J Membrane Biol (2014) 247:147–154
DOI 10.1007/s00232-013-9617-9

Optimization of Electric Pulse Amplitude and Frequency In Vitro for Low Voltage and High Frequency Electrochemotherapy

Zeinab Shankayi · S. M. P. Firoozabadi · Zohair Saraf Hassan

Received: 13 June 2013 / Accepted: 8 November 2013 / Published online: 23 November 2013

Abstract During standard electrochemotherapy (ECT), using a train of 1,000 V/cm amplitude rectangular pulses with 1 Hz frequency, patients experience an unpleasant sensation and slight edema. According to the patients, muscle contractions provoked by high amplitude (about 1,000 V/cm) and low repetition frequency (1 Hz) pulses are the most unpleasant and painful sensations. Recently, ECT using low voltage and higher repetition frequency (LVHF) has been shown to be an effective tool for inhibiting tumor growth. The aim of the present study was to optimize electric pulse amplitude and repetition frequency for LVHF ECT by sampling the different sets of pulse parameters on cell viability and permeabilization. In ECT, a reversible effect based on high permeabilization is desirable. For this purpose, we used bleomycin to evaluate the permeabilization of K562 and MIA-PACA2 cells caused by low voltage (50–150 V/cm) and higher repetition frequency (4–6 kHz) electric pulses. We show that the reversible effect with electropermeabilization of the cells caused by LVHF ECT is accessible; this interaction is more effective for electric pulses with 70 V/cm amplitude.

Keywords Electrochemotherapy · Low voltage · Higher repetition frequency · Cell viability · Electropermeabilization

Z. Shankayi · S. M. P. Firoozabadi (✉)
Department of Medical Physics, School of Medical Science, Tarbiat Modares University, Tehran, Iran
e-mail: pourmir@modares.ac.ir

Z. S. Hassan
Department of Immunology, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran

Introduction

Electric pulses with application in drugs and genes delivery have been used for more than two decades; this phenomenon has been named as electroporation. The use of electric field, at specific parameters, induced reversible or irreversible effects on the cell membrane (Chen et al. 2006; Gehl 2003; Kotnik et al. 2001). In the reversible electroporation method, electropores are formed on the plasma cell membrane during electroporation and are resealed a short time after the electroporation procedure. Therefore, this method lets the cells survive, while irreversible electroporation directly kills the cell (Golberg et al. 2011; Zupanic et al. 2012).

Electrochemotherapy (ECT) employed the electric pulse effect to improve the transport of chemotherapy drugs inside cancer cells. A reversible effect based on high permeabilization is desirable in ECT (Sersa et al. 2008; Zupanic et al. 2012). Therefore, a threshold effect in cell killing and permeability, which is influenced by properties of electric pulses and cell type, is required. For the transient effect, choosing the appropriate amplitude, number, and repetition frequency of pulses is important (Lebar et al. 2002a; Rols and Teissié 1998; Gothelf et al. 2003). According to these situations, ECT standard protocols are proposed. These protocols have used a train of 1,000 V/cm voltage to distance ratio resulting in few/several hundred V/cm local electric fields and rectangular pulses with 1 Hz repetition frequency for the treatment of patients (Lebar et al. 2002b). Using this protocol, patients experience an unpleasant sensation and slight edema or erythema. Most unpleasant and painful, according to the patients, are the sensations during the pulse delivery, which are mainly attributed to muscle contractions provoked by high amplitude and low repetition frequency pulses. Edema

results from high local current density (Sersa et al. 2008; Mir et al. 2006; Miklavčič et al. 2005; Pucihar et al. 2002). In order to reduce the pain sensation during ECT, application of higher repetition frequency or low amplitude electric field has been suggested (Pucihar et al. 2002; Miklavčič et al. 2005; Shankayi et al. 2010).

Recently, ECT by low intensity and high repetition frequency has been shown to be an effective tool for inhibiting tumor growth. Electrochemotherapy involving the burst of rectangular electric pulse, 100 μs duration, using low amplitude pulse (70–150 V/cm) with 5 kHz repetition frequency, has been successfully used in the in vivo condition (Shankayi and Firoozabadi 2011; Shankayi et al. 2010, 2012). The higher electric pulse repetition frequency, above the frequency of tetanic contraction, reduces the number of induced muscle contractions and is more comfortable for ECT (Miklavčič et al. 2005; Zupanic et al. 2007). But, the effect of this protocol on viability and permeabilization is not clear. Therefore, the aim of the present study was to determine the effective electric pulses for electrochemotherapy using electric pulses with high repetition frequency (4–6 kHz) and low amplitudes (50–150 V/cm) from an in vitro experiment. Because electrochemotherapy is based on enhanced uptake of chemotherapeutic drugs into tumor cells with reversible effects on viability, we examined the effect of different sets of electric pulses on the viability of cells and the uptake of bleomycin into electropermeabilized cells in vitro.

