ELECTROPORATION OF MALIGNANT CELLS FOR ENHANCED UPTAKE OF THERAPEUTIC DRUGS

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Abstract. In this work we intend to find optimal conditions (electric pulse amplitude and duration), in order to obtain high percentage of porated and viable cells by means of electroporation method. The experiments were done on B16F10 cell line. The poration yield and the viability of electroporated cells were evaluated following the ethidium bromide (EB) uptake in to the cells. Electric pulses (exponential decay) were used with the amplitude in the range of 0 – 6 kV/cm and pulse length of 100 μs and 200 μs. We obtained the highest percentage of viable porated cells for one or two pulses of 100 μs with the amplitude in the range of 1–2 kV/cm. The procedure described here offered a simple way to find optimal conditions of electroporation for malignant cells.

Key words: electroporation, electric field, cell culture.

INTRODUCTION

Electroporation is the physical process of inducing transient permeability of biological membranes to chemical species, by short pulses of electric fields [6]. The most important parameters for effective electroporation are the electrical field strength [kV/cm] and the length of time the field is applied (pulse length [μs]). One of the many uses of electroporation is that for treating tumors [1, 7] and it is also considered one of the useful techniques in gene therapy [3].

The action of a short electric pulse of appropriate amplitude is able to produce a reversible permeabilization of the membrane [2, 5]. During this state the exogenous molecules, for which normally the membrane is not permeable, can pass through the membrane barrier by diffusion. In cancer therapy the technique was successfully used to improve the efficiency of cytostatics [7, 8]. A derived method is the transdermal electroporation. The electric pulses are applied across the dermal layer increasing the diffusion of drugs through it.

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In this paper our work was focused on searching for a suitable protocol to electropermeabilize the malignant cells in culture. In view of this we were seeking for some good conditions of amplitude and length of the pulses applied. Our work aim is to increase by electroporation the aminolevulinic acid (ALA) penetration into tumor cells [5]. ALA is a precursor in synthesis of protoporphyrin IX (Pp IX) – a photo-sensitizer used in photodynamic therapy of cancer.

MATERIALS AND METHODS

B16F10 Murine metastatic melanoma cells were used as experimental object. They were cultured in 3 cm Petri dishes, at 37 °C, 5% CO2, in Dulbecco's MEM (Gibco) supplemented with 10% foetal calf serum (Gibco), 100 units/ml of penicillin and 100 μg/ml streptomycin (BioClone). The B16F10 cell line was kindly provided by Dr. L.M.Mir, Gustave-Roussy Institute (Villejuif), France (Fig. 1).

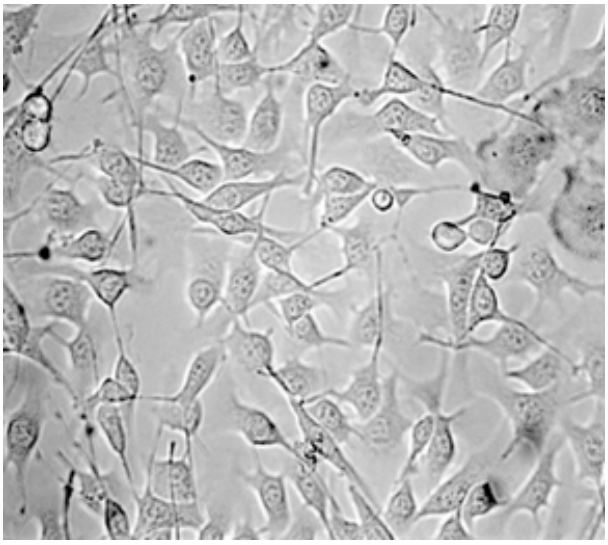

Fig. 1. The B16F10 murine metastatic melanoma cells monolayer grown in a normal cell culture flask.

The cell electroporation was done using an Eppendorf electroporator. The suspension medium was based on a mannitol solution, with a pH value of ~ 7.4 and a conductivity of ~ 4.5 μS/cm. Exponential electric pulses were applied between the electrodes of the electroporation cuvette (BioRad, 2 mm gap) where the cell suspension was introduced [4]. The electric field intensity ranged from 0.5 to 6 kV/cm. In order to evaluate the efficiency of electroporation, the uptake of a membrane

impermeable molecule (ethidium bromide, EB) was followed up (Fig. 2). The cells were electroporated in the suspension medium containing in addition EB. After 10 min the labelled cells were counted in a cell count chamber under a fluorescence microscope (Zeiss Axiovert 200) equipped with an appropriate filter set; the percentage of the coloured cells was calculated as the poration yield.

