NEW MODALITY FOR ELECTROCHEMOTHERAPY OF SURFACE TUMORS

Ivan Dotsinsky¹, Biliana Nikolova¹, Ekaterina Peycheva² and Iana Tsoneva¹
¹Bulgarian Academy of Sciences, Institute of Biophysics and Biomedical Engineering, Sofia, Bulgaria
²Specialized Hospital for Active Treatment of Oncology, Sofia, Bulgaria
Correspondence to: Iana Tsoneva
E-mail: itsoneva@bio21.bas.bg

ABSTRACT
Electrochemotherapy is based on electroporation, which creates aqueous pathways in the cell membrane as a result of applied short intensive electric field usually generated by high voltage electrical pulses. The phenomenon enhances the simultaneous administration of cytotoxic drugs. Recently, several researchers forewarn of some adverse effects that could occur with electrochemotherapy of tumors located close to the heart.

We developed equipment Chemipulse III, consisting of portable low-cost electroporator, QRS synchronizer and module for long-term recording of pulse parameters. It is designed for data collection of the pulse parameters and the impedances of treated patients with surface tumors without risk of heart complications but with enhanced means for electrical safety of both patient and physician. The new instrument is with battery supply, enhanced protection against electrical hazards both for the patient and the physician, autonomy providing for more than 200 shocks with one battery charging. The data could be used to support the optimization of the voltage shock setting and the evaluation of the procedure effectiveness.

The results obtained show high efficiency of the improved approach (new modality) for electrochemotherapy. Thirty-seven patients with a total of 47 lesions of Ca basocellulare, Ca spinocellulare and Kaposi Sarcoma were treated with electrochemotherapy using intralesional application of bleomycin and new developed equipment Chemipulse III. The electrochemotherapy was done by 16 biphasic rectangular pulses, 50+50 μs duration each, with a 20 μs interval between both phases and a pause of 880 μs between the bipolar pulses. The equipment with QRS synchronizer is mandatory in cases of electrochemotherapy of patients with pacemakers and patients with heart problems. Erythema and slight edema at the site of the treated lesions occurred in most of the patients and disappeared in a few days, one week at most.


Keywords: electrochemotherapy, skin tumors, electroporator, high-voltage pulse generation, QRS synchronization

Introduction
Electrochemotherapy became a powerful method for tumor treatment and gene delivery during the past decades. The procedure is based on electroporation (electropermeabilisation) of the cell membrane that is associated with the creation of aqueous pathways in the cell membrane as a result of applied short intensive electric field. This phenomenon speeds up the simultaneous administration of cytotoxic drugs, usually bleomycin or cisplatin.

The background of the mechanism of electroporation, including analytic description of the transmembrane voltage induced in a single cell, in cell suspensions and in tissues, may be found in a monographic book chapter by Miclavcic and Kotnik (10).

The electric field is usually generated by sequences of high voltage pulses. Many authors have been studying the influence of the pulse parameters on the electroporation level. Their optimization is designed to find these minimal electrical conditions that lead to a significant antitumor effect. Usually, the field intensity (the amplitude), the number and the duration of different shapes of pulses have been in the focus of the investigations (4, 6, 7, 8, 11, 12, 18, 23).

The electroporation occurs only at a threshold value of the transmembrane potential (18). Above this threshold the quantity of the porated cells increases approximately following an ascending sigmoid function, while the percentage of surviving cells decreases by the same but descending function.

Miller et al. (11) carried out experiments with pulses of various amplitudes. The results showed the use of multiple pulses as more effective for cancer cell ablation than the delivering of the same energy in one single pulse.

The critical pulse amplitude leading to electroporation becomes lower if the pulse number and/or the duration increase (18). Cumulative effects are observed when repeated pulses are applied. Therefore, electroporation is very often performed using bursts of rectangular pulses. Their typical durations are in the range from hundreds of μs to tens of ms, while the intervals between the pulses vary from several ms to several s.