Materials and Methods

Cell Lines

The human erythroleukemia K562 cell line as a sensitive cancer cell to ordinary chemotherapy (type one is derived from a CML (Chronic myeloid leukemia) patient) and the human pancreatic carcinoma Mia Paca-2 cell line as a resistant cancer cell to ordinary chemotherapy were grown in RPMI containing 10 % fetal bovine serum, 160 μg/ml L-glutamine (all from Invitrogen, GIBCO, USA), 100 units/ml penicillin, and 16 μg/mg gentamicin, and incubated in 5 % CO_2 at 37 °C.

Electric Pulse Exposure

Electric pulses were applied to the cells using an ECT-SBDC (designed and made in the Small Business Development Center and Bioelectromagnetic Laboratory of the Medical Physics Department of Tarbiat Modares University, Tehran, Iran). The cells suspended in RPMI were placed between two parallel plate gold electrodes 10 mm apart and exposed to 4,000 electric pulse, for 100 μs duration with different electric intensities and frequencies. Electric pulses applied in our study were as follows: 50–150 V/cm with increment of 10 V/cm in 4, 5, and 6 kHz pulse repetition frequency (In total 33 protocols). All experiments were repeated three times.

Determination of Cell Permeabilization

To determine the uptake of molecules into the permeabilized cells, bleomycin was added to the cell suspension before pulsing. In the present experiments, the anticancer drug bleomycin (Nippon Kayaku Co. Ltd., Tokyo, Japan) was used at 1 μM concentration.

After trypsinization and inactivation of trypsin (Bio Idea Group, Tehran Iran) by the serum factors of the complete medium (only for Mia Paca-2), cells were centrifuged for 5 min at 1,000 rpm and resuspended at a density of 1×10^6 cells/ml in RPMI (Invitrogen, GIBCO, USA) eventually containing bleomycin at 1 μM. 300 μl of the mixture was immediately deposited between the two electrodes and subjected to the electric treatment. After the delivery of the electric pulses, the cells were kept for 1 min in room temperature and then seeded in a 96-well plate, and the complete cell culture (RPMI containing 20 % fetal bovine serum, 320 μg/ml L-glutamine) was added to each well to measure the viability through a MTT assay.

MTT Assay

EP and ECT cytotoxicity was evaluated by the MTT assay. The viability of the cells after electric field exposure was tested by 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Invitrogen, GIBCO, USA). 20 μl of MTT solution (5 mg MTT/ml in PBS) was added to the wells 24 h after the electric field exposure for the K562 cell (mitotic cycle is about 10 h) line and 72 h after exposure for the Mia Paca-2 cell line (mitotic cycle is about 32 h). Then, the cells were incubated at 37 °C for 4 h. 100 μl DMSO was added to the well and mixed. After 15 min, the optical density was measured in a Multiscan MS ELISA reader (Labsystems Multiscan MS, U.K.) with a 540-nm filter.

Statistical Analysis

All results are given as an average of more than three times and are represented in bar graphs. Vertical bars represent the standard deviation of the mean. Statistical analyses were performed using SPSS for windows 16.0. (SPSS Inc., Polar Engineering and Consulting). All data were tested for normality. One-way ANOVA, followed by LSD, was performed and statistical difference analysis was accomplished by a t test. P values of less than 0.05 were considered significant for rejection of the null hypothesis.

Results

Toxicity of Electric Pulses Alone as a Function of the Repetition Frequency and Strength of Electric Pulses

In the present study, we varied the amplitude of pulses between 50 and 150 V/cm at the increment of 10 V/cm in 4, 5, and 6 kHz repetition frequency. The influence of 33 different sets of electric pulses on cell viability and electropermeabilization was measured. In the first step, we reported the effect of these conditions on leukemia and pancreatic cancer cells.

Leukemia Cells

The MTT test was carried out to determine the sensitivity of K562 cells for 33 electric pulses alone (Fig. 1). In this cell line, as the cells were sensitive to treatment, below 70 V/cm amplitude cell viability was desirable (more than 50 %).

Pancreatic Cancer Cell

In this part, MIA-PACA2 cells were exposed to three different repetition frequencies of 4, 5, and 6 kHz and eleven different pulse amplitudes (50–150 V/cm); cells viability was evaluated (Fig. 2). In this cell line, as the cells were resistant to treatment, 150–110 V/cm pulse amplitude led to the death of more than 50 % of the cell population, below 110 V/cm the cell viability increased, and below 80 V/cm the cell viability was partly preserved (Fig. 2).

