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Preclinical report

Toxicity of anticancer agents mediated by electroporation *in vitro*

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Electroporation is a physical event that temporarily reduces cell membrane barrier properties. Diminished membrane barrier properties are achieved by exposing cells to pulsed electric fields. When a cell has been treated with electric fields it is possible for extracellular agents to gain access to the cell interior. This process has been used *in vivo* to increase the uptake of chemotherapeutic agents by tumor cells which results in dramatically higher response rates than when drug is used alone. This type of treatment is called electrochemotherapy (ECT); bleomycin is most often used as the drug for this type of treatment. It was hypothesized that electroporation could be used to augment the cytotoxicity of other anticancer agents. Therefore, this study was performed in order to screen 44 different combinations of drug and cell type *in vitro* to identify drugs that may have higher cytotoxicity when combined with electroporation. Results from seven cell types indicate that the IC_{50} of bleomycin can be reduced by a factor of 100–5000 when electroporation is used to facilitate internalization. The IC_{50} values of cisplatin and carboplatin could be reduced by factors ranging from 3 to 13 in six different cell lines as a result of electroporation. These IC_{50} reductions in multiple cell lines suggest that cisplatin and carboplatin may be effective *in vivo* as part of ECT treatment. [© 2000 Lippincott Williams & Wilkins.]

Key words: Cytotoxicity, electrochemotherapy, electropermeabilization, electroporation, MTT.

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Introduction

The use of pulsed electric fields to allow entry of extracellular molecules into the cytosol has recently become a popular method for manipulating mammalian cells. This method is know as electroporation or, alternatively, electropermeabilization. A physical process is the underlying mechanism of imparting a permeabilized state. A transmembrane potential is induced during exposure to very brief but intense electric pulses.[1] If this potential is of sufficient magnitude then the cell membrane dielectrically breaks down and barrier properties are diminished for a period of time that is on the order of minutes.[2] When a cell is in this type of electroporated state, it is possible for molecules that do not normally pass through the membrane to gain access to the cell interior.[3–14] When electroporation is conducted properly, cells will recover from their permeable state and retain their viability. One advantage of this physical method is that it is not specific to particular cell types. It can be applied to any type of cell; therefore, it has very broad applicability.

Electroporation has been used to facilitate the internalization of fluorescent molecules,[4,5] drugs[5–12] and DNA[13,14] by cells *in vitro*. Electroporation has also been performed *in vivo* as a delivery method for both chemotherapeutic agents[12,15–18] and DNA.[19,20] It has been established that increased tissue levels of delivered molecules result from electroporation.[21,22] The use of electric fields to mediate the uptake of chemotherapeutic agents, electrochemotherapy (ECT), has been used to treat tumors in clinical trials. Very high objective and complete response rates have been obtained for melanoma, basal cell carcinoma, and head and neck squamous cell carcinoma.[23–26] The majority of ECT

studies have utilized the drug bleomycin combined with electric fields; however, cisplatin has also been used successfully as the drug component of ECT.[27–29] Given the success of ECT with two drugs it was envisioned that enhanced cytotoxic effects could result from using other chemotherapeutic agents when they are used in combination with permeabilizing electric fields. Therefore, a study was initiated to screen anticancer drugs for enhanced cytotoxicity resulting from electropermeabilization. This study was conducted *in vitro* using drugs that are currently used in the clinic and have intracellular sites of action. Seven different cell lines were used for testing.

Materials and methods

Cell lines and culture

T47D human breast ductal carcinoma cells (HTB 133), Capan-2 human pancreatic adenocarcinoma cells (HTB 80), N1-S1 rat hepatocellular carcinoma cells (CRL 1604), murine Lewis lung carcinoma cells (CRL 1642) and B16 murine melanoma cells (CRL 6323) were obtained from the ATCC (Rockville, MD). LN1 human ovarian cancer cells[30] and Ishikawa well-differentiated human endometrial adenocarcinoma cells[31] were a gift from Dr Jeanne Becker (University of South Florida). All cell lines were maintained as monolayers in 225 cm^2 tissue culture flasks (Costar, Cambridge, MA) at 37°C in a humidified atmosphere containing 5% CO_2.

