Review Article

Electrochemotherapy: An enhancement of cytotoxicity of anticancer drugs

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ABSTRACT

Electrochemotherapy (ECT) is a local treatment of cancer employing electric pulses to improve transmembrane transfer of cytotoxic drugs. In this article, we discuss ECT and review the steps needed to move such a treatment from initial prototypes into clinical practice. The delivery of electric pulses at the time of when a chemotherapeutic drug reaches its highest extracellular concentration considerably increases the transport through the membrane toward the intracellular targets of cytotoxicity. The two most commonly used drugs are bleomycin and cisplatin which do not freely cross the intact cell membrane and directly affect the nuclear DNA structure, adversely interfering with mitosis leading to cancer cell death. ECT, in general, can be considered as a palliative option for cancers for which standard treatments have failed or proved to be insufficient. Sarcomas, carcinomas, or melanoma tumors responded with a high percentage of complete responses when the drugs were injected intravenously or intratumorally. Due to high effectiveness of ECT in the treatment of cutaneous and subcutaneous tumors regardless of histological origin, there are now attempts to extend its use to the treatment of internal tumors. To advance the applicability of ECT to treatment of internal solid tumors, new technological developments are needed that will enable treatment of these tumors in daily clinical practice.

Keywords: Cytotoxicity, electrochemotherapy, electroporation, tumor

Introduction

A proportion of patients who have undergone extensive tumor treatment, including all conventional treatment modalities, face the problem of metastases. Progression of the disease in cutaneous tissue, without dissemination to other organs, raises the problem of which treatment to choose. In these cases, a simple and effective treatment approach, with no or minimal systemic side effects and good antitumor effectiveness is desirable. There are several reasons why many of the chemotherapeutic drugs presently used are not sufficiently effective. One reason is the hampered transport of the drugs through the plasma membrane. Therefore, new ways to facilitate chemotherapeutic drug delivery into cells are being sought to potentiate their effectiveness, while lowering systemic toxicity. Among the drug delivery systems presently under investigation[1] is the use of electric pulses.[2] In electrochemotherapy (ECT), electric pulses are used as a means of increasing chemotherapeutic drug delivery into cells.[2] Exposure of cells to electric pulses under specific conditions increases plasma membrane permeability, temporarily and reversibly, without affecting cell viability.[2,3] This technique, termed electroporation, has been used successfully for the insertion of drugs, dyes, genes, oligonucleotides, and monoclonal antibodies into cells.[4] Among several chemotherapeutic drugs tested for potentiation of their cytotoxicity by electroporation, bleomycin and cisplatin have been

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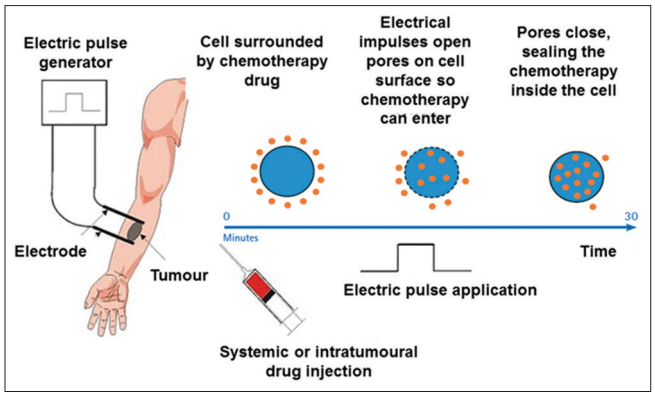

Figure 1: Basic steps in electrochemotherapy treatment

found to be very suitable, due to their limited transport through the plasma membrane.[5-7] The aim of this document is to define the basis for understanding the mechanisms of the ECT as well as its possibilities as antitumor treatment (Figure 1).

Principle Mechanisms of ECT

The vascular lock

The electric pulses produce a transient state of hypoperfusion by local reflex vasoconstriction at the arteriolar level (lasting 1-2 min) and a phase of interstitial edema (that resolves with membrane resealing). However, the effect may last longer (12 h to 5 days) in rapidly dividing tumor cells and is more prominent in tumors with a less mature endothelial lining and higher interstitial pressure. This phenomenon mediated by the sympathetic nervous system is termed the "vascular lock" and it has implications for timing of drug administration.[8] After application of the electric current, there is retention of drugs already in the tumor but there is also an impairment of entry of drugs from the circulation.[9,10]