Toxicity of Bleomycin as a Function of the Repetition Frequency and Strength of Electric Pulses

Leukemia Cells

Comparisons were also performed between the ECT group and the group of electric pulse alone and bleomycin uptake. Figure 4 shows the viability of the K562 cells treated with BLM at 24 h after the electric pulse exposure as a function of pulse repetition frequency and pulse amplitude. The cytotoxic effect in the group with the LVHF ECT in the presence of the BLM was significantly higher than that in the group with BLM alone. This effect significantly increased on LVHF ECT with 70 V/cm at all frequencies (Fig. 3).

Pancreatic Cancer Cell

These experiments were repeated in the presence of bleomycin and applying electrical pulses for pancreatic cancer cells. Bleomycin generates some decrease in cell viability (Fig. 4). In contrast to the K562 cell line, viability reduction, in comparison to EP, significantly increased below 110 V/cm (Figs. 3, 4).

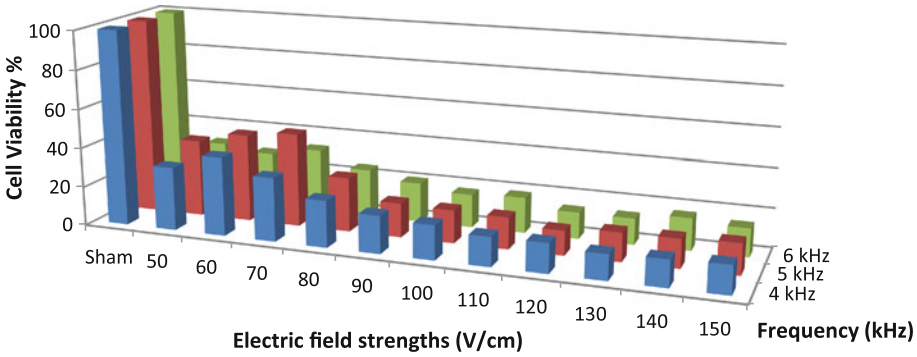

Fig. 1 Viability of the K562 cells at 24 h after the LVHF EP

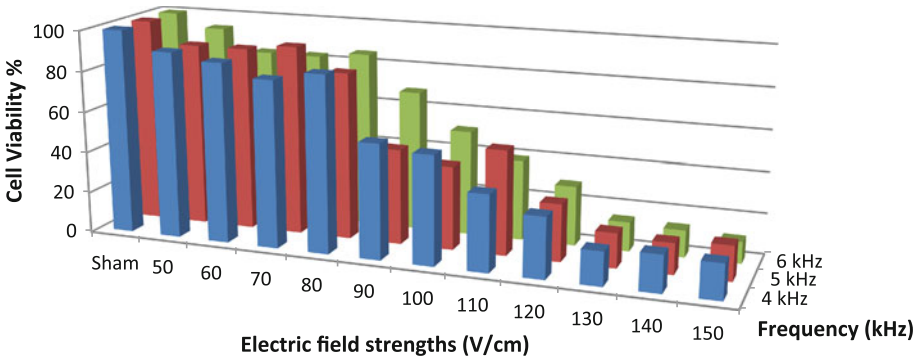

Fig. 2 Viability of the MIA-PACA2 cells at 72 h after the LVHF EP

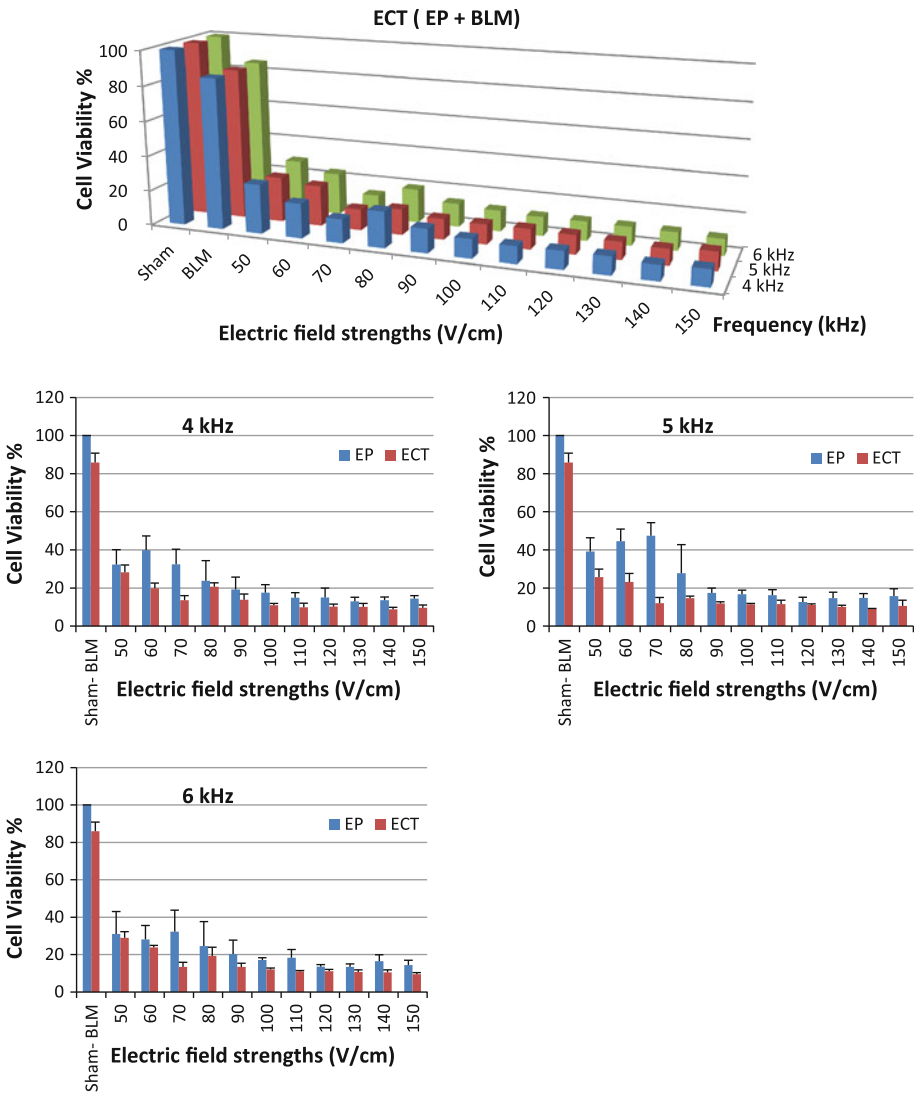

Fig. 3 Viability of the K562 cells at 24 h after the LVHF ECT. Results are presented as mean ± SD

