

Requirements for a clinical electrochemotherapy device - electroporator

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In the paper we discuss requirements for a clinical electrochemotherapy (ECT) device. These requirements are discussed in the light of the hardware that is needed for ECT. The hardware needed for ECT consists of an electroporator and a set of electrodes. The electroporator is a device that has to fulfill both electrical and safety requirements. Under electrical requirements we understand output characteristics of the electroporator that make the treatment efficient. This is why they have to be consistently fulfilled. On the other hand, the electroporator has to be built and operate in a way that the safety requirements defined by IEC standards are met. Safety requirements are intended to protect both the patient and the personnel from an accidental electric shock. In addition, these safety requirements have to define who can use the electroporator, a device which is similar to a defibrillator with respect to its high voltage output. The second hardware component that is needed for ECT are the electrodes. We classified them as internal and external, based on whether they are used for treatment beyond the skin or superficially. Both types have been studied since the start of ECT application. We also describe the electroporators that are currently being used in clinical situation today. Notwithstanding the availability of some electroporators, we have to conclude that a true clinical electroporator is still needed, since the currently used electroporators do not fulfill all requirements.

Key words: electroporation-instrumentation; neoplasms-drug therapy

Introduction

The combined treatment in which delivery of chemotherapeutic agent is followed by pulsed high electric fields has been termed electrochemotherapy (ECT). This treatment relies on the physical effect of locally applied electric fields that cause permeabilization of cell plasma membrane. This permeabilization of plasmalemma allows increased entry of the drug molecules into the cell. The comparison of ECT and conventional chemotherapy shows that much lower amounts of drugs are needed in ECT to

achieve equal antitumor effect. Thus the effectiveness of chemotherapeutic drugs with intra-cellular target which do not readily cross the plasma membrane can be greatly potentiated.

Since the first report of this type of treatment, many studies have been conducted with encouraging results. Studies focused on the optimization of ECT by testing various chemotherapeutic agents, electrodes and electric pulse parameters. In most *in vivo* studies, however, the same electrical parameters were used, i.e. 4 or 8 square-wave electric pulses of 100 μ s duration, delivered at 1Hz repetition frequency and 1000-1500Vcm⁻¹ voltage to electrode distance ratio.¹ The ECT parameters from *in vivo* studies were then transferred to experimental clinical trials and, in all cases, the same pulse generators were used. For experimental clinical trials, laboratory equipment can be used, but it needs to be

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emphasized that safety requirements for clinical devices are much more severe than for laboratory devices. If we want the ECT to become a cancer treatment of choice, appropriate clinical electroporation devices have to be developed.

In this paper, we explore all the important parameters for a clinical electroporator and electrodes that are currently available. Every clinical electric device has to fulfill safety requirements, and in the same time has to be efficient. We can meet these requirements with appropriate electronic design, assuring the output parameters that are based on pre-clinical studies.

Currently there are many electroporators available, but only few of them could be used in clinical situation. One of the electroporators that could be used in clinical situation is BTX's T820. It generates square wave pulses and can work in high voltage or low voltage mode. In the high voltage mode T820 can generate pulses of 5 μ s to 99 μ s and 100V to 3000V. In the low voltage mode it can generate pulses of 0.3 ms to 99 ms and 50V to 500V. The BTX T820 can generate up to 99 consecutive pulses with repetition frequency of 1Hz. This instrument is designed in a way that electrodes are floating all the time.²

A new clinical electroporator is also being developed by Genetronix, USA.

Parameter description

The hardware that is used in ECT consists of an electroporator and a set of electrodes. Our classification of parameters is based on such a division of hardware equipment. The parameter values refer to preclinical and clinical ECT studies as well as to the theoretical analysis.

The electroporator

The electroporator is an electronic device that has to meet several requirements. Appropriate choice of electrical parameters makes ECT efficient by facilitating the uptake of chemotherapeutic drugs by cell. Therefore, it is of extreme importance to be able to deliver pulses of specific width, amplitude, and to meet the power requirements. Table 1 shows the parameters, used in representative ECT studies.

Table 1. Parameters of representative electrochemotherapy.

