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Electro-endocrine combination therapy for aggressive breast tumors

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Abstract

Obesity is an epidemic in the US and an established risk factor for breast cancer incidence and morbidity. In obese patients breast cancer is commonly more aggressive and associated with poor prognosis. The goal of our research is to develop safe and efficient novel therapies to treat aggressive breast tumors, like those in obese patients. To enhance drug uptake we intend to develop electro-endocrine therapy, an electrically mediated hormone (endocrine) delivery system. In this study, we optimize parameters for electrical pulses and tamoxifen (anti-hormone drug) dosages in an *in vitro* model of breast cancer, as these parameters vary for each cell type. For this purpose, MCF-7 human breast adenocarcinoma cells were used. Results of our experiments show that treatment of electroporation combined with tamoxifen has an enhanced effect on suppressing tumor cell growth compared to tamoxifen treatment alone. The long-term goal is to translate application of this method to clinical practice.

Keywords: Obesity; Breast cancer; Tamoxifen; MCF-7 cells; Electroporation; Electropermeabilization; Endocrine; Estrogen; Hormone; Fluorescence microscopy

1. Introduction

Endocrine (hormonal) therapies are generally easier to treat with fewer side effects than chemotherapy [1]. For the past 20 years, tamoxifen has been the drug of choice in hormonal therapy for breast cancer in women. It is a 20 mg/day oral pill that works as a selective estrogen receptor modulator (SERM) blocking estrogen, a hormone that can promote breast cancer. In clinical studies or experiments with human cells in culture, it has been demonstrated that tamoxifen exhibits anti-estrogenic (inhibition) and in some cases estrogenic (stimulation) activity depending on tissue type. In breast cancer cells, tamoxifen suppresses estrogen stimulated gene expression that leads to cytostatic (cessation of growth) or apoptotic (cell death) effects, with higher tamoxifen concentrations triggering apoptosis [2]. Tamoxifen is the world's largest selling breast cancer drug used for treatment of primary and metastatic diseases in pre- and post-menopausal women. On October 29, 1998, it was also approved by the FDA for prevention, to reduce the incidence of breast cancer in high-risk women. For tamoxifen to be effective, tumors should be sensitive to estrogen (estrogen receptor positive, ER+) [1,3]. The majority of breast cancer patients are diagnosed with ER+ tumors, and many of these women benefit from tamoxifen. However, for up to 60% of patients tamoxifen is not an effective treatment. Patients receiving tamoxifen experience significant side effects that include hot flashes, increased risk of thromboembolic events and increased risk of endometrial cancer [1]. These side effects result from estrogenic affects of orally delivered tamoxifen that travels via the blood stream and acts on estrogen receptor containing tissues other than the breast, such as the uterus.

Recent epidemiological data have shown that obese individuals have increased incidence of breast cancer. Moreover, breast tumors of obese patients are commonly more aggressive and are associated with poorer prognosis

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and drug resistance [4,5]. Considering that aggressive mammary tumors over express extracellular matrix (ECM), which can interfere with local drug penetration [6,7]; our work focuses on testing strategies for improving response in aggressive tumors, such as those of obese subjects. Towards reducing toxicity and enhancing drug delivery, we propose, for the first time, the development of a technique that couples low volume electroporation with tamoxifen. Electroporation or electropermeabilization (EP) is a physical, non-viral technique utilizing precisely controlled electric fields of short duration and high intensity to open up transient aqueous pathways through semi-permeable membranes, allowing targeted delivery of therapeutic molecules including drugs, antibodies, and nucleotides. EP offers a 100–1000-fold improved therapeutic benefit compared to using a drug alone. It is a very efficient technique to enhance the efficacy of drug delivery for cancer treatment, gene transfer and similar applications in biology, biotechnology and medicine [8,9]. EP is a local, site-specific, physical technique with minimal side effects, if any at all.