In continuation of the procedure, we subtracted the cells' viability after EP, from ECT, to find the best treatment protocols between the treatment groups (This number will represent the cell permeability). Based on this method, each group which had the highest value was selected as the best group (Table 1). Our data, which are presented in Table 1, demonstrate that LVHF ECT using 70 V/cm amplitude and 5 kHz pulse repetition frequency is the best protocol.

Discussion

The present study has demonstrated sufficient cell permeability with LVHF ECT. Our results show that LVHF ECT can efficiently enhance cells' permeability (Figs. 1, 2, 3, 4). Among the treatment groups, the system gives the optimal permeabilization when cells are exposed to a train of 4,000 square pulses with 70 V/cm amplitude and at all three frequencies (4, 5, and 6 kHz).

ECT with standard protocols is already used for cancer treatment in clinics (Sersa et al. 2012; Mir et al. 2006). Despite the benefits of this approach, some side effects were reported. The most unpleasant and painful side effects of ECT are the muscle contractions and related sensations during pulse delivery (Sersa et al. 2008; Mir et al. 2006; Miklavčič et al. 2005; Pucihar et al. 2002). It would be possible to reduce these painful sensations using pulse frequencies higher than tetanic contractions or lower electric field strengths (Miklavčič et al. 2005; Zupanic et al. 2007; Ikehara et al. 2005). In vitro and in vivo experiments have demonstrated that chemical uptake at the highest frequencies is achievable (Sersa et al. 2010; Miklavčič et al. 2005; Pucihar et al. 2002; Shankayi and Firoozabadi 2012). Also, with the increase of the number or duration of pulses, an additional increase in uptake can be