Electroporation

Under physiological conditions, the cell plasma membrane is subjected to a transmembrane potential difference caused by a system of ion pumps and channels in the membrane. This voltage, termed the resting transmembrane voltage, is in the range of tens of millivolts and is present in every cell. Exposure of a cell to an external electric field results in an additional component of the voltage across the membrane. This component, termed the induced transmembrane potential difference, is superimposed onto the resting voltage and exists only as long as the external field is present. An external electric field also alters ionic currents and ion distributions in the extracellular space and activates a cascade of signaling pathways that upregulate transcription and translation levels.[11] The induced transmembrane voltage is proportional to the strength of the external electric field, and consequently, exposures to sufficiently strong fields can lead to transmembrane voltages far exceeding their physiological range.[12,13] An electric field leads to a formation of hydrophilic (aqueous) pores, in which the lipids adjacent to the aqueous inside of the pore are reoriented in a manner that their hydrophilic heads are facing the pore, while their hydrophobic tails are hidden inside the membrane (Figure 2). As electric field amplitude increases, the presence of

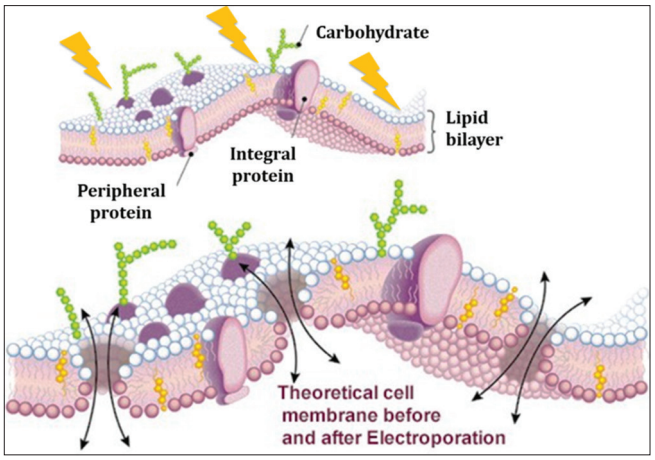

Figure 2: Formation of an aqueous pore. The situation is shown for transmembrane voltage increasing from top to bottom: A nonporated membrane, formation of a hydrophobic pore, transformation into a hydrophilic pore (electroporation)

hydrophilic pores becomes energetically ever more favorable, which leads to the formation of pores with an average radius. This can lead to structural rearrangements of lipids in the membrane bilayer due to large build-ups of oppositely charged ions on either side of the cell surface resulting in formation and stabilization of nano-sized pores.[14,15] This physical phenomenon was termed electroporation or electropermeabilization because it was observed that molecules that do not normally pass the membrane gain intracellular access through diffusion after the cells were treated with electric fields.[16,17]

Types of drugs

Several chemotherapeutic drugs were tested *in vitro* on cells for potential application in combination with electroporation; some of them are daunorubicin, doxorubicin, etoposide, paclitaxel, actinomycin D, adriamycin, mitomycin C, 5-fluorouracil, vinblastine, vincristine, gemcitabine, netropsin, cytarabine, oxaliplatin, methotrexate, melphalan, ancitabine, Taxotere, nimustine, cyclophosphamide, carboplatin, cisplatin, and bleomycin. Electroporation of cells increases the cytotoxicity of some of these drugs ranging from two to several thousand folds.[18]

Instrumentation

CLINIPORATOR™ (IGEA S.r.l., Carpi, Italy) is certified as a medical device that was prepared in 2006 during the European Standard Operating Procedures of ECT (ESOPE) project.[19] Along with the development of the electric pulse generator, also plate and needle electrodes were developed. The standard operating procedures were prepared based on the experience of the leading European cancer centres on ECT.[20] Cliniporator™ consists of two parts: A console (industrial PC compatible computer) for local collection of treatment data and user-friendly interface; and an electroporator. Electroporator consists of a control unit, high voltage amplifier, and low voltage amplifier.[21]

A user controls the electroporator through the graphical display and a keyboard of the console unit. He/she can enter relevant patient

data, choose appropriate electrodes, and define pulse parameters such as number (e.g., 8 pulses), amplitude (up to 1000 V), duration (e.g., 100 μs), and repetition frequency (e.g., 1 Hz) of pulses. By pressing a foot switch, the user triggers pulse generation. Square-shaped pulses are delivered. During the pulse delivery, control unit measures voltage and current through the load (a cell suspension or a tissue). After the pulse application voltage and current measurements are stored in the local database. Based on collected data, we intend to develop an algorithm, which will allow device to adjust pulse voltage according to the current and voltage measurements in the real time and thus prevent irreversible changes in the cell membranes.[22,23] The treatment planning consists of several phases: Image pre-processing, three-dimensional model generation, electrode placement, implementation of the mathematical model of electroporation, and optimization of the results to define the voltage applied which is dictated for each couple of electrodes by the number of electrodes and the electrodes positions.

