgery or radiation if confined within the prostate. Given the long natural history and the increasing number of cancers detected at early stages, concern has arisen regarding overtreatment. The unwelcome side effects of radical prostatectomy (incontinence and erectile dysfunction) and radiation therapy (radiation cystitis or radiation proctitis) have significant adverse effects on quality of life [1]. Many are advocating that lower risk cases do not require radical intervention. Active surveillance is an attractive option to avoid the morbidity of radical therapies. Unfortunately, approximately 30% of patients progress and need definitive treatments [2,3]. Interest in exploring new forms of focal treatments, such as high intensity focused ultrasound [4], photosensitizers [5,6], laser-induced interstitial thermotherapy, cryotherapy [7] and electroporation [8] has grown to find modalities with low morbidity while providing oncologic outcomes similar to surgery and radiotherapy.

Electricity-based approaches enter in the category of local therapy. They may use both alternating and direct current. For instance, low dose alternating current is growth-inhibitory in xenografts derived from glioblastoma [9] and PCa (C4-2B and LuCaP) cell lines [10], the drawback being significant local heating in tissues. Direct electric current application can be interval-based or continuous. In the former, short pulses of high voltage current lead to PCa cell death without heat effects and result in irreversible electroporation and pore formation within cell membranes [8,11]. In contrast, the continuous electrochemical therapy (EChT) or electrochemical red-ox therapy operates at a low voltage (<15 U) with application of the direct current (mA) using thin electrodes embedded within tumors and destroying them in less than 2 h. Its killing capacity relies on electrochemical reduction–oxidation reactions or "electrolysis" events [12,13] rather than electrical "shocks" due to ohmic or heating effects, electrical charge accumulation and electroporation of cell membranes.

The proposed principles and conceptual basis of EChT are illustrated below.

Electrolysis is initiated by electro-osmosis due to water migration from the anode to the cathode, causing dryness in the tissue surrounding the anode and oedema at the cathode (Scheme 1a). Electrochemical reactions then taking place cause drastic changes in pH at electrode

Abbreviations: EChT, electrochemical therapy; PCa, prostate cancer; PSA, prostate specific antigen; s.c, subcutaneous; U, voltage; T, temperature; Pt–Ir, Platinum–Iridium; CD, current density; RFS, recurrence-free survival; HR, hazard ratio.

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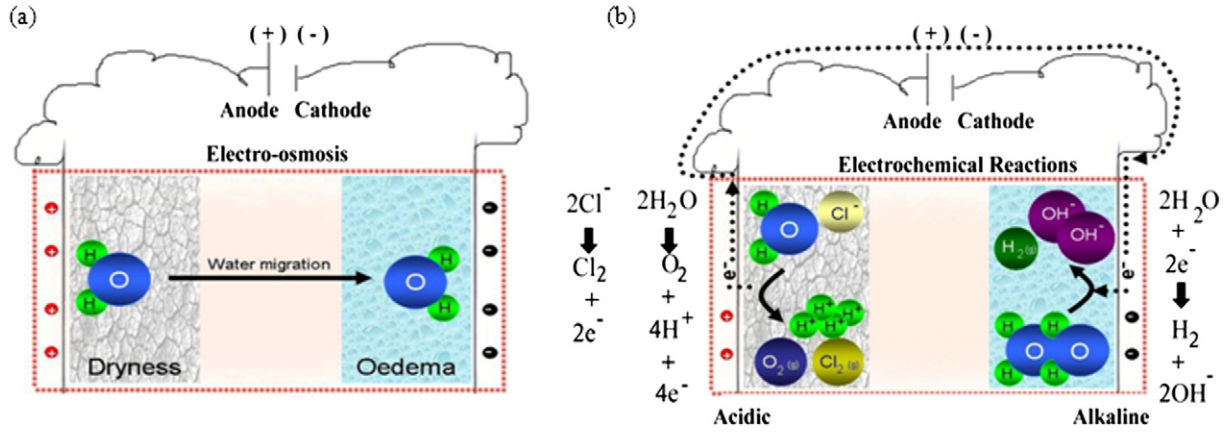

Scheme 1. EChT mechanism of action. EChT is based on water migration (a) and electrolysis (b) resulting in electrochemical reduction–oxidation reactions leading to acidity and alkalinity at the tumor-electrode junctions and extending to the entire treatment field. Highly toxic ions rapidly kill cells.

sites. More specifically, the evolution of hydrogen (H_2) generated at the cathode (Scheme 1b, right side) leads to a build-up of alkalinity (OH^-) and produces local cavitations in the tissue that becomes strongly alkaline. At the anode, the evolution of oxygen (O_2) causes an increase in acidity due to produced protons (H^+) that immediately undergo hydration and form H_3O^+ entities. Concomitant to the evolution of O_2, chlorine evolution at the anode causes local tissue bleaching (Scheme 1b, left side). Monovalent ions (Na^+, K^+) reach higher concentrations around the strongly alkaline cathode while multivalent cations (Ca^{+2}, Mg^{+2}) remain unchanged. Macromolecules (proteins, nucleic acids) are altered in an environment progressively becoming cytotoxic for cells comprised within the treatment field [12–15], implying that surrounding structures remain intact.