T47D cells were cultured with RPMI medium (Mediatech, Washington, DC) supplemented with 10% (v/v) fetal bovine serum (FBS; HyClone, Logan, UT) and 90 μg/ml gentamicin (Gibco, Grand Island, NY). Capan-2 and B16 cells were grown in McCoy's 5A medium (Mediatech) with 10% FBS (v/v) and 90 μg/ml gentamicin. N1-S1 cells were grown in Swimm's medium (Sigma, St Louis, MO) supplemented with 10% (v/v) FBS, 1.8 mM $CaCl_2$, 0.05 mM g/l L-cystine hydrochloride, 26.2 mM sodium bicarbonate, 1 g/l pluronic F-68 NF (BASF, Parsippany, NJ), 4 mM L-glutamine and 0.02 M phenol red. Lewis lung carcinoma cells were cultured in Dulbecco's modification of Eagle's medium (Mediatech) supplemented with 10% (v/v) FBS and 90 μg/ml gentamycin. Ishikawa cells were grown in a mixture of 50% minimum essential medium (Mediatech) and 50% Ham's F12 medium (Mediatech) that was supplemented with 10% (v/v) FBS and 90 μg/ml gentamycin. LN1 cells were maintained using L15 medium (Mediatech) supplemented with 10% FBS (v/v), 90 μg/ml gentamicin, 20 mM HEPES buffer and 2 mM L-glutamine.

Cells used for experimental purposes were detached using trypsin–EDTA (0.05% trypsin–0.53 mM EDTA; Mediatech), collected by centrifugation at 215 *g* for 5 min. Cells were then washed 3 times with media, resuspended in media and enumerated using a hemacytometer (Fischer Scientific, Pittsburgh, PA). Cells with a viability greater than 95%, as determined by the Trypan blue dye method (0.4% Trypan blue; Sigma), were used for all experimental purposes.

The size of each cell type was determined using cells harvested, washed and enumerated as described above. The diameters of 20 cells of each type were measured using a standard reticle as a measurement device. The reticle was calibrated using a stage micrometer prior to making measurements for each cell type. The time required to prepare the cells insured that all cells were in a rounded-up state during measurement. A mean and standard deviation of the diameter measurements was computed.

Chemotherapeutic agents

The following anticancer drugs were used: bleomycin (Blenoxane; Sigma), paraplatin (carboplatin; Sigma), ancitabine (Sigma), mitomycin C (Mutamycin; Sigma), cytarabine (Cytosar; Sigma), vincristine (Oncovin; Sigma), vinblastine (Velban; Sigma), doxorubicin (Sigma), Etoposide (Sigma), gemcitabine (Gemzar; Eli Lilly, Indianapolis, IN), paclitaxel (Taxol; Bristol-Meyers Squibb, Princeton, NJ), taxotere (Docetaxel; Rhone-Poulenc Rorer, Collegeville, PA), fluorouracil (Solopak Laboratories, Elk Grove Village, IL) and cisplatin (Platinol; Sigma). Fluorouracil and paclitaxel were used in liquid form as supplied by the manufacturers. Drugs that were received as solids were dissolved in sterile 0.9% bacteriostatic saline (Solopak Laboratories) with the exception of vincristine, mitomycin C and cisplatin, which were mixed with sterile distilled deionized water for cell culture (Mediatech).

Electroporation apparatus

A BTX T820 DC generator (BTX, San Diego, CA) was used to generate rectangular direct current pulses. Electrically treated samples received eight 99 μs pulses. Electric field strengths were varied by adjusting the output voltage of the generator. Cell suspensions were placed into standard 2 mm gap electroporation cuvettes (BTX PN 620; BTX). The cuvettes were constructed with parallel aluminum plates which served as electrodes. Aliquots containing 1×10^6 cells (in 200 μl) were placed into the cuvettes and electric pulses of a specific electric field strength were applied using the generator.