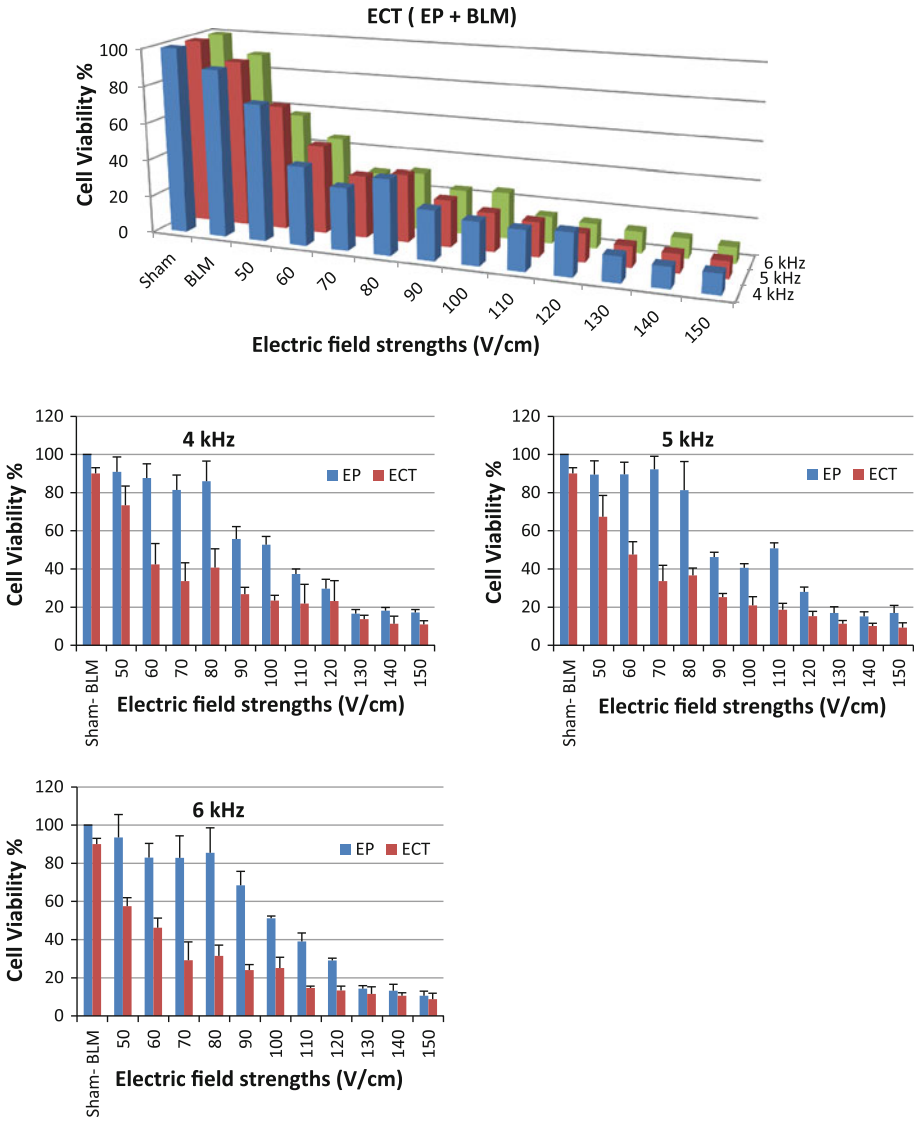

Fig. 4 Viability of the MIA-PACA2 cells at 72 h after the LVHF ECT. Results are presented as mean ± SD

Table 1 Subtraction of viability (%) of cells treated with EP compared to ECT (EP–ECT) %

Frequency/cell line		Electric amplitude (V/cm)										
		50	60	70	80	90	100	110	120	130	140	150
4 kHz	K562	4.17	20.04	18.87	3.17	5.42	6.66	5.08	4.85	2.78	4.83	4.74
	MIA-PACA2	17.56	**45.31**	**47.81**	**45.33**	28.84	29.36	15.50	6.49	2.82	6.79	6.30
5 kHz	K562	13.39	21.33	**35.41**	13.02	5.60	5.29	4.58	1.54	4.59	5.72	5.21
	MIA-PACA2	22.13	41.92	**58.67**	**44.69**	20.99	19.75	32.13	12.67	5.60	5.15	7.72
6 kHz	K562	2.08	4.22	18.90	5.25	6.82	5.06	7.32	2.52	2.68	6.09	4.91
	MIA-PACA2	36.07	36.83	**53.67**	**54.08**	44.38	25.99	24.47	15.72	2.69	2.64	1.77o

The bold numbers show the best response to ECT

obtained (Pucihar et al. 2002; LEBAR et al. 2002b). Several articles have reported that a low voltage electric field could inhibit tumor growth (Entin et al. 2003; Horiuchi et al. 2000). Recently, by changing frequency, pulse amplitude, and the number of pulses, sufficient results in LVHF ECT were obtained (Mansourian et al. 2013; Shankayi and Firoozabadi 2011; Shankayi et al. 2010, 2012). In a hybrid approach, we combined electric pulses using 5 kHz repetition frequency and low voltage amplitude (70, 100, and 150 V/cm) with 500, 2000, 4000, and

5,000 number of pulses. In the conditions that we examined, the best protocol was that utilizing 70 V/cm amplitude, 4,000 pulses of 100 μs duration, and 5 kHz pulse repetition frequency (Shankayi and Firoozabadi 2011; Shankayi et al. 2010). Furthermore, we showed that despite standard ECT, frequency is an important parameter in LVHF ECT. This study showed significant differences in tumor volumes between mice treated with 70 V/cm with 5 kHz repetition frequency and 1 Hz repetition frequency; but, the inhibited tumor growth in high amplitude with 1 Hz and 5 kHz was comparable (Shankayi et al. 2012).