Lebar et al. (7) examined how the inter-pulse interval of a train of rectangular pulses influences the electroporation of some bilayer lipid membranes. They used 100 μs pulse duration and found that the threshold voltage decreased linearly with the logarithm of the interpulse interval and dropped to that of a single pulse with an interval of 1 ms.
Kotnik et al. (6) compared the efficiency of three sequences of rectangular pulses consisting of: 1) eight 1 ms unipolar pulses; 2) eight 1 ms symmetrical bipolar pulses; 3) four 2 ms symmetrical bipolar ones. All sequences were generated at intervals of 1 s. The conclusion is that the concentrations of ions released by bipolar pulses are in an order of magnitude higher than those delivered by unipolar pulses of the same amplitude and duration.

The electrochemotherapy is accompanied with unpleasant sensations because of the contraction of muscles around the electrodes. These sensations are reproduced with every pulse of a train of pulses if the frequency of repetition is low. Daskalov et al. (4) drew attention that they can be smoothed down to only one if the pauses between the pulses are shorter than the duration of a tetanic contraction. In such case the nerve axon cannot be excited for the refractory period.

This pulse sequence has been optimized during long series of clinical tests with companion animals (20, 21, 22) and human patients and was implemented in a family of instruments currently used in the medical practice (14, 16).

Daskalov et al. (4) considered also that the lack of detail on the tissue impedance hinders the accurate assessment of the properties of different pulse shapes. Thus, they suggested in general terms patient data collection during electroporation.

Recently, several researchers forewarn of some adverse effects that could occur with electrochemotherapy of tumors located close to the heart. Music et al. (13) gave a warning of fibrillation risk if the electroporation pulses are delivered during the vulnerable period or coincide with arrhythmias that may lower the fibrillation threshold.

Sersa et al. (19) carried out histological evaluation and physiological measurements of tumors based on prediction of a mathematical model to confirm that electroporation and electrotherapy of tumors have a vascular disruption action.

Mali et al. (9) examined the influence of electroporation pulses on heart functioning. They observed that the transient interval between two consecutive R waves decreases after the treatment. Although no noxious effects due to electroporation have been reported so far, the probability for complications could increase in cases of internal tumors, in tumor ablation by irreversible electroporation and with pulses of longer durations. Therefore, the authors introduced pulse generation synchronized with the QRS complex of the electrocardiogram. It is known that the most appropriate time for such intervention is before the onset of the vulnerable period of the ventricles, since it may be prolonged, e.g. after premature heart beats. Finally, the authors concluded that a synchronized electroporation would increase the patient safety in cases of anatomical locations presently not accessible to the existing devices and electrodes.

Evidently, a pulse generation immediately after the QRS detection is the most reasonable approach. The time reserve for safe delivery after the detection and before the onset of the vulnerable period is approximately 60 ms.

In general, the QRS detection is a permanent goal as a part of different approaches in the electrocardiology. Numerous hardware and software solutions have been published, each of them contributing to the specific objective of the research (1, 2, 3, 5, 15, 17).

Research concept

The developed new equipment is aimed to contribute to data collection during electrochemotherapy of surface tumors that could be of importance for analysis and evaluation of the procedure effectiveness. It includes portable low-cost electroporator, QRS synchronizer and module for long-term recording of pulse parameters, thus giving an opportunity to new modality for electrochemotherapy of cancer.

Materials and Methods

Apparatus

Portable electroporator. The portable electroporator named by us Chemipulse III (Chemipulse-best) is an improved version of the high voltage generator that was introduced by Daskalov et al. (4).

The pulse transformer was optimized after studying the allowed compromise between the technologically provoked 1) non-ideal leading and trailing edges of the pulses, and 2) unavoidable pause between the positive and negative parts of the biphasic pulses on the one hand, and the efficiency of the biphasic sequence on the other.

Exceptional means warranting the electrical safety of both patient and physician are provided. The electroporator is battery supplied. Additional circuit automatically disconnects the applied part from the instrument as soon as the charging device (adaptor) is coupled. The pulse sequence is generated by high voltage capacitor, which is charged from the battery immediately before the shock. The amplitude is selected gradually from 100 V through 2200 V but the pulse sequence cannot be activated if the voltage choice does not start from the lowest level. Thus the risk of involuntary provoked electroshock with unsuitable energy is eliminated. Once selected, the electrical charge is available to be applied on the patient not more than 10 s. After that an automatic discharge is accomplished, if the physician has not started the shock.