First author; year of publication	Shape of the pulses	Duration	Number of pulses	Electrode to distance ratio	Electrodes
Okino; 1987, 1990 ^{8,11}	exponential	2 ms	1	5 kVcm ⁻¹	external
Kanesada; 1990 ¹²	exponential	4 ms	1	3 kVcm ⁻¹	external
Mir; 1991, ⁹	rectangular	100 μ s	8	1.5 kVcm ⁻¹	external
Belehradek; 1991 ¹³					
Salford; 1993 ¹⁴	exponential	325 μ s [*]	8 to 12	400V or 600V [*]	needle
Belehradek; 1994 ¹⁵	rectangular	100 μ s	8	>1050Vcm ⁻¹	external
Serša; 1995 ¹⁰	rectangular	100 μ s	8	1.3 kVcm ⁻¹	external
Heller; 1995 ¹⁶	rectangular	99 μ s	8	1.5 kVcm ⁻¹	external
Heller; 1997 ¹⁷	rectangular	99 μ s	8	1.3 kVcm ⁻¹	external
Jarozeski; 1997 ¹⁸	rectangular	99 μ s	6	1 kVcm ⁻¹	needle array
Mir; 1997 ⁴	rectangular	100 μ s	4+4 \emptyset	800 Vcm ⁻¹	needle array

^{*} Pulse amplitude

^{*} Time constant

\emptyset Four pulses of each polarity

The second one is Jouan's Cellular Electropulsator PS 10 (or newer model PS 15) that generates square wave pulses of 5 μ s to 24 ms and 0V to 1500V. The design of the older model is a bit awkward, but it could be improved so that it would become safer.^{3,4}

The third one, Antony's CELTEM MKO, is still a prototype and is able to deliver electric pulses to needle arrays. So far it has only been used in France.⁴

From Table 1 it is evident that the electrical parameters of ECT have been optimized since the first trials. According to the *in vivo* and clinical studies performed so far, a clinical electroporator should generate pulses with amplitude up to 3000V, but probably not much higher, since excessive strength of electric field strength diminishes the viability of cells,⁵ thus killing the cells around electrodes, causing necrosis. On the other hand, the electric field has to be strong enough to induce

sufficient transmembrane voltage change $\Delta\Phi_m$ to cause permeabilization of the cell membrane. The equation which defines induced transmembrane voltage reads:⁶

$$\Delta\Phi_m = f_s ER \cos\theta \cdot \left[1 - \exp\left(-\frac{t}{\tau}\right) \right] \quad (1)$$

where E is the magnitude of electric field, t is the time, R is the cell radius, θ is the polar angle measured with respect to the direction of the field, f_s is a function reflecting the electric and dimensional properties of the cell and the surroundings, and τ is the time constant of the membrane (for detailed description, refer to reference 6).

The rate of transmembrane voltage change depends on the ratio in the exponential term in the equation (1). Under physiological conditions, τ is in the microseconds range. In case where the electric pulse duration (T) is much longer than τ (i.e. $T \geq 3\tau$), $\Delta\Phi_m$ reaches its peak value during of the duration of the pulse and at that point the membrane permeabilization occurs.⁶ Therefore, an electroporator should generate electric pulses that have duration of $10\mu\text{s}$ or more.

In addition, the electroporator needs sufficient energy for its operation. Estimated energy W is calculated according to the equation (2), where U is the voltage amplitude of the pulse, I is the electric current, Z is the impedance of the tissue between the electrodes (including the tumor), and T is the pulse width:

$$W = U \cdot I \cdot T = \frac{U^2}{Z} \cdot T \quad (2)$$

Since the electroporator provides constant voltage, the energy load increases with decrease of impedance Z , which is evident from equation (2). Impedance Z is given by the equation (3), where ρ is specific resistivity of the tumor, l is the distance between the electrodes, and S is the effective surface of the electrodes.

$$Z = \frac{\rho \cdot l}{S} \quad (3)$$

The easiest way to calculate energy is to measure the electric current, the voltage amplitude of the pulse, and the pulse width during the treatment. According to the study of Rudolf et al.¹⁹ the electric current was estimated to be 4A, at the voltage amplitude of 910V, and the pulse width of $100\mu\text{s}$. These values give, according to the first part of equation (2), energy of 0.37J. If we consider that maximum of 8 pulses are currently delivered in one session and that during this treatment the electroporator does not obtain any energy, then it has to store at least 2.96J of energy at the beginning of the treatment.