An oscilloscope (PM3375; Philips, Eindhoven, Netherlands) was used to monitor duration, voltage and current associated with each delivered pulse.

MTT assay for cell survival

After electroporation alone or electroporation in the presence of a drug, aliquots containing 1×10^4 cells (in 2 μl) were seeded into 96-well tissue culture plates (Falcon, Lincoln Park, NJ). Wells were then filled with 98 μl of the appropriate media. Plates were incubated at 37°C for 24 h for the case of electroporation alone and for 48 h when cells were porated in the presence of anticancer drugs. Then 15 μl of 5 mg/ml MTT solution (Thiazolyl Blue; Sigma) in sterile water was added to each well of the plates at the end of the incubation period. The plates were incubated for 2 h and then aspirated. Finally 100 μl of dimethyl sulfoxide (Sigma) was added to each well and colorimetric analysis was performed using a microplate reader (MR700; Dynatech). A reference filter set at 630 nm and a test filter set at 570 nm were used for the colorimetric analysis. Absorbance readings were analyzed and compared to results from wells seeded on the same plate to generate standard curves. Results were graphed as percent survival of cells versus treatment conditions.

Survival after electrical treatment

Three aliquots of 1×10^6 cells (in 200 μl) were placed into separate electroporation cuvettes. Cells in each cuvette were electroporated at a specific electric field strength. This triplicate sample procedure was conducted for electric field strengths of 0, 500, 750, 1000, 1250, 1500, 1750, 2000, 3000 and 4000 V/cm. Five minutes after electric field exposure, three aliquots of 1×10^4 cells were plated from each cuvette into a 96-well tissue culture plates that also contained wells seeded for standard curves. The plates were incubated for 24 h at 37°C, then an MTT survival assay was performed. Experiments were run in triplicate and the electric field strength which maintained 90% survival was determined by examining mean plots of survival versus electric field strength for each cell line.

Flow cytometric confirmation of electroporation

FluorX (PA 28000; Biological Detection Systems, Pittsburgh, PA) was conjugated to bleomycin according to the manufacturer's instructions and purified on a P-2 gel column (BioRad, Hercules, CA). Cells were placed into suspension and exposed to 1×10^{-4} M bleomycin–FluorX. Cells were then pulsed at various electric field strengths including the electric field strength at which maintained greater than 90% survival. Unpulsed cells that were exposed to the same concentration of bleomycin–FluorX were also included. After treatment, cells were washed 3 times in media and analyzed by flow cytometry. Histograms of the resulting flow cytometric data were plotted as number of cells versus fluorescence intensity.

Drug screening with electroporation

Standard curves were established in 96-well tissue culture plates with values ranging from 0 to 3.5×10^4 cells/well. A 1×10^{-2} M stock solution was mixed for each drug, except Taxol, from which serial dilutions were made. Eight drug concentrations ranging from 1×10^{-10} to 1×10^{-3} M were tested. A 1×10^{-3} M stock solution of Taxol was used; for this drug, seven concentrations ranging from 1×10^{-10} to 1×10^{-4} were used. An aliquot of the appropriate diluent was used in place of drug solution for the samples that received no drug.

For each drug concentration, mixtures of 1×10^6 cells and each drug at the appropriate concentration were placed into six separate electroporation cuvettes. Three of these samples were not pulsed to represent cells treated with drug alone and no electric pulses (D+E−). The remaining three samples were pulsed at the electric field strength that maintained 90% survival for the particular cell line under investigation. This group represented cells treated with drug and electric pulses (D+E+). During the testing of each drug, three samples were also treated with no drug and no pulses (D−E−), and an additional three samples were treated with pulses and no drug (D−E+). Drug and cell mixtures were prepared immediately prior to filling each set of six cuvettes so that the cells had minimal drug exposure time prior to electroporation. Pulsed samples were incubated for 5 min before removing the cells from the cuvettes; unpulsed samples were incubated for an identical time period. Three aliquots of 1×10^4 cells were then plated from each sample onto a 96-well tissue culture plate. Plates were incubated for 48 h and then analyzed using the MTT assay. Results were graphed as percent survival versus the log of the drug concentrations.