QRS synchronizer. The specificity of implementation suggests the elaboration of simple but reliable hardware QRS detector. The ECG amplifier is a mandatory single lead circuit with additional means of patient protection. Usually, a peripheral lead is used. An iput notch filter is inserted for an unconditional suppression of the power-line interference. Since the instrument is currently used both for experiments with small animals and for tumor treatment of animals and human beings, a next band pass filter is designed in two commutative versions with central frequencies of 18 Hz (for human beings) and 28 Hz (for animals). Thus, the statistically optimal frequency bands of the QRS complexes, concentrating maximum signal energy, is better selected and amplified.
The QRS detection is based on a well-known approach (15) consisting of comparison between the ongoing ECG signal and an adaptive threshold. The final hardware version was preliminarily checked in MATLAB environment. Fig. 1 shows the result of the simulation with ECG recording taken from the widely accepted American Heart Association (AHA) database. The upper subplot shows a short epoch of original ECG signal. Two traces are presented in the middle subplot: the normal line stands for the differentiated ECG signal; the dash line describes the adaptive threshold. The last subplot draws the pulses coinciding with the detected QRS complexes, which are used in the hardware version for synchronizing the start of the pulse sequence generation.

![Fig. 1. Simulated QRS detection in MATLAB environment; first trace: original 1007d1 ECG signal obtained from The AHA Database; second trace: adaptive threshold; third trace: differentiated ECG signal; fourth trace: marks coinciding with the detected QRS complexes.](image1)

Residual power-line interference, if any, is removed from the selected ECG signal. As can be seen, the QRS complexes in the differentiated ECG are enhanced, while the T- and P-waves are reduced in amplitude. The adaptive threshold decreases gradually after each detected complex, thus disregarding eventual nearly located high amplitude T-waves. The drop down of the threshold is stopped at 400 μV above the zero line to eliminate the influence of low amplitude noise usually accompanying the real ECG signals.

The example shown in Fig. 1 is too simple for QRS detection. It is rather chosen to illustrate the principle of the algorithm comparing differentiated ECG signal with adaptive threshold. Fig. 2 presents a complicated case with four low amplitude normal QRS complexes and one ectopic beat with a compensatory pause. One may observe a successful detection of all ventricular beats.

**Module for long-term recording of pulse parameters during electrochemotherapy.** Fig. 3 (A, B) shows the new developed electroporator coupled with the module for long-term recording of pulse parameters during treatment of surface tumors (A) and QRS synchronizer embedding the hardware detector (B). The first trace in Fig. 3B visualizes real going ECG signal and the lower graphic consists of recognized complexes, which are marked by vertical straight lines.

![Fig. 3. Chemipulse III coupled with the module for long-term recording of pulse parameters during treatment of surface tumors (A) and (B) Hardware QRS detection accomplished in real time by the QRS synchronizer.](image2)

The current signal is obtained by low-resistance resistor introduced in series to the patient; the voltage is taken from a high-voltage resistor divider connected in parallel to the patient. Both signals are digitized by independent A/D converters contributing to synchronized current and voltage samples recording in corresponding buffers. They are further on used for calculation of the patient impedance, which could support the optimization of the voltage shock setting and the evaluation of the procedure effectiveness.

**Patients**

The application and study of electrochemotherapy in patients was approved by the ethics committee of the hospital and all patients gave written information consent before beginning treatment. Patients were cured according to the following criteria: clinically and cytologically confirmed carcinoma basocellulare, age >18 years, appropriate for electrochemotherapy.

All treatments were carried out at The Dermatologic Clinic at The Specialized Hospital for Active Treatment of Oncology, Sofia. All patients were treated under local anesthesia (lidocain 1 %). A pair of parallel flat electrodes...
with an adjustable interelectrode distance (calliper type) in the range of (5–30) mm were used. The electrotreatment was done by 16 biphasic rectangular pulses, 50+50 μs duration each, with a 20 μs interval between both phases and a pause of 880 μs between the bipolar pulses. The size of the lesion was measured by calliper instrument (Arimedex). Good contact between electrodes and the skin was ensured by a conductive gel. Electric pulses were delivered 10 min after intralesional injection of cytostatic bleomycin. The treatment response has to be evaluated at least 4 weeks after the treatment according to WHO (World Health Organization) guidelines as follows: 1) Complete response (CR): the absence of any trace of tumor; 2) Partial response (PR): decrease in the tumor volume by 50 % or greater; 3) No response (NR): decrease of < 50% or an increase of < 25% in the tumor volume; 4) Progressive disease (PD): tumor volume enlarged more than 25 %.

