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 \textit{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...
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/Streptomycin (Invitrogen, Carlsbad, CA). Cells were grown in an incubator at 37 °C at 95% humidity and 5% CO₂.

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 \( 1 \times 10^6 \) 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% CO₂ 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.
2.4. Fluorescence microscopy

Aliquots of 500 μL containing 5 × 10^5 cells were pulsed at either 400 or 1200 V/cm in the absence or presence of 1 μM tamoxifen. Cells were then cultured on sterile cover slips, in the bottom of 35 mm Petri dishes containing 1.5 ml of growth media. Control cells were not pulsed. Two days after electroporation and culture, cells were washed with PBS and stained with fluorescein diacetate (green marker). The fluorescein diacetate stock was made of media and placed in another 35 mm Petri dish with 2 ml of media for cover slip mounting.

Cells were observed with a 60 × Plan Apo objective on a Nikon TE 2000 inverted microscope equipped (Nikon, Melville, NY) with a Lambda LS Xenon arc lamp (Sutter Instrument, Novato, CA) and a standard FITC filter cube. Images were taken with a CoolSnap HQ CCD (Roper Scientific, Tucson, AZ) using Metamorph 7.0 software (Universal Imaging, Molecular Devices, Downingtown, PA). Cells were visualized using a 480–510 nm excitation filter and 535–585 nm emission filter for fluorescein diacetate.

2.5. Effect of tamoxifen on MCF-7 cells

The effect of tamoxifen alone, without electroporation, on MCF-7 cell growth and viability was determined. Cells suspended in 500 μl aliquots (1 × 10^6 cells/ml) containing RPMI media, 10% FBS and either 0.5, 1, 2.5, 5 or 10 μM tamoxifen were cultured in 24 well plates. Control cells were treated with media devoid of tamoxifen. Cells were cultured at 37 °C for either 24 or 48 h, stained with Trypan Blue (1:1 ratio) and counted via hemocytometer.

2.6. Statistical analysis

Results are expressed as mean ± S.E.M. Analyses were performed using one way and repeated measures of ANOVA with Tukey’s post-test using Proc Mixed (SAS Institute 1994) [17].

3. Results

3.1. Effect of electrical pulses on MCF-7 cell viability

Optimal EP parameters that do not compromise viability were determined for MCF-7 breast cancer cells incubated for 48 h (Table 1). The pulses chosen were based on previous reports and calculations based on \( ET = K \), a constant [11,15,16]. No tamoxifen was used in this set of experiments. Resulting viabilities from these pulses are illustrated in Fig. 2. The only decrease in % viability was observed with the 1200 V/cm, 100 μs, 8 pulse conditions. These results indicate that low EP parameters do not affect MCF-7 cell viability, as compared to non-EP treated controls at 48 h. No difference in viability at any EP parameter tested was observed at 2 h after electroporation.

3.2. Effect of tamoxifen on MCF-7 cell growth (no electroporation)

Cells were incubated without or with 0.5, 1, 2.5, 5 or 10 μM tamoxifen. Fig. 3 shows the total number of cells for each treatment after 24 and 48 h incubation. Tamoxifen at concentrations between 2.5 and 10 μM significantly decreased tumor cell growth at 24 h, compared to non-tamoxifen treated controls (Fig. 3a). At 48 h tamoxifen significantly decreased cell growth at all concentrations except 0.5 μM (Fig. 3b). At this time point 10 μM tamoxifen had the most robust effect on suppressing cell growth (9.1 × 10^5 ± 0.49 × 10^5 cells), as compared to no tamoxifen (13.8 × 10^5 ± 0.55 × 10^5 cells). No significant differences in % viability were observed at any of the incubation times and tamoxifen concentrations used in this set of experiments (data not shown). These data reflect the cytostatic mechanism of action of tamoxifen [2].

3.3. Effect of EP on MCF-7 cell growth

MCF-7 cells were pulsed using high intensity, short duration or low intensity, long duration conditions (Table 1). The influence of these EP conditions on cell growth was determined as described in Section 2. The effect of EP on total cell counts after 2 and 48 h incubation are presented in Fig. 4. Compared to non-EP treated cells, at 2 h, there were no significant differences in total cell number with any of the EP conditions tested (Fig. 4a). At 48 h post-EP, a significant reduction in cell number occurred with 400 V/cm(13.2 × 10^5 ± 0.5 × 10^5 cells) and 1200 volts/cm (9.1 × 10^5 ± 0.5 × 10^5 cells), as compared to non-EP controls (16.8 × 10^5 ± 0.9 × 10^5 cells) (Fig. 4b).
Fig. 2. Effect of EP on MCF-7 cell viability. MCF-7 cells were electroporated and cultured for 48 h (a) or 2 h (b), and the percent viability determined as described in Section 2. Counts for live, dead and total numbers of cells 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. 3. Effect of tamoxifen on MCF-7 cell growth at 24 h (a) and 48 h (b). MCF-7 cells were cultured and counts for live, dead and total numbers of cells were done for each tamoxifen treatment as described in Section 2. EP conditions were not used in this set of experiments. Three replicates for each treatment were counted four times and averages calculated. Results are means ± S.E.M. from three experiments. Mean values statistically significantly different from control samples (no tamoxifen) are indicated by an asterisk (*). Statistical significance was determined by ANOVA and Tukey's tests where the threshold p value < 0.05.