One example of enhanced efficacy with EP is its use in skin cancer clinical trials, where an electric field intensity of 1300 V/cm and duration of 100 μs were applied [8–10]. In these studies, the number of pulses varied from 6 to 8, with an interval of 1 s (1 Hz). In a similar study, an electric field intensity of 1200 V/cm for 99 μs and 8 pulses was used by Gehl and Geertsen to treat a 68-year-old male patient with ulcerated malignant melanoma metastases on the chest [11]. EP is cell specific [12,13] and depends on a number of parameters, such as conductivity of the media, type of drug and size of drug [12–14]. This necessitates the optimization of pulse parameters for each type of cell/cancer.

While, in general, EP treatment is safe and well tolerated by patients, there is transient muscle contraction that occurs during pulsing and occasionally limited discomfort is felt by some patients. Transient small burns or marks in the areas contacting electrodes have also been observed in some cases. However, these consequences are benign, reversible and treatment is quite safe and tolerable [12]. Nevertheless, it is always desirable to use the lowest effective voltage possible in these treatments. Effective use of two 450 V/cm, 20 ms pulses for regression of high-grade malignancy in mice was reported by Torrero et al. [15]. These studies motivate the use of low intensity pulses. To calculate the reduced field strengths, the concept that $ET = K$, a constant [16] was used, where E is the electric field strength (V/cm) and T is the pulse duration, μs.

Based on the potential usefulness of EP methods towards breast cancer, the main objectives of our research are to identify the optimal EP pulse parameters and to investigate the effect of reduced field strengths on MCF-7 human breast cancer cells. It is hypothesized that electro-endocrine therapy will be more effective with improved therapeutic outcome due to the combination of local toxicity of tamoxifen and the increased permeability of cell membranes exposed to electrical pulses.

2. Materials and methods

2.1. MCF-7 cancer cell line

ER + MCF-7 (human, Caucasian, breast adenocarcinoma) cells were used. Cells were cultured in 90% RPMI 1640 media + 10% FBS (ATTC, Manassas, VA) and 1% Penicillin/Streptomysin (Invitrogen, Carlsbad, CA). Cells were grown in an incubator at 37 °C at 95% humidity and 5% CO_2.

2.2. Electroporation of MCF-7 cells

Cells were dissociated from flask by treatment with 0.25% trypsin/EDTA solution (Invitrogen, Carlsbad, CA). Cells were counted using a hemocytometer and resuspended in RPMI culture media to a final concentration of 1×10^6 cells/ml. Aliquots of 500 μL were used for electroporation.

A BTX 830 square wave electroporator (Genetronics, San Diego, CA) along with 0.4 cm cuvettes were used for electroporation (Fig. 1). Various voltages, pulse durations and number of pulses at 1 s intervals were studied (details in Table 1). Cells were pulsed in media without tamoxifen or in media containing 1, 5 or 10 μM tamoxifen. Cells were undisturbed for 30 min after pulsing, then removed from cuvettes and seeded in 24 well plates. Cells were then incubated at 37 °C in a 5% CO_2 atmosphere at 95% humidity for 2, 24 or 48 h.

2.3. MCF-7 cell viability and growth assay

Following electroporation and incubation, cells suspended in media were stained with a 1:1 ratio of Trypan Blue and counted using a hemocytometer. Counts for live and dead cells (viability), and total numbers of cells (growth) were done for each electroporation treatment. Three replicates for each treatment were counted four times and the averages calculated. Each experiment was repeated three times.

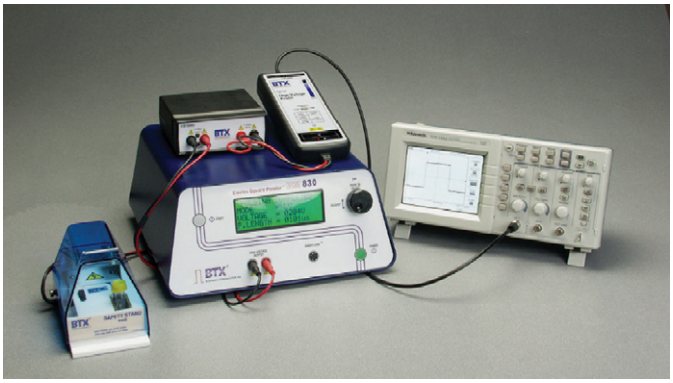

Fig. 1. Square wave electroporator used for electro-endocrine therapy.