Results and Discussion
The portable electroporator Chemipulse III improved by the introduced synchronization and module for long term recording (Fig. 3) is currently tested with domestic animals in the Regina Elena Cancer Institute (Rome, Italy) and is applied on human patients in the Specialized Hospital for Active Treatment of Oncology, Sofia.

A total of 37 patients with 47 lesions of Ca basocellulare, Ca spinocellulare and Kaposi Sarcoma were treated with the new equipment using intralesional application of bleomycin. The age of the treated patients was in a wide range of 27 to 89 years. The gender distribution was relatively uniform: 20 males and 17 females. All the patients were in stage I T1, N0, M0. This study was prospective and nonrandomized. The electrochemotherapy was performed by using Chemipulse III electroporator (Fig. 3).

Fig. 4. Three representative cases: patient 1: before treatment (A), one week after treatment (B), one month after electrochemotherapy (C); patient 2: before treatment (D), one week later (E), one month follow-up (F); patient 3: before treatment (G), 24 h after treatment – edema at the site of the treatment (H), 18 months follow-up (I).
We give three representative patients with heart problems (Fig. 4). Patient 1: 54-year-old woman, with Ca basocellulare, stage T1N0M0. The new onset round lesion on the forehead with a diameter of 10 mm and thickness of 2 mm was registered in June 2010. After intralesional injection of local anesthetic (lidocaine) and 1.5 ml of bleomycin, 1000 V electric pulse was applied. The status of the patient is presented in Fig. 4 (A, B, C): A – before treatment, the lesion is round, active with subcutaneous infiltrate; B – one week after treatment the lesion is secreting with erythema and edema; C – one month later the treated area is not active without any traces of erythema and edema.

Patient 2: 82-year-old woman with Ca basocellulare, stage T1N0M0, registered in November 2010. The new onset lesion on the right cheek with dimensions of 20 mm×25.2 mm×1 mm. The applied electric field was 1000 V; and 2 ml intralesional bleomycin. The status of the patient is presented in Fig. 4 (D, E, F): D – before treatment, E – one week after treatment, F – one month after treatment.

Patient 3: 57-year-old man with Ca basocellulare, stage T1N0M0 with a new onset round lesion on the right cheek with a diameter of 10 mm. Electric field 900 V and 1 ml bleomycin were applied to the lesion. In Fig. 4 one of the side effects of electrochemotherapy is shown. Erythema and slight edema (Fig. 4G, H, I) occurred in most of the patients at the site of the treated lesions and disappeared in a few days or one week, marks of electrodes were noted in almost every case, followed by scars healing within a month of electrochemotherapy. Patients did not report local pain or other subjective symptoms.

The equipment with QRS synchronizer is mandatory in cases of electrochemotherapy of patients with pacemakers and patients with heart problems. The post-treatment follow-up period for patients is at least 12 months. Single side effects after electrochemotherapy are erythema and slight edema at the site of the treated lesions, which occurred in most of the patients and disappeared in a few days, one week at most.

The drug delivery conditions and the dose of cytostatic drugs are personal for every single case even if they are applied to one and the same patient but with different lesion locations.

Conclusions
The new modality of electrochemotherapy is an effective, personalized, inexpensive and mostly single procedure for treatment of skin tumors without hospitalization of the patient. The new electroporator with battery supplier is safe for patients and physicians. Possible risk of involuntary provoked electroshock with unsuitable initial energy is eliminated. The equipment with QRS synchronizer is suitable for patients with different heart problems.

Acknowledgements
This study was supported by the National Science Fund of Bulgaria, grant DO 02/178. The study was conducted in the scope of the EU-COST Action TD1104. We are thankful to A. Dashinov for the critical editing of the English text.

REFERENCES