Results

Before investigating how electroporation can enhance the cytotoxicity of the anticancer agents used in this study, electrical conditions that induced electroporaton with a high percentage of cell survival had to be determined for each cell line. Therefore, each of the seven cell lines used for this study was exposed to nine different fields ranging from 500 to 4000 V/cm. Three different samples were exposed to each field. Twenty-four hours after exposure an MTT survival assay was performed. Three replicate experiments were performed for each cell line. Survival data was pooled from all three experiments to obtain a mean and SEM for each electric field. Figure 1 shows the resulting data for the B16 cell line. Note that a negligible effect on survival resulted when fields in the range of 0–1000 V/cm. However, survival was effected for fields of 1250 V/cm and higher. The remaining cell lines had similar survival profiles with respect to lower fields having negligible effect on survival, but the threshold value at which survival began to decrease differed from cell line to cell line. Field strengths that resulted in 90% survival were identified for each cell line. These conditions were identified for further investigation as they were likely to result in a high degree of electroporation while maintaining viability. Table 1 lists the field strength identified for each cell line. Table 1 also lists the mean diameter of each cell type along with the standard deviation associated with each mean.

In order to establish that the field identified for each cell line induced an electroporated state, each cell type was exposed to 1×10^{-4} M bleomycin–FluorX and pulsed using the respective field indicated in Table 1. Five minutes after pulsation, the cells were washed twice with phosphate-buffered saline and analyzed by flow cytometry. Figure 2 shows resulting histograms for the Capan-2 cell line. One sample in Figure 2 was exposed to the fluorescent conjugate alone, and the other was exposed to the conjugate and then pulsed. Samples that were exposed to bleomycin–FluorX and pulsed had cell populations that were markedly more fluorescent than their respective samples that were treated with the fluorescent tracer alone. The increased fluorescence indicated that electroporation had been achieved. Typically, about 5–15% of the cell populations overlapped on the histograms, which indicated that 85–95% of the cells had higher levels of the tracer relative to controls.

After establishing that the identified electrical conditions would result in electroporation, pulsed electric fields were then used to facilitate drug entry into the cells. Each cell line was exposed to anticancer agents with and without electric pulses. A range of exposure concentrations was used for each drug as noted in the

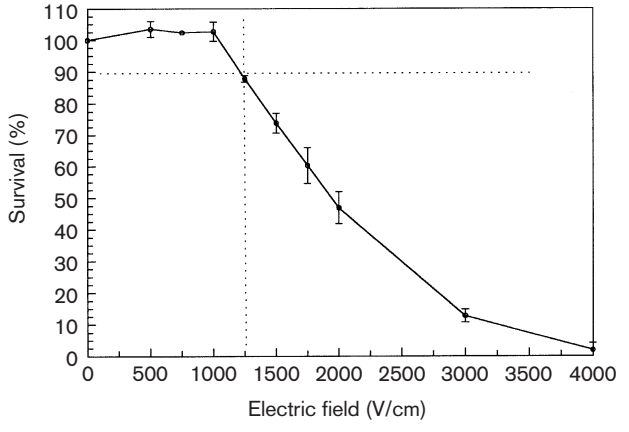

Figure 1. Survival of B16 cells after treatment with eight 99 μs pulses at the field strength specified on the *x*-axis. Each point represents the mean of three independent experiments with three replicate samples per experiment. Bars represent the SEM.