In ECT, the electric pulses are used to induce transient permeabilization in the cells plasma and to deliver nonpermeant drugs inside the cells (Chen et al. 2006; Kotnik et al. 2001; LEBAR et al. 2002b). Therefore, to reach this optimization level, we investigated the effect of LVHF ECT on cell viability and the uptake of bleomycin into electropermeabilized cells. Bleomycin is a nonpermeant chemotherapy drug with a high cytotoxicity. Indeed, if molecules of bleomycin can enter the cell, less than 500 molecules are needed to kill it (Mir 2006; Silve et al. 2011; Silve and Mir 2011). Therefore, the detection of cell death indicates that at least 500 molecules have been able to cross the plasma membrane and allows us to quantitatively determine plasma membrane permeabilization(Silve et al. 2011; Silve and Mir 2011). This study, in continuation of a prior study (Mansourian et al. 2013; Shankayi et al. 2013; Silve et al. 2011; Tofani et al. 2003), confirms the possibility that bleomycin is a very sensitive cell marker of plasma membrane permeabilization, and in spite of other markers such as YoPRO, Lucifer yellow, or Propidium iodide which do not allow for quantitative data achievement, BLM has this advantage. The other disadvantage of fluorescence permeabilization markers is that fluorescence intensity will change because of the marker's interaction with the cell inside components, like DNA (Silve et al. 2011).

Type of cells and electrical parameters like the pulse amplitude, pulse duration, number of pulses, and pulse repetition frequency are the major factors that affect electrochemotherapy (Lebar et al. 2002a; Rols and Teissié 1998; LEBAR et al. 2002b; Kotnik et al. 2001; Chen et al. 2010). The optimum conditions for membrane electropermeabilization are obtained by the interaction between pulse duration and pulse amplitude. So, the same permeabilization efficiency can occur either at low number and duration of pulses but high amplitude or at a low amplitude and high number and duration of pulses (Lebar et al. 2002a). The last electric field conditions preserved the viability. Rols and Teissié (1998) reported that increasing the number of pulses (N) enhanced the rate of permeabilization. Also, the same permeabilization efficiency was obtained at a low value for E and high values for N and T. Lebar et al. (2002a) experimented on lipid bilayer membranes and demonstrated interpulse intervals shorter than 250 ms; the second pulse resulted in an immediate jump in membrane conductance, while for longer interpulse intervals, the conductance behavior was similar to that of the first pulse. Furthermore, Pucihar et al. (2011) determined the relations among the basic parameters of electroporation resulting in the same effectiveness of EP. They demonstrated that longer pulses or higher number of electric pulses are needed to lower amplitudes for the same electroporated results (Mikirova et al. 2001). In accordance with these studies, we showed that LVHF ECT could permeabilize cancer cells. In standard protocols (1 Hz repetition frequency, 100 μs duration, and 8 pulses), the threshold amplitude for electropermeability is about 300–400 V/cm (Teissié et al. 2008; Cemazer et al. 1998) But, our data, using LVHF protocols (4–6 kHz repetition frequency, 100 μs duration, and 4,000 pulses) demonstrated that electropermeability occurred at a lower electroporation threshold (Figs. 3, 4).

The type of cells is other factor that affects electroporation. Cells size, shape, membrane structure, and composition, and transmembrane potential are important for in vitro experiments (Gehl 2003; Kotnik et al. 2012). Therefore, we chose two cell lines: one sensitive to treatment (K562) and another resistant to treatment (MIA-PACA2); our results, in accordance with that of other research, showed the importance of this. Interestingly, between the two cell lines, there is an increase in penetration of bleomycin in the resistant cell line. Two hypotheses can be considered for the fact. Although the electric pulses have an effect on both cell types, these membrane effects are lethal for sensitive cell, whereas resistant cells have the ability to repair these damages during two mitoses. In the presence of plasma membrane damage, BLM enters the cytoplasm, stabilizes the damage, and induces DSBs in the both cell lines. Moreover, with a small number of these fragmentations, the cell cannot divide; at the next mitosis, the daughter cells will be completely impaired, resulting in cell death (Silve and Mir 2011). Another hypothesis is that receptor-mediated endocytosis can play a role in addition to membrane damage, and it explains the increasing cell death in the MIA-PACA2 cell line. In previous studies, low amplitude electric field exposure was reported to enhance the uptake of cells. The reason for the uptake increase was suggested to be the stimulating endocytotic-like process named electroendocytosis (Antov et al. 2004; Antov et al. 2005; Shankayi et al. 2013; Towhidi et al. 2012). Therefore, based on these results, we suggest that maybe the electroendocytosis can facilitate the passage of external substances right through the membrane.

Conclusions

In conclusion, our experiments show the reversible effect of electropermeabilization on the cells caused by LVHF ECT in optimum parameters. We used bleomycin as a

marker to analyze permeabilization of the cells. The quantitative characteristic of this method lets us perform a comparison between the electric amplitude and repetition frequency of pulses and the type of cells.

