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Effectiveness of tumor electrochemotherapy as a function of electric pulse strength and duration

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Abstract

The aim of this study was to evaluate the effectiveness of electrochemotherapy (ECT) as a function of various combinations of pulse strength and duration. C57Bl mice bearing LLC tumors were injected i.p. with bleomycin (BLM) at doses 5 mg/kg in 0.2 ml of physiological saline. Thirty minutes later, tumors were positioned between plate electrodes and were pulsed with eight-square wave electric pulses with an individual pulse strength of 900, 1100, 1300 or 1500 V/cm and duration of 0.1, 0.25, 0.5 or 1 ms. Effectiveness of ECT was estimated by measuring inhibition of tumor growth and by estimating extent of necrosis in histological slices of the treated tumors. At pulse strength of 900 V/cm and duration of 0.1 ms, electrochemotherapy was ineffective. Noticeable inhibition of tumor growth (threshold of ECT) was obtained when pulse duration at this field strength was increased up to 0.25 ms. Further increase of pulse strength and/or duration resulted in progressive enhancement of antitumor effects. Using tumor doubling time (DT) as a criteria, we showed that the same efficacy of ECT could be achieved using various pairs of values for pulse strength and duration. Largest antitumor efficacy of ECT was obtained at pulse strength of 1500 V/cm and duration of 1 ms. These pulse conditions applied alone neither significantly suppressed tumor growth nor induced noticeable side effects of the surrounding tissues. The results of this study thus suggest that the effectiveness of electrochemotherapy can be enhanced (in comparison to widely accepted conditions of electrochemotherapy—8 pulses of 1300 V/cm, 0.1 ms) if 1500-V/cm, 1-ms electric pulses are used. Our study also implicates that other pulse conditions could be found for this enhanced ECT.

Keywords: Electroporation; Electropermeabilization; Electrochemotherapy; Tumor treatment; Bleomycin; Lewis lung carcinoma

1. Introduction

When the cell in suspension or in tissue is exposed to sufficiently strong and long lasting electric field pulse(s) the permeability of the cell membrane temporarily increases providing access of exogenous molecules into the cytosol of the cell. The phenomenon is known as electroporation or electropermeabilization [1]. It has been successfully exploited for different biomedical applications in vivo [2]. The most promising between these applications are DNA electrotransfer into tissues for gene therapy [3–5] and antitumor electrochemotherapy [6–9]. Antitumor electrochemotherapy (ECT) is much more progressed and currently entered clinical trials. The treatment consists of the injection of highly cytotoxic drug (like bleomycin or cysplatin) followed by delivery of high voltage electric pulses to the tumor. The key point of ECT is that tissue permeabilization allows local targeting of cytotoxic drugs into the cells of the tumor exposed to electric field. This enables one to obtain effective antitumor responses with highly reduced doses of cytotoxic drugs.

Effectiveness of ECT basically depends on two main requirements of the treatment: (i) effective distribution of the highly cytotoxic drug (at appropriate doses) in the tumor and (ii) permeabilization of the vast majority of the tumor cells. Since in ECT trials most usually bleomycin, a hydrophilic drug, is used, it is assumed that the drug injected systemically or intratumorally prior to delivery of electric pulses distributes freely in the volume of tumor tissue. To our knowledge, dynamics and pattern of this distribution in whole tumor are

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still unknown. Nevertheless, effectiveness of ECT is dose dependent [6,8,10], which clearly shows that appropriate amounts of the drug are needed for the effective electrochemotherapy.

The second requirement has been an object of more intense studies. In vitro cell permeabilization depends on many parameters, but all above, on the parameters of electric pulses, that is: shape, strength, duration, number and frequency at which the pulses are delivered [11–16]. In vitro, it was established that high percentage of permeabilized cells, with minor percentage of killed cells, can be obtained using eight-square wave pulses with individual pulse strength of 1300 V/cm, duration of 0.1 ms [17,18]. These pulses were also effective in vivo conditions to permeabilize cells in tumor tissues [6,19,20]. Consequently, already in the first experiments on in vivo electrochemotherapy, high responses of the treatment were obtained [6]. These findings suggest that majority of the tumor cells in tissue were permeabilized by the pulses and destroyed by bleomycin internalized into the cells. Therefore, eight square-wave pulses of 1300 V/cm, 0.1 ms were widely accepted in the world and applied for electrochemotherapy of many tumor models in preclinical as well as in clinical trials [6–9,21–24].