Table 1. Electric field strengths that resulted in 90% survival

Cell line	Mean cell diameter (μM) (SD)	Field Strength (V/cm)
Capan-2	13.38 (1.44)	900
LN1	12.69 (1.76)	1100
T47D	16.10 (2.31)	900
Lewis lung 2	12.37 (1.91)	750
N1-S1	14.58 (2.48)	1250
B16	16.49 (3.06)	1200
Ishikawa	12.62 (1.88)	1200

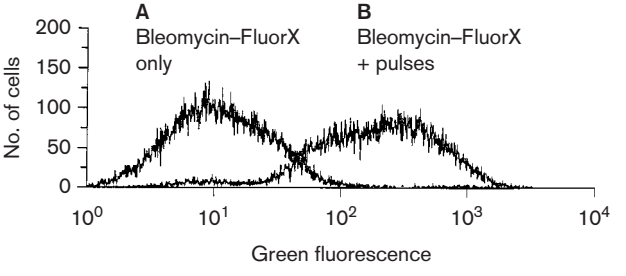

Figure 2. Flow cytometric histogram overlay from Capan-2 samples that were treated with (A) bleomycin–FluorX and (B) bleomycin–FluorX followed by 900 V/cm pulses. An increase in flourescence for samples treated with the labeled drug followed by eight 99 μs pulses at a field strength inicated in Table 1 was obtained for all cell lines in this study indicating that electroporation was achieved.

Materials and methods. The magnitude of the applied field for each cell line was determined to result in electroporation with a high percentage of survival. The

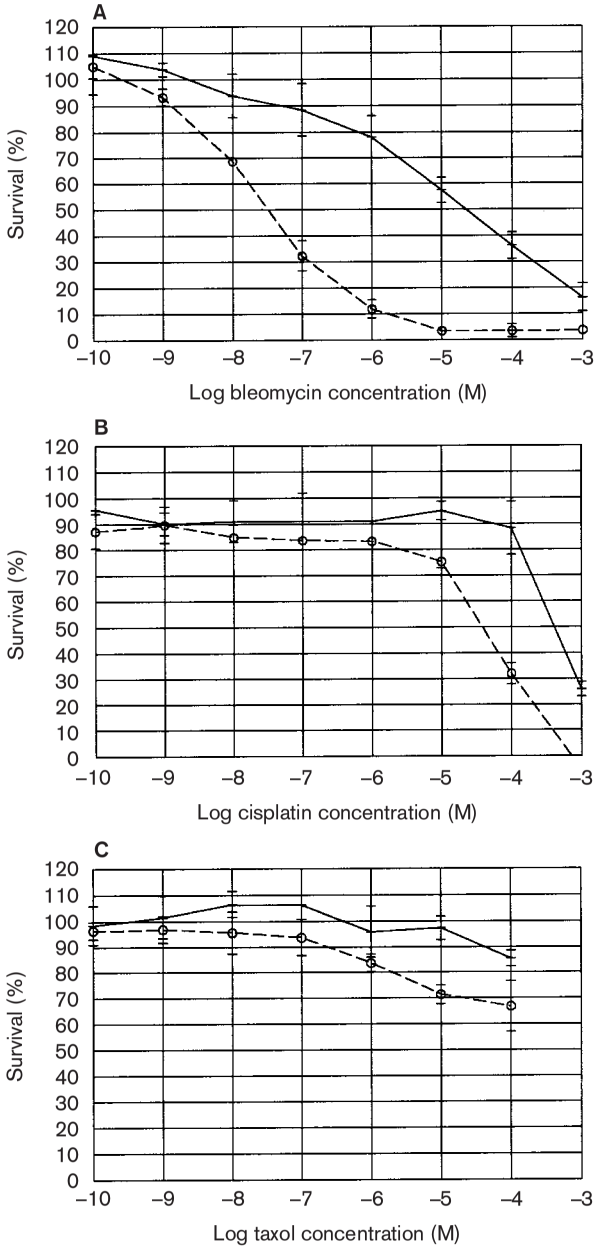

Figure 3. Representative plots of the cytotoxic effects of chemotherapeutic agents alone and combined with pulsed electric fields. Solid lines indicate exposure to drug; dashed lines indicate exposure to drug followed by electric pulses. (A) Effects of bleomycin on the Capan-2 cell line showing a large decrease in IC_{50} when pulsing was used to electroporate the cells. (B) Effects of cisplatin on T47D cells showing a moderate decrease in IC_{50} when the cells were treated with the drug and electric fields. (C) Effects of Taxol on N1-S1 cells indicating that the IC_{50} was not reached for either treatment shown.