Fig. 4. Effect of EP on MCF-7 cell growth. MCF-7 cells were electroporated, cultured for 2 h (a) and 48 h (b), and total numbers of cells were counted for each EP treatment, as described in Section 2. Tamoxifen was not used in this set of experiments. Three replicates for each treatment were counted four times and averages calculated. Results are means ± S.E.M. from three experiments. Mean values statistically significantly different from control samples (no EP) are indicated by an asterisk (*). Statistical significance was determined by ANOVA and Tukey’s tests where the threshold p value < 0.05.
3.4. Effect of tamoxifen and EP combination on MCF-7 cell growth

To determine the effects of combining tamoxifen with EP, MCF-7 cells were exposed to tamoxifen at 1, 5 or 10 μM and different EP conditions, as described in Table 1. A significant reduction in cell growth occurred with each tamoxifen concentration that was combined with 400, 100 and 1200 V/cm treatment (Fig. 5). At each tamoxifen concentration tested, the largest reduction of cell growth was observed at 1200 V/cm (Fig. 5a–c).

3.5. Summary of EP–tamoxifen combination therapy on MCF-7 breast cancer cell growth

Table 2 summarizes results for combinations of electrical pulse conditions (400 V/cm, 2 pulses at 20 ms; 100 V/cm, 8 pulses 1 ms; 1200 V/cm, 8 pulses 100 μs); and 1, 5 and 10 μM tamoxifen treatments that were most effective in suppressing MCF-7 cell growth. The use of EP only, in the absence of tamoxifen, produced a 15–20% reduction in cell number at both 400 and 100 V/cm. The most substantial reduction in cell number was observed at the highest voltage of 1200 V/cm. Tamoxifen alone, with no EP exposure, showed a dose-dependent decrease in cell number ranging from 10% to 45%. At every tamoxifen concentration, use of EP produced an additive or slightly enhanced therapeutic effect over tamoxifen alone.

In this Table, 48 h cell count data from Fig. 3 (tamoxifen), Fig. 4 (EP) and Fig. 5 (tamoxifen–EP) were used to determine percent decrease in cell number, as compared to control cells not treated with EP or tamoxifen.

3.6. Fluorescence microscopy

Fig. 6a shows fluorescence micrographs of live MCF-7 cells, incubated for 48 h, which were either non-EP treated or exposed to electric fields of 1200 or 400 V/cm. The non-EP treated control cells showed maximum viability whereas cells exposed to 1200 V/cm were least viable. Consistent with data in Figs. 2 and 4, micrographs reflect that the 1200 V/cm pulse condition yields the most substantial effect on cell number reduction as compared to 400 V/cm and non-EP control. Micrographs also provide details about cell morphology. Control cells are round in shape with visibly distinct plasma membranes and the areas of separation between cells are more consistent, each of these characteristics indicating healthy cells. Cells treated with the highest voltage (1200 V/cm) are amorphous and lack a clearly defined plasma membrane, indicative of dying cells. Cells receiving 400 V/cm are similar in morphology to cells receiving no EP.

![Fig. 5. Effects of tamoxifen and EP combination on MCF-7 cell growth. MCF-7 cells were suspended in media containing 1 μM (a), 5 μM (b) or 10 μM (c) tamoxifen and electroporated using conditions described. Subsequently, cells were cultured for 48 h and total numbers of cells were counted for each EP–tamoxifen combination treatment, as described in Section 2. Cells treated with tamoxifen but not exposed to EP were included for each tamoxifen concentration and served as EP control samples. Three replicates for each treatment were counted four times and averages calculated. Results are means ± S.E.M. from three experiments. Mean values significantly different from EP controls are indicated by an asterisk (*). Statistical significance was determined by ANOVA and Tukey’s tests where the threshold p value < 0.05.](image-url)
with the area between cells consistent in size. However, these cells appear to have compromised plasma membrane integrity (not well delineated) and are much larger compared to control cells. These morphological characteristics are representative of the cytostatic (growth suppression) actions of tamoxifen. Cells receiving a combination of tamoxifen and EP at 400 or 1200 V/cm are amorphous and severely lack clear definition of a plasma membrane, indicative of apoptotic (dying) cells.