In spite of these reports, there is a lack of more systemic studies on the effectiveness of ECT in dependence of various parameters of electric pulses. This study was thus designed to investigate the effectiveness of ECT in dependence of various combinations of electric pulse strength and duration. Along this, we also aimed to determine relation of pulse strength and pulse duration resulting in the same efficacy of electrochemotherapy.

2. Materials and methods

2.1. Animals and tumor model

Eight- to twelve-week-old female C57Bl mice (Institute of Immunology, Vilnius, Lithuania) were employed in the experiments. Animals were maintained at steady room temperature (22 °C) with natural day–night cycle. To obtain solid tumors, the tumor tissue was removed from the mice bearing Lewis Lung carcinoma (LLC) tumors and minced with a pair of scissors after addition of sterile 0.9% NaCl with ratio 5 ml to 1 g of tumor tissue. Then 0.2 ml of the suspension was injected subcutaneously in the right hind limb of the experimental mice. When tumors reached approximately 200–400 mm^3 in volume, the mice were divided into experimental groups (at least six mice in each group) and subjected to specific treatment.

2.2. Electrochemotherapy

Bleomycin (BLM) (Nippon Kayaky, Japan) in 0.2 ml was administered by intraperitoneal injection 30 min before delivery of electric pulses. Treated mice received 5 mg/kg of the drug. Electric pulses were delivered using distance-adjustable plate (two flat stainless steel plates, 8 mm in width, 20 mm in length) electrodes. The electrodes were placed at the opposite sides of the tumor and the distance of the plates was adjusted according the size of the tumor. To reach good contact between the skin and the electrodes, the fur was clipped off from the tumor area, and conductive gel was used. Square-wave electric pulses were generated by an electroporator constructed and manufactured in our laboratory. The tumors were pulsed with eight-square wave electric pulses of variable strength (900, 1100, 1300 and 1500 V/cm) and duration (0.1, 0.25, 0.5 and 1 ms) delivered at 1 Hz. Shape, voltage and duration of each individual pulse were controlled by means of storage oscilloscope (C8-13, Russia). ECT treatment was performed without anesthesia and was well tolerated by the mice. Mice were sacrificed either 14 days after the treatment or 2 days after tumor in mouse doubled in volume.

2.3. Assessment of response and treatment of the data

The tumor's longest diameter (a) and the next perpendicularly longest diameter (b) were measured with a caliper every second day, starting from the experimental day '0'. The tumor volume (V) was calculated by the formula $V=ab^2\pi/6$ [25]. From the measurements, tumor doubling time (DT) was determined for each individual mice. From the values, the mean and standard errors of the mean of tumor volume and DT were calculated. Similarly, extent of tumor necrosis (see bellow) in individual groups was expressed as a mean±standard error of the mean. To evaluate statistical difference between experimental groups, two-tailed Student's t-test for paired values was used. Statistical difference was taken to be significant when $p<0.05$.

Experimentally defined DT in dependence of pulse duration for each pulse strength (900, 1100, 1300 and 1500 V/cm) were fitted to a two parameter logarithmic curve $DT(\tau)=a+b\times\ln(\tau)$, where τ is pulse duration, a and b are parameters that determine shape of the logarithmic curve. Thereby, four fitted curves exposing tumor doubling time as the functions of pulse duration were obtained. The line crossing these curves in parallel to τ axis gave us at least four pulse strength–duration values defining the same tumor doubling time, and consequently the same efficiency of ECT. By plotting these values on strength–duration coordinates and fitting them to a three parameter exponential curve $E(\tau)=a+b\times e^{-c\tau}$ (E is pulse strength, τ is pulse duration, a, b and c are parameters that determine shape of the exponential curve), the set of strength–duration relations, which define the same DT, was determined. All fits were obtained by least-squares nonlinear regression using Sigma Plot 6.10 (SPSS, Richmond, CA, USA).