The electric pulses may be applied to the tumors either by plate electrodes on the skin surface or by needle electrodes to be inserted into the lesion. The electric field distribution is determined by the geometry of the electrodes.[24] Current pulse generators in use are the Cliniporator (IGEA, Carpi, Italy) (Figure 3) and the Medpulser™ (Inovio Biomedical Corporation, CA, USA). It appears that plate electrodes are more suitable for use in superficial skin lesions, while needle electrodes are used for deeper seated lesions, such as exophytic and thick lesions (maximum depth 3 cm).[19] A disadvantage of plate electrodes over the needle type is the potential skin damage that may be generated by the higher impedance/resistance of the skin, especially when treating larger affected areas.[25] Care must be taken to avoid inserting the needle electrodes into the healthy tissue surrounding the tumors, which may also result in local subcutaneous burns.[26] There are four types of electrodes in common usage (Figure 4). Type I electrodes consist of two parallel stainless-steel plate electrodes, used for superficial lesions and do not penetrate the skin. Type II electrodes are used for smaller lesions and consist of two rows of eight needles with 4 mm distance between them. Type III electrodes are recommended for larger lesions (>1 cm), with the needles in a hexagonal configuration. Type IV electrodes, called finger electrodes, allow the treatment of narrow body cavities not accessible by standard electrodes; they exhibit a good operator sensitivity and tactile feedback during the procedure. The needles are inserted encircling the tumor and down to the subcutaneous tissue, slightly deeper than tumor depth.[8,19,24] Each electrode is coded, during the treatment session. The code is typed into the Cliniporator which automatically sets the pulse characteristics including amplitude, number, and frequency of the impulses. Type I and II electrodes are used to deliver 8 pulses lasting for 100 μs, and the pulses can be delivered at 1 Hz or 5 kHz frequency. Type III electrode delivers up to 24 series of 8 pulses (to limit patient discomfort pulses are delivered at 5 kHz only). Cliniporator is the only device that allows high-frequency pulse delivery for clinical use.

The overview of the implementation of ECT in the treatment of deep seated tumors demonstrates that new electrodes are being developed and used in the treatment of various tumor types and

Figure 3: Cliniporator™ and Type III electrode (needle array)

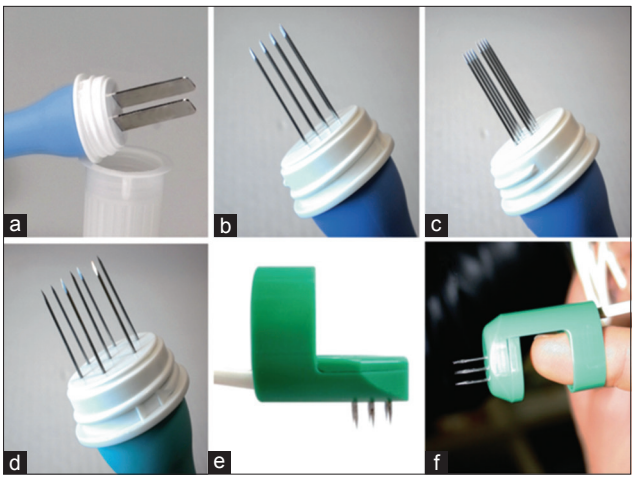

Figure 4: Fixed geometry electrodes (a) plate electrodes (b) linear needle electrodes (c) parallel needle electrodes (d) hexagonal needle electrodes (e) finger electrodes and (f) finger electrodes with axial needles

locations. Basically, three types of electrodes have been developed and are being used: The first ones are long needle electrodes that are inserted into the tumor and surrounding tissue to safely cover the tumor and achieve an appropriate margin. Such electrodes are being tested for the treatment of liver metastases of colorectal tumors, hepatocellular carcinoma or cholangiocarcinoma, soft tissue sarcomas and for bone metastases based on solid preclinical evidence.[27,28] The second is endoluminal electrodes that are being tested for the treatment of colorectal cancer but could be used also for the treatment of esophageal tumors.[29] The third are the electrodes that are aimed at the treatment of brain tumors. The electrodes have proven to be suitable for the treatment of the brain tumors, through the skull.[30]

Patient Selection

This part covers the criteria that must be checked during the pre-inclusion visit for the treatment by ECT of patients with cutaneous and subcutaneous nodules.

History

A full history should be taken from the patient, and special attention should be paid to the following: If the patient has sensitivity to

bleomycin or a history of pulmonary fibrosis, then this will preclude the administration of intravenous bleomycin. The presence of a pacemaker precludes treatment on the anterior chest wall. If any previous difficulties with local anesthesia (lidocaine) or general anesthetic this will determine treatment method.[31]

Electrocardiogram (ECG)

ECG before treatment depends on the location of the nodules, i.e., it is less important if the nodules are on the limbs and more important if on the trunk. It also depends on the patient's cardiac history. It is highly recommended in the case of manifest cardiac arrhythmia or previous cardiac event.

