MAŁGORZATA KOTULSKA

Electrochemotherapy in Cancer Treatment
Elektrochemioterapia w leczeniu nowotworów

Institute of Biomedical Engineering and Instrumentation, Wrocław University of Technology, Poland

Abstract
An electric field of high intensity changes the organization of lipids in the cell membrane, creating transient hydrophilic pores through which ions can freely permeate. This phenomenon, called electroporation (EP), greatly facilitates molecular transport across the membrane and permits enhanced delivery of biologically active molecules, such as drugs and nucleic acids, into the cell. Currently, EP is commonly used for in vitro cell transfection as the cleanest method available. It can also be applied in genetic therapy or immunotherapy in vivo. The most recent application of EP, becoming increasingly popular in the EU, the USA, and Australia, is electrochemotherapy (ECT), in which electroporation significantly increases the intracellular concentration of a cytotoxic agent. ECT applied with a drug that can hardly permeate through the plasma membrane without EP, such as bleomycin, allows localized treatment of a tumor, thereby reducing the side effects of systemic chemotherapy. In some cases, ECT eliminates the necessity of surgery (Adv Clin Exp Med 2007, 16, 5, 601–607).

Key words: electroporation, electrochemotherapy, drug internalization, cancer.

Streszczenie

Słowa kluczowe: elektroporacja, elektrochemioterapia, rak.

Cell membranes are sensitive to physicochemical conditions such as the biochemical environment, temperature, mechanical stress, and electromagnetic field. It was discovered that under certain conditions the plasma membrane of a cell loses its continuity. This has been observed mainly under an electric field of high intensity (Fig. 1) and is called electroporation (EP) (reviewed in [4, 13, 30, 39]). The mechanism of electroporation is still not sufficiently explored and not fully understood. Due to their small size, combined with their very high dynamics, electropores cannot be visually observed. Currently, only indirect experimental methods based on increased membrane conductivity, supported by models and computer simulations, are employed to study electroporation. The main objectives of studying electropores include a better understanding of the mechanisms leading to electroporation and its course, the sensitivity of this process to the physicochemical conditions of the environment, and the development of methods for a better control of electropore size and opening time [14–22].
Pores significantly increase membrane conductance and open a transport pathway for molecules. Opening a new pathway through the plasma membrane has severe consequences for the cell, which is no longer separated from its environment. Various molecules normally kept outside the cell can get into the cytoplasm, avoiding the usual strict control. Although this is undesirable under the physiological conditions, it can be useful in therapy. Typically, molecules are transported into cells by channels or pumps located in the plasma membrane. Lipophilic molecules, which are soluble in the lipid bilayer, can diffuse across the plasma membrane directly. Molecules that are neither lipophilic nor transported by channels and pumps cannot be easily delivered into the cell. In genetic therapy, all targets are inside the cell. Therefore, a very important application of electroporation is gene therapy or gene vaccination, where it has become a standard method for delivering DNA or RNA into cells since the 1980s. Electroporation is currently used in the electrochemotherapy (ECT) of cancer [29], gene therapy, and DNA vaccination (reviewed in [11]).

Tests show that it can be applied in intraocular therapy [32], and the application of EP in photodynamic therapy is also considered [24]. In current applications, the electroporermabilization of the tissue enhances the internalization of extracellular molecules under 30 kDa. Results from chronomperometry after current clamp (CACC) electroporation [22], the authors’ novel combinatory method using electropores with the edge gradually stabilized, show that creating large pores with a controllable diameter and lifetime is possible. Such electropores may facilitate the delivery of large molecules, e.g. plasmid DNA, into cells. It was recently shown that ultra-short nanosecond pulses alone are capable of eradicating melanoma cells with no use of cytostatic drugs. The studies indicate the induction of apoptosis in tumor cells subjected to nanosecond pulses [1].

Electrochemotherapy in Cancer Treatment

Electrochemotherapy (ECT) is a novel therapeutic approach enabling the delivery of nonpermeant drugs directly into the cell. The first study of ECT in vivo on an animal tumor model was reported in 1987 [31]. The effectiveness of electrochemotherapy was first tested on animal models and then on humans in preclinical and clinical trials. ECT is a very promising treatment method in the case of localized tumors. It may increase treatment effectiveness for various cancers treated by chemotherapy, increasing the permeability of the cell membrane for the cytotoxic agent. ECT was envisaged as a very promising method in tumors resistant to conventional cancer therapies, such as melanoma, where the typical response rate is low, with partial response (PR) of 20–45% and complete response (CR) of less than 5% [2, 10]. The ECT tests included cutaneous and subcutaneous tumors, treated mainly by means of the non-lipophilic cytotoxic drug bleomycin. Preclinical trials with bleomycin, especially efficient in the treatment of head and neck cancer, showed that cytotoxicity increased 300 to 700-fold if ECT was applied [7]. Patients with tumors located in the facial/cervical region, oral cavity, pharynx, larynx, and sinus showed 73% objective response (CR+PR) when treated with bleomycin by ECT [5]. No response was obtained with similar doses of bleomycin or electroporation alone. Tests have also been conducted using cisplatin, where a 2- to 8-fold enhancement of citotoxicity was reported [7], and actinomycin D, with a 3 to 5-fold cytoxicity increase [32]. ECT does not increase the cytotoxicity of amphi- and lipophilic drugs, such as danorubicin, doxorubicin, etoposide, and paclitaxel [7, 12].