To evaluate the cells 'death' (at high electric field intensity a large number of cells die) the EB was added to the electroporated cell suspension after 10 min. It was considered that during this period the membrane permeability of surviving cells was recovered and only the irreversibly permeabilized cells will take up the dye. The coloured cells were again counted, resulting this time the dead cells percentage.

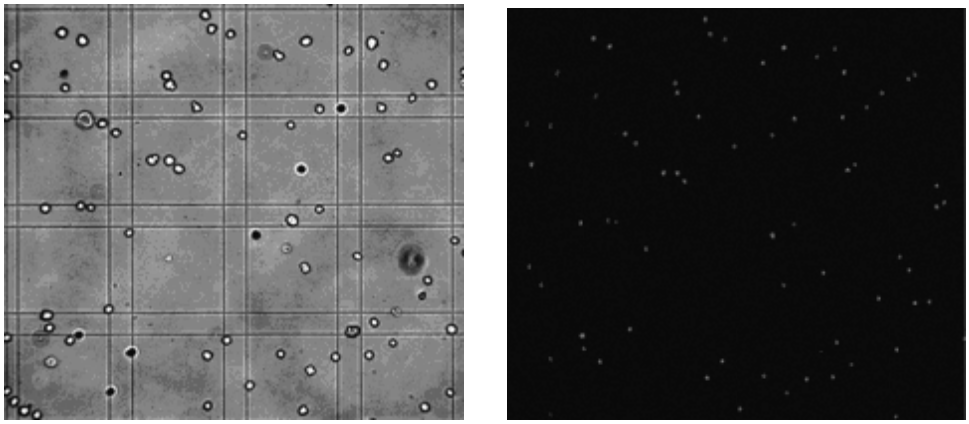

Fig 2. B16F10 cells treated with EB visualized by: a) transmitted light (left); b) fluorescence (right).

RESULTS

Percentage of porated and viable cells after treatment by 100 μs pulses of different voltages was evaluated by ethidium bromide uptake, as described above. An example of the results obtained in one experiment (for each electric field value three different samples were tested) are presented in Figures 3 and 4. The percentage of cells where the EB was uptaken (the porated cells) increases rapidly with the electric field intensity (Fig. 3) and after 2 kV/cm the number of porated cells is almost constant, approximately 90%. Adding the EB later, 10 min after the poration, the most of the electrically induced pores resealed and only the irreversible porated cells were able to take in the EB. These cells are actually dead. In Figure 4 the percentage of viable cells after poration is represented as function of electric field intensity and one can observe that the irreversible poration occurs for a field higher than 4.5–5 kV/cm.

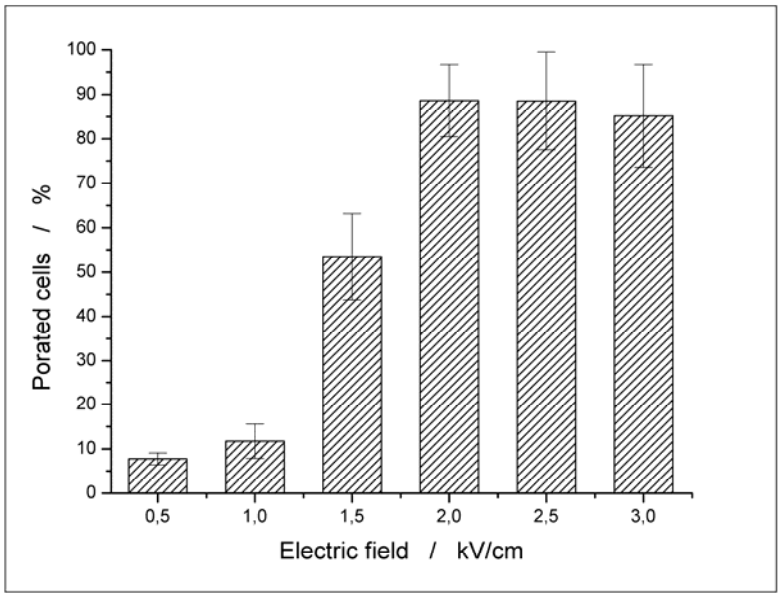

Fig. 3. Percentage of porated cells *versus* electric field values.