If these power requirements are not met, the amplitude of pulses will decrease and with it the electric field magnitude. Therefore, we have to assure that the electroporator stores enough energy.

The purpose of safety precautions when using electrical devices in medicine is to protect the patient, the person who is operating the device – a medical doctor, and any other medical worker who can come in touch with the device. In general, we have to assure that electric devices are used by qualified, responsible, and authorized personnel only.

Electric current used for electrochemotherapy helps the patient, but if not properly controlled and handled, it can become dangerous. The treatment of internal tumors is especially critical. In such cases, the natural barrier, i.e. skin, is by-passed and with it the body's natural protection.

Reaction to electric current varies from person to person. Sensitivity depends on many parameters, such as the state and the level of contact with electric wire, moistness and thickness of skin, etc. Regarding those parameters, the designer of the clinical electric device has to consider levels of protection and provide written rules.

If a malfunction of clinical electric device occurs, it must occur in a controlled maneuver. This means that in case of a single malfunction, which can be caused by a defect of one component in the protection chain, the device must not cause any danger either to the patient or to the person who is operating it. We can achieve this with double protection construction, redundant checks, and special protection components. The state of the single malfunction has to be time limited, because otherwise the electric device could become dangerous to the patient and the operator in case of a second malfunction. It is therefore important that the personnel is notified in case of the single malfunction. In

most cases, this is not achievable immediately and the malfunction is discovered indirectly by means of periodical test measurements. Such control of medical equipment is performed by a clinical engineer.

If the clinical electric device has a single malfunction, and then the second malfunction occurs, the clinical electric device must stop operating automatically, and must stay in that state until it is repaired.

The detailed classification of clinical electric devices is defined by IEC standards. This classification refers to: the level of electric shock protection, the level of protection in presence of flammable and explosive gases and fluids, the level of electric connection between the patient and the electric device, and the operation of the device according to the time.

The electroporator is a device that can produce electric pulses with amplitude up to 3000V, although the pulses usually are as short as 100 μ s, they can be delivered rapidly. It is important to stress that the electroporator is similar to a defibrillator, with the respect to the high voltage output, so the safety requirements for the electroporator have to be as severe as they are for the defibrillator. The electroporator must be galvanically separated from the main supply voltage, the electrodes must float, the output should be monitored during the treatment in case of any malfunction, etc. Finally, we have to stress that the electroporator should only be used by authorized and qualified personnel as it is in the case of defibrillator.

Electrodes

Electrodes represent the second part of the hardware that is needed for ECT. Basically, there are two types of electrodes, external and internal. Both types of electrodes have been tested first in preclinical studies, where they showed their advantages and disadvantages. For all types of electrodes it is important that they deliver electric field and to cover effectively as large volume of a tumor as possible.

At the beginning of electrochemotherapy studies, external parallel plate electrodes were used. These electrodes represent the only external type of electrodes currently used, there are different designs of them, but basic principle is the same. One of possible designs is presented in Figure 1A. The parallel plate electrode is mounted on a plastic vernier cali-

per. The faces of the electrodes measure 1 cm by 1 cm. The movement of the caliper allows adjustment of the distance between the electrodes to accommodate tumors of different sizes. The usual voltage to electrode distance ratio for this type of electrodes is 1500 Vcm⁻¹.⁷ The main disadvantage of surface electrodes is small electric field penetration. Consequently only superficial tumors can be effectively treated.

The advantage of internal electrodes is that they allow treatment of the deepest parts of the tumors even in the thickest skin nodules, as well as internal tumors. With the implanted needles, the electric field will be delivered approximately to the same depth as the electrodes. The first treatment with internal needle electrodes was reported in 1993, by Salford et al.¹⁴ Part B of Figure 1 shows the needle electrodes that were used. Electrodes were 1.5 cm long and 0.070 cm in diameter. Both needles were inserted on either side of the tumor.⁷

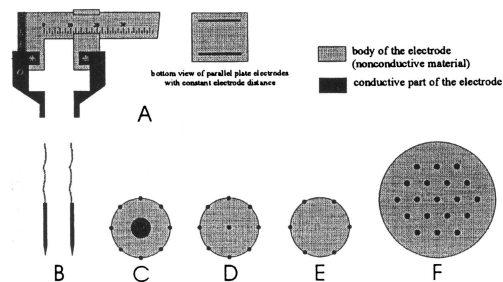


Figure 1. Different electrodes used for electrochemotherapy: (A) Parallel plate electrodes, (B) needle pair electrode, (C) 8+1 solid electrode, (D) 8+1 needle electrode, (E) 3 × 3 and 2 × 2 arrays, (F) 'honeycomb' array. Electrodes are not drawn to a scale.