An important feature of electroporation in vivo is its influence on blood flow, demonstrated by decreased blood circulation [28]. This phenomenon is called a vascular lock. The vascular system reacts to an electric field by constriction (1–2 min), which is enhanced by an increase in the interstitial pressure (30 min) related to the release of fluids from the electroporated cells into the extracellular space [9]. Therefore, for a few minutes following the application of electrical pulses the cells under treatment are not accessible for molecules in the blood. Vascular constriction is
most conspicuous in tumor tissue due to its irregular microvasculature and lack of a well-defined boundary separating the vascular system from the interstitial fluid. In tumor tissues the vascular lock lasts up to one hour. This effect is very favorable in terms of chemotherapy, where it prevents washing out of the drug from the cancerous tissue. The drug should be provided before the application of the electric field so that it can enter the cells before the lock appears.

On the other hand, the tumor hypoxia associated with the vascular lock may result in resistance to radiotherapy, opposing the radiosensitizing effect of bleomycin and cisplatin used in ECT. It was proved [23], however, that ECT-induced hypoxia of the tumor does not counteract the favorable effect of bleomycin. Therefore, the ECT-enhanced uptake of bleomycin and cisplatin into cancerous cells results in significantly higher sensitivity of the tumor to radiotherapy. Moreover, the temporal decrease in tissue oxygenation may be utilized in the therapy with hypoxic cytotoxins, such as tirapazamine. Animal model studies showed an up to six-fold increase in tumor growth delay when ECT with tirapazamine was combined with radiotherapy compared with tirapazamine with radiation alone [3, 25].

The combination of electrochemotherapy with immunotherapy increasing the host’s immune response by the administration of biologically active modifiers might be an attractive modification of standard ECT [26]. Preclinical trials with interleukin-2 have been performed on a non-metastasizing murine tumor model [27], a metastasizing murine tumor model [33], and a carcinoma model transplanted into rabbit liver [35]. The studies showed improvement in the local effects combined with the systemic antitumor effect.

**Principles of Electropermeabilization in ECT**

Several tests on animal models have been conducted in search of the optimal parameters for the ECT procedure, such as the field frequency, amplitude, and the electrode layout. It was estimated that the intensity of the electric field used in ECT should fall between 900–1500 V/cm [10], with the optimum in cancer therapy being 1300 V/cm. Typically, the electric field is delivered by four to eight square pulses, each 100 µs in width, administered at one-second intervals. Rectangular signals are more effective than the initially used exponential pulses since their duration can be controlled independently of the pulse intensity; thereby, less intense fields can be applied. It was shown that rotating the electrodes by 90° after the first four pulses enhances the result by better coverage of the tumor tissue [37]. One sequence of electric pulses is sufficient for small tumors. For large tumors the therapy is repeated at a different position of the electrodes so that the tumor surface can be better covered. The most unpleasant side effect of the pulse wave protocol is a painful muscle contraction appearing with each train of pulses and caused by the high amplitude of the pulses. Since the amplitude cannot be reduced, it was proposed to increase the frequency of the pulse repetition, which would diminish the number of painful individual muscle contractions. As results showed that an increase in the repetition frequency even to 8.3 kHz does not affect drug uptake [34], it was proposed that the repetition frequency may be set above the frequency of tetanic contraction (100 Hz).

The effective electric field in the cells depends on the voltage amplitude and the configuration of the electrodes. Several different electrode layouts have been analyzed: parallel-plate electrodes and needle electrodes located in linear, circular, and hexagonal arrays [8]. It was found that needle electrodes are more efficient for subcutaneous tumors. A study published by Gehl and colleagues [6] recommends linear eight-needle electrodes in which the needles are placed in two rows 4 mm apart. Such electrodes distribute the electric field more homogeneously, which permits applying lower voltages than in a circular arrangement. Other authors recommend six electrodes arranged hexagonally, with changing polarity [5]. Although plate electrodes are noninvasive, the electric field cannot be applied deeply. They can be successively used for cutaneous cancers. Application of plate electrodes may lead to the occurrence of superficial burns on the patient’s skin.