Table 2. IC_{50} data for drug exposure alone and drug exposure followed by electroporation

Drug	Capan-2			LN1			T47D			Lewis lung 2		
	IC_{50} drug only[a]	IC_{50} drug + EP[b]	Ratio[c,d]	IC_{50} drug only[a]	IC_{50} drug + EP[b]	Ratio[c,d]	IC_{50} drug only[a]	IC_{50} drug + EP[b]	Ratio[c,d]	IC_{50} drug only[a]	IC_{50} drug + EP[b]	Ratio[c,d]
Bleomycin	3.5×10^{-5}	5.0×10^{-8}	700	7.5×10^{-6}	3.0×10^{-8}	250	3.5×10^{-4}	5.0×10^{-7}	700	2.0×10^{-5}	2.0×10^{-7}	100
Carboplatin	$>1.0\times10^{-3}$	6.0×10^{-4}	>1.6	$>1.0\times10^{-3}$	6.0×10^{-4}	>1.7	4.0×10^{-3}	4.0×10^{-4}	10	$>1.0\times10^{-3}$	$>1.0\times10^{-3}$	ND
Cisplatin	7.0×10^{-4}	6.0×10^{-5}	12	7.8×10^{-4}	6.4×10^{-5}	12.2	6.0×10^{-4}	6.0×10^{-5}	10	5.0×10^{-4}	5.0×10^{-4}	1
Vincristine	4.0×10^{-6}	5.0×10^{-6}	0.8	4.0×10^{-6}	3.0×10^{-6}	1.3	3.5×10^{-7}	2.5×10^{-7}	3.4			
Vinblastine	4.5×10^{-7}	4.0×10^{-7}	1.1	5.5×10^{-7}	6.2×10^{-7}	0.9	5.0×10^{-7}	4.0×10^{-7}	1.3			
Cytarabine	2.0×10^{-4}	1.0×10^{-4}	2	$>1.0\times10^{-3}$	$>1.0\times10^{-3}$	ND	$>1.0\times10^{-3}$	$>1.0\times10^{-3}$	ND			
Mitomycin C	1.5×10^{-4}	1.5×10^{-4}	1	5.5×10^{-5}	4.0×10^{-5}	1.4				$>1.0\times10^{-3}$	$>1.0\times10^{-3}$	ND
Doxorubicin				2.0×10^{-4}	1.0×10^{-4}	2				7.0×10^{-5}	5.5×10^{-5}	1.27
Gemcitibine	$>1.0\times10^{-3}$	$>1.0\times10^{-3}$	ND									
Paclitaxel				5.0×10^{-6}	5.0×10^{-6}	1	5.0×10^{-7}	4.0×10^{-7}	1.3			
Taxotere				6.0×10^{-7}	6.0×10^{-7}	1	4.0×10^{-7}	4.0×10^{-7}	1			
Fluorouracil				$>1.0\times10^{-3}$	$>1.0\times10^{-3}$	ND						
Ancitibine							$>1.0\times10^{-3}$	$>1.0\times10^{-3}$	ND			
Etoposide										$>1.0\times10^{-3}$	$>1.0\times10^{-3}$	ND

[a]IC_{50} (M) for exposure to drug alone. [b]IC_{50} (M) for drug exposure followed by electroporation. [c]Ratio of IC_{50} for exposure to drug alone to IC_{50} for drug exposure followed by electroporation. [d]ND, not determined because IC_{50} values for drug exposure alone and drug exposure followed by electroporation were higher than the maximum concentration tested.