Acknowledgments This study was supported by Tarbiat Modaresm University as part of the requirements of a PhD thesis.

References

Antov Y, Barbul A, Korenstein R (2004) Electroendocytosis: stimulation of adsorptive and fluid-phase uptake by pulsed low electric fields. Exp Cell Res 297(2):348–362. doi:10.1016/j.yexcr.2004.03.027

Antov Y, Barbul A, Mantsur H, Korenstein R (2005) Electroendocytosis: exposure of cells to pulsed low electric fields enhances adsorption and uptake of macromolecules. Biophys J 88(3):2206–2223. doi:10.1529/biophysj.104.051268

Cemazer M, Jarm T, Miklavcic D, Lebar A, Ihan A, Kopitar N, Sersa G (1998) Electrosensitmty of various tumor-cell lines in vitro. Elecfro Magnetobiol 17(2):263–272. http://lbk.fe.uni-lj.si/pdfs/em1998mc.pdf

Chen C, Smye S, Robinson M, Evans J (2006) Membrane electroporation theories: a review. Med Biol Eng Comput 44(1–2):5–14

Chen C, Evans JA, Robinson MP, Smye SW, O'Toole P (2010) Electroporation of cells using EM induction of ac fields by a magnetic stimulator. Phys Med Biol 55(4):1219–1229. doi:10.1088/0031-9155/55/4/021

Entin I, Plotnikov A, Korenstein R, Keisari Y (2003) Tumor growth retardation, cure, and induction of antitumor immunity in B16 Melanoma-bearing mice by low electric field-enhanced chemotherapy. Clin Cancer Res 9(8):3190–3197

Gehl J (2003) Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research. Acta Physiol Scand 177(4):437–447

Golberg A, Laufer S, Rabinowitch HD, Rubinsky B (2011) In vivo nonthermal irreversible electroporation impact on rat liver galvanic apparent internal resistance. Phys Med Biol 56(4):951–963

Gothelf A, Mir L, Gehl J (2003) Electrochemotherapy: results of cancer treatment using enhanced delivery of bleomycin by electroporation. Cancer Treat Rev 29:371–387

Horiuchi A, Nikaido T, Mitsushita J, Toki T, Konishi I, Fujii S (2000) Enhancement of antitumor effect of bleomycin by low-voltage in vivo electroporation: a study of human uterine leiomyosarcomas in nude mice. Int J Cancer 88(4):640–644.

Ikehara T, Yamaguchi H, Hosokawa K, Houchi H, Park KH, Minakuchi K, Kashimoto H, Kitamura M, Kinouchi Y, Yoshizaki K, Miyamoto H (2005) Effects of a time-varying strong magnetic field on transient increase in Ca^{2+} release induced by cytosolic Ca^{2+} in cultured pheochromocytoma cells. Biochimica et Biophysica Acta (BBA) 1724(1):8–16. doi:10.1016/j.bbagen.2005.03.008

Kotnik T, Mir LM, Flisar K, Puc M, Miklavčič D (2001) Cell membrane electropermeabilization by symmetrical bipolar rectangular pulses: part I. Increased efficiency of permeabilization. Bioelectrochemistry 54(1):83–90

Kotnik T, Kramar P, Pucihar G, Miklavcic D (2012) Cell membrane electroporation: Part 1: the phenomenon. Electr Insulation Mag IEEE 28(5):14–23

Lebar A, Troiano G, Tung L, Miklavcic D (2002a) Inter-pulse interval between rectangular voltage pulses affects electroporation threshold of artificial lipid bilayers. IEEE Trans Nanobiosci 1:116–121

Lebar AM, Sersa G, Kranjc S, Groselp A, Vcic DM (2002b) Optimisation of pulse parameters in vitro for in vivo electrochemotherapy. Anticancer Res 22:1731–1736

Mansourian M, Firoozabadi S, Shankayi Z, Hassan Z (2013) Magnetic fields with frequency of 217 Hz can reduce cell apoptosis caused by electrochemotherapy. Electromagn Biol Med 32(1):70–78

Mikirova N, Jackson JA, Casciari JJ, Riordan HD (2001) The effect of alternating magnetic field exposure and vitamin C on cancer cells. J Orthomol Med 16(3):177–182

Miklavčič D, Pucihar G, Pavlovec M, Ribarič S, Mali M, Maček-Lebar A, Petkovšek M, Nastran J, Kranjc S, Čemažar M, Serša G (2005) The effect of high frequency electric pulses on muscle contractions and antitumor efficiency in vivo for a potential use in clinical electrochemotherapy. Bioelectrochemistry 65(2):121–128. doi:10.1016/j.bioelechem.2004.07.004