Pacemaker

If the patient has a pacemaker, verify:

- If the only nodules of the patient are located in the anterior chest wall, the patient is not eligible for treatment by ECT.
- If there are nodules located elsewhere, only these nodules will be eligible for treatment by ECT.[32]

Hematology

If platelets <70000/μL then hematological opinion regarding the risk of bleeding versus the benefit of the therapy. The clinician can then make an informed decision. If the patient has coagulation problems, verify if Type II or Type III electrode are supposed to be used. If the nodules of the patient are all deep (more than 1 cm below the skin) nodules, the patient is not eligible for treatment by ECT.[32]

Biochemistry

If using intravenous bleomycin then creatinine should be <150 μmol/l to ensure adequate renal clearance.[32]

Operating Modality

Anesthesia

Lay a rectangular infiltration of local anesthetic (lidocaine, 2%, with adrenaline 0.5%) around the area to be treated, by injecting along four lines so that the nodule is 'fenced in' by local anesthetic, so that pain transmission is completely blocked.[33] Small cutaneous tumors may be anesthetized by local infiltration of lidocaine just below the tumor, however, still covering the area of electroporation. General sedation may be decided, following the decision tree, when nodules are too numerous, too big or too painful to be anesthetized by local anesthesia or when they are inside a previously irradiated area. Analgesia is started at least 3 min before intratumoural injection: Remifentanil 0.5 μg/kg bolus then 0.1-0.15 μg/kg/min then adjusted to the response to the first pulses or target controlled infusion (target 2-4 ng/ml). It may also be replaced by alfentanil boluses 250-750 μg. Sedation is then started: Propofol 0.5 mg/kg then 2-4 mg/kg/h or target controlled infusion (target 1-2 μg/ml).[31]

Injection of chemotherapy

Intratumoural treatment can be performed either with cisplatin or bleomycin, according to the instructions below.

- Cisplatin injected dose should be 0.25 ml (0.5 mg) per cm^3 of tumor tissue for tumors larger than 1 cm^3, and 0.5 ml (1 mg) per cm^3 of tumor tissue for tumors smaller than 1 cm^3 but larger than 0.5 cm^3. For tumors smaller than 0.5 cm^3 injected dose should be 1 ml (2 mg) per cm^3.
- Bleomycin injected dose should be 0.25 ml (250 IU) per cm^3 of tumor tissue for tumors larger than 1 cm^3, and 0.5 ml (500 IU) per cm^3 of tumor tissue for tumors smaller than 1 cm^3 but larger than 0.5 cm^3. For tumors smaller than 0.5 cm^3 injected dose should be 1 ml (1000 IU) per cm^3. 15000 IU is approximately equal to 8-9 mg or 15 mg of bleomycin depending on the activity of the drug and the manufacturer.[20,34]

Choice of electrodes

Choose the appropriate electrode. If the tumor is less than 1 cm, consider using either plate or parallel array electrodes. In case the tumor is more than 1 cm, consider using the hexagonal array electrodes. Plates or needle electrode designated for superficial or more deeply located small nodules respectively.

Electric current

Electropermeabilization occurs in the cell when the internal transmembrane potential has surpassed the critical value between 200 and 300 mV. The extent of the electroporative effect depends on the number and duration of the electric pulses. Two pulse parameters have been evaluated - exponentially decaying pulses and square wave pulses. The pulse parameters selected for the treatment depend on the type of electrode.[10,19,35]

Pulse parameters

Ideally for Type I (plate) electrodes, pulse parameters of 8 square waves with an amplitude of 1300 V/cm, duration of 100 μs, and frequency of 1 Hz are used. For Type II (needle) electrodes the voltage amplitude may be reduced to 1000 V/cm. The pulses are administered simultaneously between the needle pairs. For Type III, the needles are positioned hexagonally, and 96 pulses are given together (12 pairs × 8 pulses) at a frequency of 5 kHz.[36] Due to the already described vascular lock phenomenon, it is recommended that the electric current is applied between 8 and 28 min after IV administration of the drug or immediately (within 2-10 min) after intratumoral administration.[37,38]

Pulsing procedure

- Make a test run with the Cliniporator™ and electrodes.
- Disinfect the area to be treated. Inject the drug into the tumor, and note the time. For small tumors, choose to inject either directly into the nodule or to tunnel the hypodermic needle under the skin to the nodule before injection. For larger tumors, several injections are needed. Make a pattern of injections using parallel insertions points.
- Within 10 min of drug injection, electric pulses must be applied. Take the gauze pad in one hand, and the electrode in the other. The gauze pad can enable you to grasp the skin around the lesion to be treated, to lift the lesion up from the underlying

musculature. In this way, muscle contractions and associated discomfort can be considerably reduced.