Figure 1 also shows four different geometries for needle array electrodes that have been designed in last few years. Parts C and D of figure show 8+1 solid and 8+1 needle electrodes. The two designs are similar in their construction - they are both composed of eight electrically connected 30 gauge stainless steel needles equispaced around 1 cm annulus. They differ with respect to the type and location of the center electrode. The 1 mm diameter solid electrode is located 0.465 cm from the circumferential needles in the 8+1 solid design. The central needle (30 gauge) of 8+1 needle electrode is positioned 0.50 cm from the circumferential electrodes. All needles in these two designs extend 0.5 cm from their electrode bodies. The circumferentially arranged needles are inserted into the prime-

ter of the tumor. The central electrode of the 8+1 solid configuration extends 0.15 cm from the electrode body and is in contact with the tumor during the treatment. The central needle in the 8+1 needle configuration is inserted into the tumor. The central electrode is usually anodic during pulsation for both designs, and the voltage to electrode distance ratio is 1500 Vcm⁻¹.⁷

Figure 1E illustrates the electrode geometry for the 3 × 3 and 2 × 2 needle arrays. This design comprises six 28 gauge stainless steel acupuncture needles. The needles are spaced at 60° intervals around a 1 cm diameter circle and extend 1 cm from the electrode body. Both arrays were constructed so that each needle has an independent electric connection. Although both arrays utilize the same fundamental geometry, their operation differs with respect to the sequence in which voltages are applied to the needles. The usual voltage to electrode distance ratio is 1500Vcm⁻¹. To determine the voltage amplitude that has to be applied to the needles, minimal distance between the oppositely polarized needles is used.⁷

Figure 1F illustrates internal electrodes consisting of seven parallel equidistant needles, 1.5 cm long, 6 mm apart, arranged as a centered 'honeycomb'. Electric pulses are delivered to each pair of closest electrodes. The usual voltage to electrode distance ratio is 800Vcm⁻¹.⁴

All types of electrodes have proved to be effective to a certain extent in preclinical studies, but it is difficult to state which type of electrodes are better. Namely, the electric field distribution in the tissue is not known so it is difficult to compare them, especially with respect to efficient coverage of the tumor with sufficiently high electric field magnitude, which is their prime objective.

ECT studies of electrode design have shown that internal or needle electrodes are much more effective than the external ones. With the insertion of needle electrodes into a large tumor, the electrodes split the tumor in smaller volumes, and electric pulses are delivered to each of these volumes. Smaller volume requires lower voltage and thus a safer treatment in comparison to the one in which electric pulses are delivered to the whole tumor using only two external electrodes. Two external electrodes placed at each edge of the tumor nodules have large interelectrode distance that requires higher voltage. Therefore, needle electrodes seem to be more appropriate for the treatment of large tumors. In addition, needle electrodes allow penetration into

the body so that in principle any location can be reached.

General considerations

ECT has shown promise in treating a variety of tumors in humans. The basic principles for its effectiveness are reasonably well understood. At present, there is enough hardware to evaluate ECT in clinical trials. There are at least six different designs of electrodes, and there are at least three different electroporators which can be used in clinical treatment.

Nevertheless, there is a demand for a clinical electroporator with the following expectations to meet: safety requirements, pulse amplitude up to 3000V, pulse width from few microseconds up to few hundreds of microseconds, variable repetition frequency of pulse delivery, enough power to allow very small impedance between electrodes, connections for any kind of electrodes, onboard computer which would suggest parameters of treatment for different electrodes and could control the process during treatment. In addition, electroporator needs to be easy and practical to use. Based on the above specifications, we have to conclude that currently no known electroporator meets these requirements.

Acknowledgment

Our research has been supported through various grants by the Ministry of Science and Technology of the Republic of Slovenia. The authors want to thank Alenka Maček-Lebar, M.Sc. and Tadej Kotnik, B.Sc., both from Faculty of Electrical Engineering, University of Ljubljana, for fruitful discussion during the preparation of manuscript.

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