**Bleomycin: the Most Typical Drug in ECT**

Bleomycin is a nonpermeant drug with extremely high intrinsic cytotoxicity [9]. It belongs to the family of 13 water-soluble glycopeptidic antibiotics 1.5 kDa in size and can be isolated from the fungus *Streptomyces verticillus*. Bleomycin is genotoxic and has been used in the treatment of head and neck squamous cell carcinoma, Hodgkin’s and non-Hodgkin’s lymphomas, and testicular carcinoma. It has very few side effects. However, without EP its internalization is very weak. The effects are more pronounced in cancerous cells, which was utilized in the “bleomycin
test” [36] to recognize the genetic instability characteristic of cancerous cells. Bleomycin does not cause myelotoxicity, cardiotoxicity, diarrhea, vomiting, or nausea; only very high cumulative doses can lead to lung fibrosis. Importantly, bleomycin does not restrict the cellular immune response. However, the low permeability of bleomycin into the cell, leading to its low effectiveness, hampered its use and it could not be administered alone. In electrochemotherapy, this feature can be regarded as an asset. A high intracellular concentration of the drug can be limited to only the regions undergoing treatment.

At therapeutic doses, which can be significantly decreased if ECT is used, bleomycin kills cells by generating free radicals which cleave DNA and RNA. There is no agreement on whether the cell death is due to apoptosis or necrosis. In necrotic death, the damaged ion pumps and channels of the cell do not perform their selective transport, which leads to swelling of the cell and its organelles, leakage of the material into the extracellular space, and an inflammatory response. In apoptosis, which is a genetically programmed cell death, the gradual dismantling of cell function is very orderly. Mir et al. support the idea of non-apoptotic cell death caused by bleomycin [28]. They claim that bleomycin triggers a slow death process of the cell which resembles mitotic cell death. Therefore, a favorable inflammatory immune response is present. Nonetheless, other authors who observed chromatin condensation and nuclear fragmentation, which is typical for apoptosis, claim that the cell disintegrates in an apoptotic way with some elements of necrosis [5]. Bleomycin is known to release or induce the production of cytokines such as interleukin-2, interleukin-6, and tumor necrosis factor, which additionally contributes to the healing process [5]. The half-life of bleomycin in the blood of patients with good renal function is 2–4 hours. The drug is eliminated mainly by renal excretion. Cells with increased repair mechanisms may be less sensitive to bleomycin. No rapid induction of resistant cells has been observed [9]. Similarly, bleomycin is not subject to multidrug resistance (MDR), which is a common problem with other cytotoxic agents. The MDR gene increases the activity of P-glycoprotein, which transports many anti-neoplastic drugs but it does not transport bleomycin [9]. Bleomycin can be degraded by bleomycin hydrolase present in the cytosol of all cells as a part of the cells’ proteosome disintegrating many cellular proteins. Bleomycin hydrolase has a lower concentration in the lungs and skin, which probably explains the higher cytotoxicity of bleomycin in these tissues. Also, bleomycin can be disabled by bleomycin resistance proteins (BRP), which are found in microorganisms producing bleomycin or bleomycin derivatives.

The low transport rate of the bleomycin molecules into the cell is the main problem of an effective treatment. Typically, when no electropermeabilization is induced, bleomycin is bound by specific membrane proteins located on the cell surface and transported into the cell by endocytosis. Autoradiography with C14-labeled bleomycin demonstrated that only 0.1% of the bleomycin added to the medium was associated with cells after a few hours. When cellular electropermeabilization is applied, the drug’s internalization is massively increased. Moreover, bleomycin in electropermeabilized cells produces DNA breaks very rapidly, usually in less than 30 sec. The concentration of bleomycin in the electropermeabilized cell is proportional to its external concentration in a well-defined manner. On average, an external concentration of 10 nM relates to 3000 bleomycin molecules inside the cell. This helps to predict the precise dose and cellular reaction.

Most favorably, bleomycin in ECT can be administered intravenously or intratumorally ([9] and references therein) as a bolus or infusion [10]. The optimal intravenous doses related to the patient’s approximate body surface area were estimated to be 5.6–15 mg/m² (10–27 U/m²; one unit (U) of antimicrobial activity contains 0.56–0.66 mg of bleomycin [9] and corresponds to 1000 international units (IU)). The optimal infusion time at this dose is 30–45 s. The doses for intravenous treatment are similar to the doses used in conventional chemotherapy, i.e. 18–27 U/m² [9]; the injection is applied only once. Intratumoral treatment is suggested in the case of a small number of distinct subcutaneous tumors, especially when the tumors are poorly vascularized. The doses depend on the tumor volume V, calculated as:

\[ V = \frac{ab^2\pi}{6} \]