- Inform patient when the first pulse is to be delivered. Several pulse applications may be needed to cover the tumor volume. The electric field drops off very quickly outside the area of the electrodes, so the area to be treated by pulses must be encompassed within the electrodes. After treatment, a loose dry dressing can be applied, or the lesion can be left without dressing.
- Patients can be retreated. Even though bleomycin doses are low, it is recalled that the cumulative bleomycin dose must not exceed 400000 IU/m^2 due to risk of lung fibrosis.
- To avoid potential pain at the end of the procedure (some localizations can be uncomfortable), paracetamol can be given in a prophylactic way, but can also be offered to patients after the procedure.[20]

Follow-up session

See patient again at 4 weeks post-treatment or earlier if required. At that time, treatment efficacy can be determined in most cases. For larger lesions, more healing time may be necessary. Retreatment can be considered on the evaluation at 4 weeks post-treatment, but also later. A healing time of up to 10 weeks is possible for lesions over 1.5 cm. For smaller lesions, healing time is in the order of 4-8 weeks.[20]

Clinical Applications

The first clinical study on ECT was published in 1991 by Mir *et al*.,[39] and demonstrated the feasibility, safety, and effectiveness of ECT. This first study stimulated groups in the United States (Tampa), Slovenia (Ljubljana), and France (Toulouse and Reims) to perform further clinical studies.[26] The coalescence of the field was marked by the report of a European project called the ESOPE. Based on the results of a clinical study[19] together with the standard operating procedures, ECT was widely accepted for clinical use throughout Europe. Clinical indications were published in 2008,[40] and a systematic review and meta-analysis recently analyzed the results of all the published studies through 2012.[41] Data analysis confirmed that delivery via ECT has a significantly ($P < 0.001$) higher effectiveness (by more than 50%) than bleomycin or cisplatin injection alone. The overall effectiveness of ECT was 84.1% objective responses (which include both complete and partial responses), and from these, 59.4% were complete responses (which indicate complete regressions of tumors after therapy) after a single ECT treatment. The procedure can, however, be repeated with similar effectiveness.[42] Another recent review and clinical study suggested that the SOP may need refinement, as the current SOP for ECT may not be suitable for tumors bigger than 3 cm in diameter, which require multiple, consecutive ECT treatments.[43] Several ongoing ECT studies are targeting superficial tumors, predominantly melanoma but also chest wall breast cancer recurrences[44] and head and neck cancers.[45] Furthermore, the technology is also being adapted for the treatment of deep-seated tumors, such as colorectal tumors, soft tissue sarcomas, and brain, bone, and liver metastases.[24] At present, ECT is being used for the treatment of metastases and primary tumors in more than 130 cancer centers in Europe and has been accepted in line with other local tumor treatments.[46] In addition, ECT is also used in veterinary oncology, for the treatment of metastases as well as primary tumors.[47-50] The success rate of primary tumor treatment with ECT also provides good evidence for translation of ECT into the treatment of less-advanced tumors in humans.

Combinations of ECT with other therapies

The combined application of ECT with other treatment modalities may provide an additional tool for cancer treatment since their combination is expected to exert an amplified effect regarding the efficient control of cancer. Preclinical studies have showed that ECT acts synergistically with radiotherapy, exerting a radiosensitizing effect on different types of tumors, which results in toxicity enhancement.[51] Furthermore, ECT combined with photodynamic therapy has been in research with the intention to enhance efficacy and achieve synergistic cell death. In addition, several studies suggest that the immune system is involved in the mechanisms of response to ECT treatment and that this could be exploited for systemic disease control. However, regression of untreated distant metastases has never been reported. Immunotherapy along with ECT would have the potential not only for local but also for distant treatment of tumors such as those of malignant melanoma, by stimulating a self-driven immune response to achieve systemic control of the disease.[52,53]

Advantages of ECT

ECT is easy and quick to perform and is inexpensive. The requirements are a suitable room for patient preparation and treatment, and an electric pulse generator with different sets of electrodes that are used for different sizes of tumor nodules. After the treatment, patients do not require special attention or post-treatment medication. They can wait for a while in the hospital to be in the position to obtain medical attention, if needed, but so far no side effects were observed or medical attention of the patients required. A good indicator that ECT is not a stressful or painful procedure is that the majority of the patients interviewed during a clinical study aimed at defining the ESOPE would be willing to accept the treatment another time if it would be necessary. The effects are localized to the target area (only electropermeabilized cells are affected), therefore, no systemic side effects occur of the drug used. In the case of the bleomycin administered intravenously, it has been shown that ECT will selectively eliminate the tumor cells because their division rate is much faster than the division rate of the normal cells surrounding the tumor. The absence of systemic side effects and the low impact on the immune system also make this treatment suitable for elderly people and patients with poor physical condition, even with repeated courses.[54]