4. Discussion and summary

In this work, we predicted that EP could enhance the efficacy of the breast cancer drug tamoxifen. Herein, we show that using electroporation in combination with tamoxifen results in a significant additive or an enhanced effect on suppression of tumor cell growth, greater than tamoxifen treatment alone. This increased effect with EP occurred at each pulse/tamoxifen dosage combination employed. Interestingly, the largest increase in efficacy (above respective tamoxifen alone treatment) was observed at 400 V/cm + 5 μM tamoxifen, a low-voltage long-duration pulse condition. The enhanced effect of EP–tamoxifen combination can be attributed to the improved entry of tamoxifen into the permeabilized cell, leading to increased local tamoxifen concentrations. It has been well established that in breast cancer cells, tamoxifen inhibits estrogen-stimulated gene expression resulting in cell cycle (proliferation) suppression. However, recent studies have shown that at higher dosages tamoxifen can stimulate apoptotic molecular signaling pathways or effect plasma membrane fluidity to promote cell death [2,19]. We speculate these additional actions, associated with higher local tamoxifen concentrations, are achievable through EP use and responsible for the enhanced tamoxifen effects in our experiments.

In additional experiments, fluorescence microscopy images correlated well with tamoxifen and EP effects observed in our cell viability and growth assays. Micrographs revealed that high voltages have a profound impact on cell death (reduced numbers and compromised cell membrane integrity), whereas low voltages do not affect cell viability. Furthermore, tamoxifen alone treatment of cells causes morphological changes characteristic of cell cycle arrest (cytostatic), while EP+tamoxifen causes changes indicative of cell death. These imaging studies support (1) low-voltage longer duration pulses can be a valid alternative to high voltage pulses and that (2) EP combined with tamoxifen can lead to activation of cell death mechanisms, as opposed to merely stopping cell growth/proliferation.

EP of cells allows drug molecules access to the cytosol [18], thus increasing efficacy. Diffusion of molecules across the electropulsed cell membrane can be described using the Fick equation [18]:

$$\phi(S) = 2\pi N \Delta S \frac{X(N, T)}{E_p/E} \exp(-k(N, T)t)$$  (1)

Table 2
Summary of the effect of tamoxifen–EP combination on MCF-7 growth

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tamoxifen concentration</th>
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<tbody>
<tr>
<td></td>
<td>0 μM</td>
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<tr>
<td>400 V/cm, 2 pulses at 20 ms</td>
<td></td>
</tr>
<tr>
<td>EP only (%)</td>
<td>15–20</td>
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<tr>
<td>Tamoxifen only (%)</td>
<td>10–15</td>
</tr>
<tr>
<td>EP + tamoxifen (%)</td>
<td>30</td>
</tr>
<tr>
<td>100 V/cm, 8 pulses at 1 ms</td>
<td></td>
</tr>
<tr>
<td>EP only (%)</td>
<td>15–20</td>
</tr>
<tr>
<td>Tamoxifen only (%)</td>
<td>10–15</td>
</tr>
<tr>
<td>EP + tamoxifen (%)</td>
<td>25</td>
</tr>
<tr>
<td>1200 V/cm, 8 pulses at 100 μs</td>
<td></td>
</tr>
<tr>
<td>EP only (%)</td>
<td></td>
</tr>
<tr>
<td>Tamoxifen only (%)</td>
<td>10–15</td>
</tr>
<tr>
<td>EP + tamoxifen (%)</td>
<td>75</td>
</tr>
</tbody>
</table>

Fig. 6b shows micrographs of live MCF-7 cells, incubated for 48 h, that were exposed to tamoxifen only (1 μM) or in combination with electric fields of 1200 and 400 V/cm. Cells treated with tamoxifen only are similar in shape to control cells receiving no tamoxifen and no EP treatment (panel a). Tamoxifen-only treated cells are round

Fig. 6b shows micrographs of live MCF-7 cells, incubated for 48 h, that were exposed to tamoxifen only (1 μM) or in combination with electric fields of 1200 and 400 V/cm. Cells treated with tamoxifen only are similar in shape to control cells receiving no tamoxifen and no EP treatment (panel a). Tamoxifen-only treated cells are round
for a given molecule $S$ and a cell with radius $r$. Here, $\varphi(S)$ is the flow at time $t$ after $N$ pulses of duration $T$ (the delay between pulses being short compared to $t$). $P_s$ is the permeability coefficient of $S$ across the permeabilized membrane and $\Delta s$ is the concentration gradient of $S$ across the membrane. $E_p$ is the critical value of electric field, and $E$ is the applied electric field intensity. $E_p$ also depends on $1/r$.