EChT has been applied in China initially. Promising results were obtained in patients, with higher response and survival rates compared to patients treated by conventional approaches [16,17]. Various types of superficial and non-superficial cancers were treated, including skin, mouth, esophagus, lung, pancreas, liver, vulva, breast and a few cases of prostate [18–20]. EChT was more recently applied to patients with localized PCa in Europe to control tumor growth, paralleled by a decrease in blood levels of prostate specific antigen (PSA) [21]. Nonetheless the lack of standardization tempers further EChT development. The present investigation was designed to establish proof of principle of EChT efficiently destroying human prostate tumor xenografts in nude mice.

2. Materials and methods

2.1. Cell lines and in vitro treatments

Human PCa cell lines, PC-3 (androgen-independent) and LNCaP (androgen-sensitive and expressing PSA), purchased from the American Type Culture Collection (Rockville, MD, USA) were maintained in complete RPMI medium (containing L-glutamine) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin solution (Invitrogen, Burlington, ON, Canada).

Cell growth assays were performed on adherent PC-3 cells, pre-exposed for 60 min to complete medium adjusted to pH 1 to 13, while maintaining controls in complete medium kept at pH 7.4. The medium was replaced by fresh complete medium at pH 7.4 cells and cells were further cultured to perform MTT assays at 48 h and 120 h [22]. Experiments were repeated thrice. Results were analyzed by one-way ANOVA tests and differences were considered significant at $p \leq 0.05$. Cell death was monitored immediately following exposure to media at different pH values, by performing propidium iodide uptake tests for 15 min and counting dead cells (stained in red) by fluorescence microscopy (Olympus IX-81 microscope, equipped with Image-Pro Plus 7.0; Centre Valley, Ca, USA). Corresponding phase contrast images were captured to obtain total cell counts per field. Results from two experiments are shown.

2.2. Prostate cancer xenograft models

The protocol to perform EChT to prostate tumors growing subcutaneously (s.c.) in nude mice was approved by the Animal Ethics Committees of the Research Institute at McGill University Health Center (MUHC) and McGill University. Briefly, 2×10^6 PC-3 or LNCaP cells were suspended in a mixture of Matrigel (BD Biosciences, Mississauga, ON, Canada) at a 1:1 ratio in phosphate buffer saline (PBS), pH 7.4 and injected under the skin in the right flank of nude mice (6 week-old CD-1 male weighing 25–30 g; Charles River, Wilmington, MD, USA) [23]. Animals were followed daily and weighed once a week. The growth of tumors was monitored bi-weekly with calipers measuring length, width, and depth [24]. Mice were randomly assigned to treatments once tumor volumes reached 0.5–1.2 cm^3.

Table 1
Experimental conditions. Mice bearing PC-3 (regrouped as #1 to #3) and LNCaP (regrouped as #4) tumor xenografts were enrolled in EChT treatment arms to test electrodes (Stainless Steel vs. Platinum–Iridium in 2 vs. 4 configurations, respectively), CD (Current Density), and duration of EChT in min. Animals were monitored for either short periods to study tumor damages and tissue destruction (Pathology) and long-term efficacy (Follow-up) to determine recurrence-free survival or cure rate. Death noticed after EChT. The LNCaP model allowed the monitoring of blood levels of Prostate Specific Antigen (PSA). No current (CD = 0) was applied in controls. (n): number of mice.

Xenograft model	PC-3											LNCaP			
Electrodes conditions	Stainless Steel	Stainless Steel #1			Stainless Steel #2				Platinum–Iridium #3			Stainless Steel #4			
CD (mA/cm^2)	0	15–25	25–30	30–35	10–15	10–15	25–30	25–30	25–30	25–30	25–30	10–15	15–25	25–30	25–30
Duration (min)	60	60	60	60	30	60	30	60	30	60	60	60	60	30	30
Total (n)	5	4	5	2	5	10	9	9	1	4	13	2	2	3	6
Death	0	0	0	2	0	1	2	0	0	0	0	0	1	0	2
Pathology (n)	1	3	0	0	1	4	2	4	1	4	7	2	1	3	2
Follow-up (n)	4	1	5	0	4	5	5	5	0	0	6	0	0	0	2