Limitations of ECT

No arrhythmias or other pathological morphological changes in the ECG recordings during ECT have been found.[55] Tumors larger than 3 cm^2 appear to have lower response rates to ECT as compared to nodules smaller than 1 cm^2. However, they can be retreated with

no loss of the ECT efficacy. When tumor nodules are located in irradiated or fibrotic tissues, the needle electrode penetration may be problematic with a suboptimal delivery of the electrical current or drugs.[56] ECT is local treatment that can be effective in the treatment of limited number of tumor lesions that are not bigger than 3 cm in diameter. Therefore, it can be effective in those patients that have few or up to 15 skin metastases in transit. In the case of more nodules ECT cannot be performed on all nodules in one session. At present, the electrodes that are used are effective in the treatment of superficial nodules, whereas they are not quite appropriate for deeper seeded or big nodules. Bigger nodules need application of several sets of electric pulses, and also several treatment sessions, to cover the whole tumor area and to be able to remove deeper layers of the tumor.

Conclusion

Current developments and future medical applications of tissue electroporation are numerous. Because ECT with bleomycin or cisplatin has been shown to act synergistically with radiotherapy in preclinical studies, use of the technique for the radiosensitization of cutaneous tumors can be foreseen, predominantly in the palliative treatment of progressive disease. Further development is focused on the use of endoluminal electrodes to treat internal tumors, a technological development that could be used for either ECT or gene electrotransfer.