The optimal concentration of delivered drug was estimated at 0.25 ml (0.25 U)/cm³ for tumors larger than 1 cm³, 0.5 ml (0.5 U)/cm³ for tumors of medium size, i.e. 0.5 cm³ < V < 1 cm³, and 1 ml (1 U)/cm³ for tumors smaller than 0.5 cm³. The drug concentration decreases since larger tumors absorb larger quantities of the solute. The drug concentration is usually insignificantly in excess because the distribution of the injected fluid can be heterogeneous. Unlike typical chemotherapy, ECT is a once-only treatment, which significantly decreases the cumulative dose that may cause lung fibrosis if a dose of 300 mg/m² is reached [9]. Other undesirable cutaneous effects that may be sometimes observed in systemic treatment with
bleomycin, such as erythema, induration, hyperkeratosis, and peeling of the skin, are avoided due to the low cumulative doses applied in ECT.

**Effectiveness and Clinical Use of EP Therapies**

Studies of the ECT effectiveness have been provided for a wide variety of cancers; a thorough review of recent clinical results is presented in a supplement to the European Journal of Cancer entirely devoted to electrochemotherapy [29]. The review includes mostly melanoma cases, where results are the most conspicuous, hepatocellular carcinoma, breast carcinoma, fibrosarcoma and glioma, head and neck squamous cell carcinoma, Kaposi’s sarcoma, basal cell carcinoma, and adenocarcinoma. In general, very significant increases in objective response (72–100%) and complete responses (85%) following ECT application have been reported in many cases. In some situations, ECT treatment proves effective where other, conventional methods fail (e.g. refractory breast cancer [40]). In other situations it permits avoiding surgery, particularly when it may improve the patient’s comfort by saving an organ or its significant part from removal (e.g. the eye lid, rectum [38]).

Among many electroporators available on the market, two are designed specifically for the clinical use of ECT on humans. The European device was constructed following the EU-funded ESOPE (European Standard Operating Procedure for Electrochemotherapy) project. As a result of this project, a biomedical company, IGEA, introduced the Cliniporator (Fig. 2). The Cliniporator complies with directive 93/42/EEC for medical devices and is officially permitted for clinical use in all EU countries. Although the standard conditions for ECT with the Cliniporator are eight square-wave pulses each 100 µs wide, the operator can choose the number of pulses from a range of 1–20. The amplitude of the pulses ranges between 100–1000 V. The electrodes optimal for the treatment of subcutaneous tumors are either hexagonal or linear needle arrays; two plates are available for cutaneous tumors. The possibility of extending the Cliniporator with an ECG synchronizer, which would minimize the risk of heart defibrillation, is under study. Compared with the prices of advanced devices for radiotherapy, the apparatus is quite cost effective. Moreover, treatment can be performed on an outpatient basis, additionally reducing the costs of treatment and increasing the patient’s comfort. In the USA, BTX released a clinical electroporator, the MedPulser, in 2004. The MedPulser is supplied with hexagonal electrodes that apply six pulses of an intensity of 1130 V within 10 seconds. However, until now the apparatus can be applied for clinical use only outside EU countries.

In Europe, ECT treatment of skin, head, and neck cancers have successfully gone through the stage of clinical tests and have received the CE mark. The clinical use of ECT among EU countries has been reported in France, Denmark, Slovenia, Ireland, Italy, and Spain. ECT is also successfully used in the USA and Australia.

![Fig. 2. The Cliniporator by IGEA and a set of electrodes for subcutaneous (upper) and cutaneous tumors (bottom). Reprinted with permission from IGEA](image-url)
Perspectives of the Electroporation Therapy

In a few years’ time, ECT may become an important alternative therapy to the surgical resection of recurrent melanoma tumors. It could be very useful in metastatic diseases in sites where defects remaining after surgery are difficult to close, especially when nodal excision or irradiation were previously administered [20]. The next stage of research into electrochemotherapy will concern the possibility of ECT treatment of primary tumors in internal organs. Genetic vaccines, which will become increasingly popular in treatment and prophylactics, will tend to use safer physicochemical methods based on non-viral vectors, such as electroporation. The main challenge concerning EP therapies will be the development of safe and efficient methods for electroporation of tissue in internal organs.

Acknowledgements

The author would like to thank Dr. Lluis M. Mir from CNRS, France, and Dr. Maja Cemazar from Institute of Oncology in Ljubljana, Slovenia, for very stimulating discussions concerning electrochemotherapy.

References


Address for correspondence:
Institute of Biomedical Engineering and Instrumentation
Wroclaw University of Technology
Wybrzeze Wyspianskiego 27
50-370 Wroclaw
Poland
Tel.: +48 71 320 39 74
E-mail: malgorzata.kotulska@pwr.wroc.pl

Conflict of interest: None declared

Received: 27.06.2007
Accepted: 18.10.2007