Mir L (2006) Bases and rationale of the electrochemotherapy. EJC Suppl 4:38–44

Mir L, Gehl J, Sersa G, Collins C, Garbay J, Billard V, Geertsen P, Rudolf Z (2006) Standard operating procedures of the electrochemotherapy: instructions for the use of bleomycin or cisplatin administered either systemically or locally and electric pulses delivered by the CliniporatorTM by means of invasive or noninvasive electrodes. EJC Suppl 4:14–25

Pucihar G, Krmelj J, Rebersek M, Napotnik TB, Miklavcic D (2011) Equivalent pulse parameters for electroporation. IEEE Trans Biomed Eng 58(11):3279–3288

Pucihar G, Mir LM, Miklavčič D (2002) The effect of pulse repetition frequency on the uptake into electropermeabilized cells in vitro with possible applications in electrochemotherapy. Bioelectrochemistry 57(2):167–172. doi:10.1016/S1567-5394(02)00116-0

Rols MP, Teissié J (1998) Electropermeabilization of mammalian cells to macromolecules: control by pulse duration. Biophys J 75(3):1415–1423

Sersa G, Miklavcic D, Cemazar M, Rudolf Z, Pucihar G, Snoj M (2008) Electrochemotherapy in treatment of tumours. Eur J Surg Oncol 34(2):232–240. doi:10.1016/j.ejso.2007.05.016

Sersa G, Kranjc S, Scancar J, Krzan M, Cemazar M (2010) Electrochemotherapy of mouse sarcoma tumors using electric pulse trains with repetition frequencies of 1 Hz and 5 kHz. J Membr Biol 236(1):155–162. doi:10.1007/s00232-010-9268-z

Sersa G, Cufer T, Paulin SM, Cemazar M, Snoj M (2012) Electrochemotherapy of chest wall breast cancer recurrence. Cancer Treat Rev 38(5):379–386. doi:10.1016/j.ctrv.2011.07.006

Shankayi Z, Firoozabadi S (2011) Tumor growth inhibited by low-voltage amplitude and 5-kHz frequency electrochemotherapy. J Membr Biol 244(3):121–128

Shankayi Z, Firoozabadi S (2012) Antitumor efficiency of electrochemotherapy by high and low frequencies and repetitive therapy in the treatment of invasive ductal carcinoma in Balb/c mice. Cell J (Yakhteh) 15(2):110–115

Shankayi Z, Firoozabadi S, Hassan Z (2010) The effect of rectangular electric pulse number in electrochemotherapy by low voltage and high frequency on breast tumors in Balb/c mice. Cell J (Yakhteh) 12(3):381–384

Shankayi Z, Firoozabadi S, Hassan Z (2012) Comparison of low voltage amplitude electrochemotherapy with 1 Hz and 5 kHz frequency in volume reduction of mouse mammary tumor in Balb/c mice. Koomesh 13(4):486–490

Shankayi Z, Firoozabadi S, Mansourian M (2013) The effect of pulsed magnetic field on the molecular uptake and medium conductivity of leukemia cell. Cell Biochem Biophys 65(2):211–216

Silve A, Mir L (2011) Cell electropermeabilization and cellular uptake of small molecules: the electrochemotherapy concept. In:

Kee S, Gehl J, Lee E (eds) Clinical aspects of electroporation. Springer, New York, pp 69–82

Silve A, Leray I, Mir L (2011) Demonstration of cell membrane permeabilization to medium-sized molecules caused by a single 10 ns electric pulse. Bioelechemistry 87:260–264

Teissié J, Escoffre J, Rols M, Golzio M (2008) Time dependence of electric field effects on cell membranes: a review for a critical selection of pulse duration for therapeutical applications. Radiol Oncol 42:196–206. doi:10.2478/v10019-008-0016-2

Tofani S, Barone D, Berardelli M, Berno E, Cintorino M, Foglia L, Ossola P, Ronchetto F, Toso E, Eandi M (2003) Static and ELF magnetic fields enhance the in vivo anti-tumor efficacy of cis-platin against lewis lung carcinoma, but not of cyclophosphamide against B16 melanotic melanoma. Pharmacol Res 48(1):83–90. doi:10.1016/S1043-6618(03)00062-8

Towhidi L, Firoozabadi SM, Mozdarani H, Miklavcic D (2012) Lucifer yellow uptake by CHO cells exposed to magnetic and electric pulses. Radiol Oncol 46(2):119–125

Zupanic A, Ribaric S, Miklavcic D (2007) Increasing the repetition frequency of electric pulse delivery reduces unpleasant sensations that occur in electrochemotherapy. Neoplasma 54:246–250

Zupanic A, Kos B, Miklavcic D (2012) Treatment planning of electroporation-based medical interventions: electrochemotherapy, gene electrotransfer and irreversible electroporation. Phys Med Biol 57:5425–5440