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References

1. Langer R. New methods of drug delivery. Science 1990;249:1527-33.
2. Mir LM, Orlowski S, Belehradek J Jr, Teissie J, Rols MP, Sersa G, *et al*. Biomedical applications of electric pulses with special emphasis on antitumor electrochemotherapy. Bioelectrochem Bioenerg 1995;38:203-7.
3. Rols MP, Teissié J. Electropermeabilization of mammalian cells. Quantitative analysis of the phenomenon. Biophys J 1990;58:1089-98.
4. Orlowski S, Mir LM. Cell electropermeabilization: A new tool for biochemical and pharmacological studies. Biochim Biophys Acta 1993;1154:51-63.
5. Mir LM, Tounekti O, Orlowski S. Bleomycin: Revival of an old drug. Gen Pharmacol 1996;27:745-8.
6. Melvik JE, Pettersen EO, Gordon PB, Seglen PO. Increase in cis-dichlorodiammineplatinum (II) cytotoxicity upon reversible electropermeabilization of the plasma membrane in cultured human NHIK 3025 cells. Eur J Cancer Clin Oncol 1986;22:1523-30.
7. Sersa G, Cemazar M, Miklavcic D. Antitumor effectiveness of electrochemotherapy with cis-diamminedichloroplatinum(II) in mice. Cancer Res 1995;55:3450-5.
8. Möller MG, Salwa S, Soden DM, O'Sullivan GC. Electrochemotherapy as an adjunct or alternative to other treatments for unresectable or in-transit melanoma. Expert Rev Anticancer Ther 2009;9:1611-30.
9. Mir LM. Bases and rationale of electrochemotherapy. Eur J Cancer Suppl 2006;4:38-44.
10. Gehl J. Electroporation: Theory and methods, perspectives for drug delivery, gene therapy and research. Acta Physiol Scand 2003;177:437-47.
11. Hronik-Tupaj M, Kaplan DL. A review of the responses of two-and three-dimensional engineered tissues to electric fields. Tissue Eng Part B Rev 2012;18:167-80.
12. Kotnik T, Bobanovic F, Miklavcic D. Sensitivity of transmembrane voltage induced by applied electric fields-a theoretical analysis. Bioelectrochem Bioenerg 1997;43:285-91.
13. Kotnik T, Miklavcic D. Analytical description of transmembrane voltage induced by electric fields on spheroidal cells. Biophys J 2000;79:670-9.
14. Kotnik T, Pucihar G, Miklavcic D. Induced transmembrane voltage and its correlation with electroporation-mediated molecular transport. J Membr Biol 2010;236:3-13.
15. Lee EW, Wong D, Tafti BA, Prieto V, Totonchy M, Hilton J, *et al*. Irreversible electroporation in eradication of rabbit VX2 liver tumor. J Vasc Interv Radiol 2012;23:833-40.
16. Neumann E, Rosenheck K. Permeability changes induced by electric impulses in vesicular membranes. J Membr Biol 1972;10:279-90.
17. Mir LM, Banoun H, Paoletti C. Introduction of definite amounts of nonpermeant molecules into living cells after electropermeabilization: Direct access to the cytosol. Exp Cell Res 1988;175:15-25.
18. Miklavcic D, Mali B, Kos B, Heller R, Serša G. Electrochemotherapy: From the drawing board into medical practice. Biomed Eng Online 2014;13:29.
19. Marty M, Sersa G, Garbay JR, Gehl J, Collins CG, Snoj M, *et al*. Electrochemotherapy-an easy, highly effective and safe treatment of cutaneous and subcutaneous metastases: Results of ESOPE (European Standard Operating Procedures of Electrochemotherapy) study. Eur J Cancer Suppl 2006;4:3-13.
20. Mir LM, Gehl J, Sersa G, Collins CG, Garbay JR, Billard V, *et al*. Standard operating procedures of the electrochemotherapy: Instructions for the use of bleomycin or cisplatin administered either systemically or locally and electric pulses delivered by the cliniporator™ by means of invasive or non-invasive electrodes. Eur J Cancer Suppl 2006;4:14-25.
21. Pavlovic I. A web application that extends functionality of medical device for tumor treatment by means of electrochemotherapy. Radiol Oncol 2004;38:49-54.
22. Kos B, Zupanic A, Kotnik T, Snoj M, Sersa G, Miklavcic D. Robustness of treatment planning for electrochemotherapy of deep-seated tumors. J Membr Biol 2010;236:147-53.
23. Pavliha D, Kos B, Zupanic A, Marcan M, Serša G, Miklavcic D. Patient-specific treatment planning of electrochemotherapy: Procedure design and possible pitfalls. Bioelectrochemistry 2012;87:265-73.
24. Byrne CM, Thompson JF. Role of electrochemotherapy in the treatment of metastatic melanoma and other metastatic and primary skin tumors. Expert Rev Anticancer Ther 2006;6:671-8.
25. Hofmann GA, Dev SB, Nanda GS, Rabussay D. Electroporation therapy of solid tumors. Crit Rev Ther Drug Carrier Syst 1999;16:523-69.
26. Mir LM, Glass LF, Sersa G, Teissié J, Domenge C, Miklavcic D, *et al*. Effective treatment of cutaneous and subcutaneous malignant tumours by electrochemotherapy. Br J Cancer 1998;77:2336-42.
27. Edhemovic I, Gadzijev EM, Brecelj E, Miklavcic D, Kos B, Zupanic A, *et al*. Electrochemotherapy: A new technological approach in treatment of metastases in the liver. Technol Cancer Res Treat 2011;10:475-85.
28. Bianchi G, Campanacci L, Donati D. Electrochemotherapy in bone metastases: Results of a phase II study. In: 7th Conference on Experimental and Translational Oncology. Ljubljana: Association of Radiology and Oncology; 2013. p. 46-7.
29. Bourke M, Salwa S, Forde P, Sadadcharam M, Larkin J, Collins C, *et al*. P80 Endoscopically targeted electrochemotherapy for the treatment of colorectal cancer. Eur J Surg Oncol 2012;38:1127-8.
30. Linnert M, Iversen HK, Gehl J. Multiple brain metastases-current management and perspectives for treatment with electrochemotherapy. Radiol Oncol 2012;46:271-8.
31. Gothelf A, Mir LM, Gehl J. Electrochemotherapy: Results of cancer treatment

using enhanced delivery of bleomycin by electroporation. Cancer Treat Rev 2003;29:371-87.