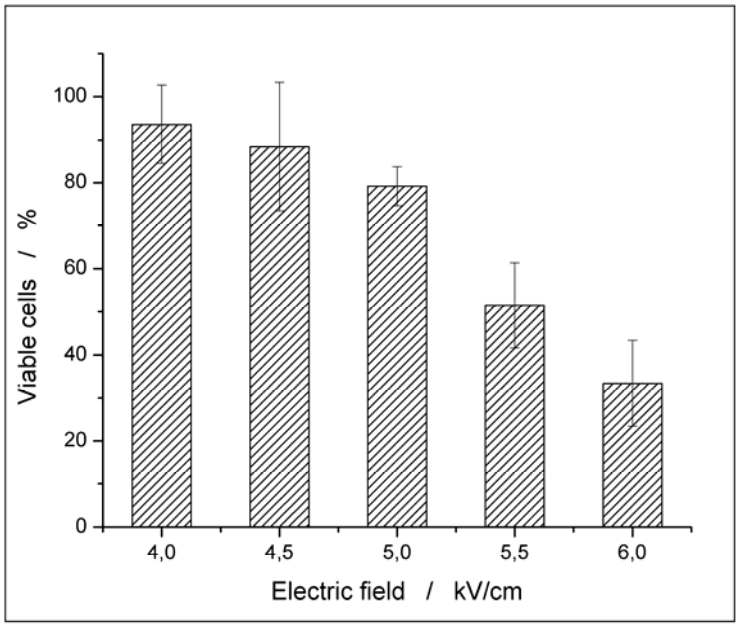

Fig. 4. Percentage of viable cells *versus* electric field values.

The global results obtained in repeated experiments for the electric field intensities in the range of 0.5–6 kV/cm representing the percentage of porated and viable cells are shown in Figure 5. At a low level of electric field intensity a rapid increase of porated cells percentage reaching a plateau for higher values of electric field is observed. On the other hand, at low field intensity the viability of cells is very close to maximum (100%) and decreases when the electric field increases. For medium values of the field intensity (between 2 and 4 kV/cm) the cells show a maximal level of poration and viability simultaneously.

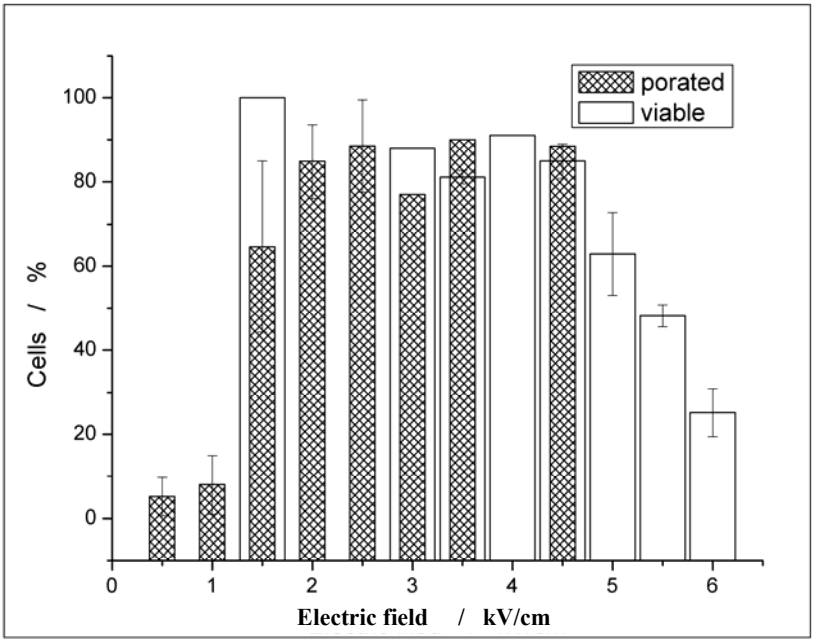

Fig. 5. Percentage of porated and viable cells as function of electric field intensity.

Another step we have followed was to study cell's behavior influenced by the pulse duration at 2.35 kV/cm (this value was considered to assure a high level of poration and viability according to Fig. 5). In this type of experiments the maximum of porated and viable cells was obtained at 100 μs. The results are presented in figure 6. The porated cells number increases very rapidly and reaches a plateau after 100 μs, while the viable cells percentage decreases after 200 μs. These results suggest an optimal range for pulse duration of 100 – 200 μs.