Table 3. IC_{50} data for drug exposure alone and drug exposure followed by electroporation

Drug	N1-S1			B16			Ishikawa		
	IC_{50} drug only[a]	IC_{50} drug + EP[b]	Ratio[c,d]	IC_{50} drug only[a]	IC_{50} drug + EP[b]	Ratio[c,d]	IC_{50} drug only[a]	IC_{50} drug + EP[b]	Ratio[c,d]
Bleomycin	9.0×10^{-6}	2.0×10^{-9}	4500	1.0×10^{-4}	2.0×10^{-7}	500	1.0×10^{-3}	2.0×10^{-7}	5000
Carboplatin	4.0×10^{-4}	3.0×10^{-5}	13						
Cisplatin	4.0×10^{-5}	5.0×10^{-6}	8	2.5×10^{-4}	4.0×10^{-4}	6.3	1.0×10^{-4}	4.0×10^{-5}	3
Doxorubicine	2.0×10^{-6}	3.0×10^{-6}	0.67						
Paclitaxel	$>1.0\times10^{-4}$	$>1.0\times10^{-4}$	ND						
Fluorouracil	7.5×10^{-5}	6.0×10^{-5}	1.25						

[a]IC_{50} (M) for exposure to drug alone.
[b]IC_{50} (M) for drug exposure followed by electroporation.
[c]Ratio of IC_{50} for exposure to drug alone to IC_{50} for drug exposure followed by electroporation.
[d]ND, not determined because IC_{50} values for drug exposure alone and drug exposure followed by electroporation were higher than the maximum concentration tested.

fields noted in Table 1 were used. Figure 3 shows representative results for three combinations of cell line and drug. Each of the plots in Figure 3 show mean survival versus drug concentration. Results from exposing a single cell line to drug alone and for the combination of drug exposure followed by electroporation are located on the same plot. Figure 3 includes examples of very high IC_{50} reduction in cells that were electroporated, moderate IC_{50} decreases from electroporation and IC_{50} values that were not reached. Tables 2 and 3 shows a complete listing of 44 combinations of drug and cell line that were examined in this study.

Discussion

The initial phase of this study focused on identifying electrical conditions that induce electroporation for all seven of the cell lines used. These fields were selected so that post-treatment viability was maintained and ranged from 750 to 1250 V/cm. These fields are similar to those used with mammalian cells in other studies.[4–12] It was necessary to identify these conditions before using electroporation to increase drug internalization as it has been established that severe electrical treatment can lead to cell death. Thus, it was assured that the cytotoxic effects of the anticancer drugs would truly be due to the agent and not due to the electrical treatment. The fields were further tested to demonstrate that an increase in intracellular fluorescence could be achieved in cells that were treated with their respective electrical conditions in the presence of bleomycin–FluorX. This testing confirmed that electrical treatment provided exogenous molecules access to the cytosol.

The diameter of the cell types did not correlate with the field that was required to electroporate the cells while maintaining cell viability. A correlation of this type is predicted by the commonly used equation $V_I=1.5\,F\,r\,\cos(\Theta)$, where V_I is the transmembrane potential induced by electric field exposure, F is the applied field, r is the cell radius and Θ reflects position on the cell surface, where $\Theta=0^\circ$ and $\Theta=180^\circ$ are normal to anodic and cathodic electrodes, respectively. This equation is widely accepted in the field of electroporation[32,33] as a simplified expression of the potential induced in a cell up to a V_I that is sufficient to cause dielectric breakdown of the membrane. It can easily be seen from this equation that cells with larger diameters, assuming that all other variables are equal, require a lower applied field for membrane breakdown and vice versa. This type of relationship was not evident for the cells listed in Table 1. It is likely that biological variables between the cell types overshadowed the theoretical relationship between cell size and the applied field required to achieve electroporation. These variables include differences in intracellular resistances and membrane conductance which are not accounted for in the above equation.

This study examined the augmentation of the cytotoxic effects of 14 different chemotherapeutic agents by facilitating their entry into the cytosol with electroporation. The ratio of IC_{50} for samples that were not treated with electroporation to the IC_{50} of samples treated with pulses was used as a measure of cytotoxicity enhancement. The cytotoxic effects of bleomycin were enhanced in all seven cell lines tested. The enhancement ranged from 100 to 5000, relative to drug exposure alone, when bleomycin was combined with pulsed electric fields. This is consistent with other studies[4–9] that also showed that electroporation markedly decreased the IC_{50} of bleomycin.