32. Mir LM. Therapeutic perspectives of *in vivo* cell electropermeabilization. Bioelectrochemistry 2001;53:1-10.
33. Testori A, Tosti G, Martinoli C, Spadola G, Cataldo F, Verrecchia F, *et al.* Electrochemotherapy for cutaneous and subcutaneous tumor lesions: A novel therapeutic approach. Dermatol Ther 2010;23:651-61.
34. Sersa G, Stabuc B, Cemazar M, Miklavcic D, Rudolf Z. Electrochemotherapy with cisplatin: Clinical experience in malignant melanoma patients. Clin Cancer Res 2000;6:863-7.
35. Sadadcharam M, Soden DM, O'Sullivan GC. Electrochemotherapy: An emerging cancer treatment. Int J Hyperthermia 2008;24:263-73.
36. Domenge C, Orlowski S, Luboinski B, De Baere T, Schwaab G, Belehradek J Jr, *et al.* Antitumor electrochemotherapy: New advances in the clinical protocol. Cancer 1996;77:956-63.
37. Heller R, Jaroszeski M, Perrott R, Messina J, Gilbert R. Effective treatment of B16 melanoma by direct delivery of bleomycin using electrochemotherapy. Melanoma Res 1997;7:10-8.
38. Cemazar M, Milacic R, Miklavcic D, Dolzan V, Sersa G. Intratumoral cisplatin administration in electrochemotherapy: Antitumor effectiveness, sequence dependence and platinum content. Anticancer Drugs 1998;9:525-30.
39. Mir LM, Belehradek M, Domenge C, Orlowski S, Poddevin B, Belehradek J Jr, *et al.* Electrochemotherapy, a new antitumor treatment: First clinical trial. C R Acad Sci III 1991;313:613-8.
40. Sersa G, Miklavcic D, Cemazar M, Rudolf Z, Pucihar G, Snoj M. Electrochemotherapy in treatment of tumours. Eur J Surg Oncol 2008;34:232-40.
41. Mali B, Jarm T, Snoj M, Sersa G, Miklavcic D. Antitumor effectiveness of electrochemotherapy: A systematic review and meta-analysis. Eur J Surg Oncol 2013;39:4-16.
42. Campana LG, Mocellin S, Basso M, Puccetti O, De Salvo GL, Chiarion-Sileni V, *et al.* Bleomycin-based electrochemotherapy: Clinical outcome from a single institution's experience with 52 patients. Ann Surg Oncol 2009;16:191-9.
43. Mali B, Miklavcic D, Campana LG, Cemazar M, Sersa G, Snoj M, *et al.* Tumor size and effectiveness of electrochemotherapy. Radiol Oncol 2013;47:32-41.
44. Matthiessen LW, Johannesen HH, Hendel HW, Moss T, Kamby C, Gehl J. Electrochemotherapy for large cutaneous recurrence of breast cancer: A phase II clinical trial. Acta Oncol 2012;51:713-21.
45. Gargiulo M, Papa A, Capasso P, Moio M, Cubicciotti E, Parascandolo S. Electrochemotherapy for non-melanoma head and neck cancers: Clinical outcomes in 25 patients. Ann Surg 2012;255:1158-64.
46. Testori A, Faries MB, Thompson JF, Pennacchioli E, Deroose JP, van Geel AN, *et al.* Local and intralesional therapy of in-transit melanoma metastases. J Surg Oncol 2011;104:391-6.
47. Kodre V, Cemazar M, Pecar J, Sersa G, Cor A, Tozon N. Electrochemotherapy compared to surgery for treatment of canine mast cell tumours. *In Vivo* 2009;23:55-62.
48. Spugnini EP, Vincenzi B, Citro G, Dotsinsky I, Mudrov T, Baldi A. Evaluation of Cisplatin as an electrochemotherapy agent for the treatment of incompletely excised mast cell tumors in dogs. J Vet Intern Med 2011;25:407-11.
49. Tamzali Y, Borde L, Rols MP, Golzio M, Lyazrhi F, Teissie J. Successful treatment of equine sarcoids with cisplatin electrochemotherapy: A retrospective study of 48 cases. Equine Vet J 2012;44:214-20.
50. Tozon N, Sersa G, Cemazar M. Electrochemotherapy: Potentiation of local antitumour effectiveness of cisplatin in dogs and cats. Anticancer Res 2001;21:2483-8.
51. Sersa G, Stabuc B, Cemazar M, Miklavcic D, Rudolf Z. Electrochemotherapy with cisplatin: The systemic antitumour effectiveness of cisplatin can be potentiated locally by the application of electric pulses in the treatment of malignant melanoma skin metastases. Melanoma Res 2000;10:381-5.
52. Mir LM, Orlowski S, Poddevin B, Belehradek J Jr. Electrochemotherapy tumor treatment is improved by interleukin-2 stimulation of the host's defenses. Eur Cytokine Netw 1992;3:331-4.
53. Heller L, Pottinger C, Jaroszeski MJ, Gilbert R, Heller R. *In vivo* electroporation of plasmids encoding GM-CSF or interleukin-2 into existing B16 melanomas combined with electrochemotherapy induces long-term antitumour immunity. Melanoma Res 2000;10:577-83.
54. Curatolo P, Quaglino P, Marenco F, Mancini M, Nardò T, Mortera C, *et al.* Electrochemotherapy in the treatment of Kaposi sarcoma cutaneous lesions: A two-center prospective phase II trial. Ann Surg Oncol 2012;19:192-8.
55. Mali B, Jarm T, Corovic S, Paulin-Kosir MS, Cemazar M, Sersa G, *et al.* The effect of electroporation pulses on functioning of the heart. Med Biol Eng Comput 2008;46:745-57.
56. Quaglino P, Mortera C, Osella-Abate S, Barberis M, Illengo M, Rissone M, *et al.* Electrochemotherapy with intravenous bleomycin in the local treatment of skin melanoma metastases. Ann Surg Oncol 2008;15:2215-22.