For electroporation to be effective, electrical parameters must be optimized. If the electric field strength is too small, permeabilization will not occur; if it is excessive, the cell plasma membranes will not resel. Thus, there is need for optimizing EP conditions for each cell type. Optimization of different electric field parameters (electric field strength ($E$), duration ($T$), and number of pulses ($N$)) and the type of pulsing conditions (buffer composition, temperature) are required for each cell line in order to preserve cell viability. Other researchers have performed studies optimizing pulse parameters for various cell types and animal models, finding that voltage and duration are dominant parameters, followed by number of pulses [12].

Our current work with MCF-7 human breast adenocarcinoma cells correlates very well with these previous studies in that voltage applied (or electric field strength which is the linear ratio of voltage to the distance between the electrodes, V/cm) to cells is a major factor affecting viability after electroporation; the lower the voltage the higher the number of living cells.

It has been reported that the use of 1300 V/cm (100 μs, 6 pulses) in skin cancer phase I/II clinical trails improves drug efficacy and in general did not cause significant pain [11]. The treatment resulted in little or no adverse side effects with patients reported as tolerating treatment well. There were slight muscle contractions observed during each pulse. An unpleasant sensation was associated with these contractions, which varied in intensity from patient to patient and was dependent on tumor location. However, in every case the unpleasant feeling subsided immediately after each electrical pulse. No residual sensations were noticed. Reactions at the site of treatment included slight edema and erythema beginning 1–2 h after treatment. These symptoms were gone by the next day. No significant modifications of cardiologic or hemodynamic parameters were noted during or after ECT treatment. Additionally, transient small burn marks at areas of electrode contact were observed in vivo in the presence of bleomycin. Collectively, these pre-clinical experiments, followed by clinical trials, show that consequences of EP are benign and reversible, and treatment is safe and tolerable [12]. In view of the above physical symptoms during electroporation, it is of practical interest to study the effectiveness of using lower voltage (electric field strength) for EP. It has been reported that electroporation transfection efficiency is proportional to both voltage (electric field strength, V/cm) and time ($\mu$s), given as $ET = K$, a constant (V/cm × $\mu$s) [16]. This gives us a choice to optimize for either $E$ or $T$. It is always desirable to use lower voltages for practical reasons. Excessive field strength may not only cause irreversible electroporation, but may also cause increased irritation, muscle contractions and non-specific tissue damage when treating patients. Hence, the voltage is to be chosen at a threshold value sufficient enough to create reversible pores. Towards this end, we tested several low voltage short duration pulses such as 500 V/cm (260 μs, 8 pulses) and 100 V/cm (1 ms, 8 pulses), keeping high voltage, 1200 V/cm (100 μs, 8 pulses) as our constant. In addition, we also studied the effect of low-voltage longer duration pulses at 400 V/cm (20 ms, 2 pulses), similar to that reported in Ref. [15].

As mentioned above, the choice of electric pulse parameters is crucial to obtain optimal permeabilization. Building on previous studies, we proposed that low-voltage longer duration pulses should be as effective towards permeabilization as high-voltage short-duration pulses, but with reduced adverse effects.

Our results demonstrate that 400 V/cm (20 ms, 2 pulses) pulses had less uptake of tamoxifen than the 1200 V/cm (100 μs 8 pulses) pulses. Other lower voltage pulses of 500, 250 and 100 V/cm also showed lower uptake and greater viability compared to 1200 V/cm pulses. Electric field intensities of much lower intensity such as 31 and 50 V/cm, and longer duration, such as 50 ms have been used effectively for gene therapy [20]. These data support the idea that low voltage longer duration pulses can considerably minimize the negative consequences on cell viability and growth that are associated with high voltage pulses. Hence, we propose to use longer pulses in our further investigation to potentially reduce non-specific in vivo tissue damage and side effects associated with EP.

In summary, this study begins to provide the conceptual foundation for the clinical use of EP towards breast cancer treatment. This method can be used to substantially increase the local concentrations of tamoxifen or several other therapeutic molecules. Combining EP + tamoxifen to improve local delivery has the potential to eliminate the adverse side affects associated with elevated systemic tamoxifen levels (hot flashes, thromboembolic events and endometrial cancer). We also provide evidence that low voltage pulses are effective towards permeabilization, and may be able to abolish clinical symptoms caused by high voltage pulses (low level pain, muscle contractions, small burn marks, etc.). Finally, an added benefit to increasing local tamoxifen via EP is that in addition to suppressing cell growth, it may actively stimulate different mechanisms that lead to cell death. Taken together, these studies suggest clinical use of low-voltage EP combined with tamoxifen, or electro-endocrine therapy, could lead to a more effective anti-tumor response, while simultaneously eliminating side effects related to high voltage EP and elevated systemic tamoxifen.

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References