Knowing that the voltage is important for an optimal electroporation, and also that the duration and number of the pulses applied rise the efficiency of electroporation, the next step was to test the influence of the number of pulses (frequency between pulses was 1 minute as established by the producer of

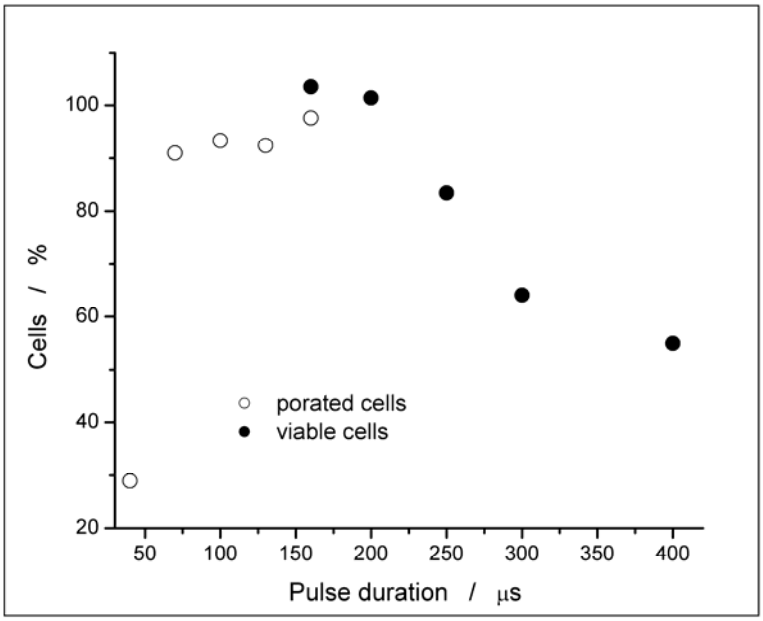

Fig. 6. Percentage of porated and viable cells *versus* pulse duration (2.35 kV/cm pulse intensity).

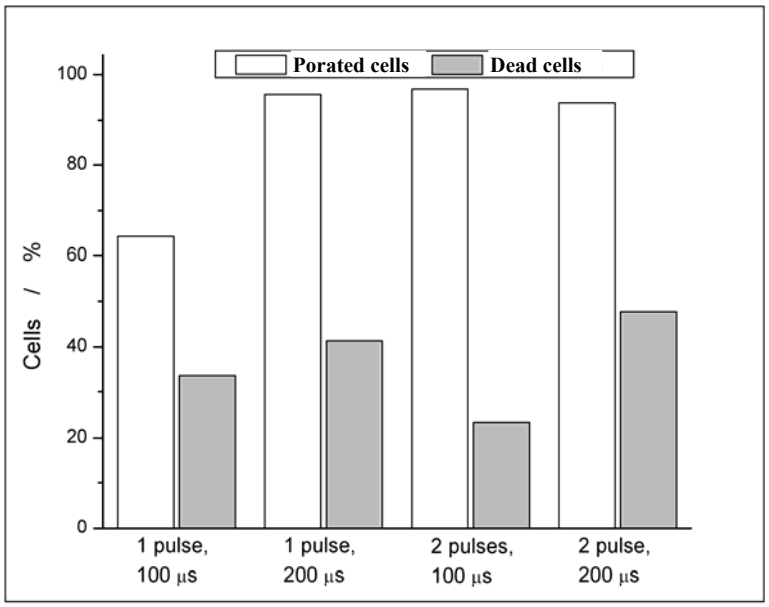

Fig 7. The effect of the number and duration of the pulses at 2.5 kV on porated and dead cells percentages.

the electroporator). This was done using one or two 2.35 kV/cm pulses with 100 μs and 200 μs duration. The results are shown in Figure 7 (as average results of two experiments). Applying one longer pulse with the duration of 100 μs more cells are porated. Instead, two pulses appear to have the same results independently of the pulse duration. Also, this experiment at 2.35 kV/cm will have a minimal effect on ulterior viability of the cells showing that using two pulses (one minute between pulses) of 2.35 kV/cm and 100 μs, is a good way for a better reversible electroporation with a good percentage of above 70% cell viability.

CONCLUSIONS

The electroporation in the presence of ethidium bromide was proved to be a very suitable way to find out the best conditions for the poration of B16F10 malignant cells. The repetition of a shorter pulse (100 μs, 1 kV/cm) allowed a higher percentage (above 70 %) of viable porated cells. The electric conditions described here can be used for entrapping also other small molecules into the B16F10 cells, but an optimization has to be done for each particular type of molecule.

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