The results of this study also indicate that carboplatin was consistently more effective when used in combination with permeabilizing pulses. The degree

of augmentation was not as pronounced as with bleomycin; however, four of five cell lines tested had reduced IC_{50} values as a result of combining pulses with the drug. T47D and N1-S1 cells were effected by a factor of 10 and 13, respectively. Capan-2 and LN1 cells had enhanced toxicities that were greater than 1.6 and 1.7, respectively. More specific determinations could not be made for these two cell types because IC_{50} values for exposure to the drug alone were greater than the highest drug concentration tested. IC_{50} values were, however, attained when pulses were used with Capan-2 and LN1 cells. Carboplatin did not have increased toxic effects in the Lewis lung 2 cells when pulses were applied.

The toxicity of cisplatin was also consistently enhanced by pulsed electric fields. The drug had increased toxicity in Capan-2, LN1, T47D, N1-S1, B16 and Ishikawa cells. Augmentation was a factor of 3–12.2. Cisplatin toxicity was not enhanced in the Lewis lung 2 cell line as a result of electrical treatment.

Electrical treatment resulted in increased cytotoxicity for two additional drugs. Each drug had enhanced cytotoxicity in one cell line. Vincristine had a 3.4-fold toxicity enhancement in T47D cells and the toxic effects of Cytarabine in Capan-2 cells was increased by a factor of 2. All other combinations of drug and cell line did not result in substantial cytotoxicity increases when electroporation was used. For these cases it is possible that the amount of drug that entered the cells by normal processes was equal to the quantity that entered as a result of drug exposure followed by electroporation. Another possibility is that any additional drug uptake that resulted from electroporation was insufficient to increase the cytotoxicity of the chemotherapeutic agent.

Bleomycin is currently the drug of choice for ECT. Thus, agreement between the present study and the literature with regard to decreasing the IC_{50} of bleomycin by electroporation was expected. The increased cytotoxicity of carboplatin in four cell lines is in agreement with the study of Gehl *et al.*[6] where a 3-fold augmentation in cytotxicity was determined for a single cell line (DCF-3 Chinese hamster lung fibroblasts). This indicates that carboplatin may be efficacious when combined with electric pulses *in vivo*; this combination warrants further investigation in the form of animals studies. Interestingly, electroporation was found to reduce IC_{50} values for cisplatin in six cell lines; a similar result was obtained for DCF-3 cells.[6] In addition, electric pulses have been shown to decrease the IC_{50} of cisplatin by a factor of 3 in NHIK 3025 human uterine cervical carcinoma cells,[11] 70 in B16 murine melanoma cells,[12] and 20 in IGROV-1 human ovarian adenocarcinoma cells[10] in other *in vitro* studies. Cisplatin has been used as the drug component of electrochemotherapy in far fewer studies than bleomycin; these *in vitro* results suggest that cisplatin may be efficacious when combined with pulsed fields for a broad range of tumor types.

Conclusions

This study demonstrates a simple *in vitro* protocol to efficiently screen/test potential drugs for ECT. The procedure involves identifying electrical conditions that result in electroporation with minimal cell losses due to electrical treatment. Identified electrical conditions are then used in combination with anticancer agents to determine changes in IC_{50} values. Bleomycin IC_{50} values were reduced by a maximum of 5000-fold when pulses were used to allow the drug access to the cytosol in seven tested cell lines. Similarly, this study showed that cisplatin has reduced IC_{50} values when combined with electric pulses in six cell lines which indicates that ECT animal studies[23,24] and a clinical trial[25] that have utilized cisplatin could be sucessfully extended to include other types of tumors. Carboplatin was determined to have cytotoxicity increases in four out of five tested cell lines. This indicates that carboplatin may also be useful as the drug component of ECT. In addition, the methods used in this study can be applied to anticancer agents that have not yet been explored for their potential cytotoxicity enhancement when coupled with electroporation.

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