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Modern medicine has a new technology: Therapeutic electroporation

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Abstract: *Electroporation is considered a new start-up in the treatment of various tumors; currently, researches are being conducted in order to develop this technology with medical applications. The technique consists in the significant increase in the electrical conductivity and permeability of the plasma membrane of cells resulting from the application of an external electric field. It is routinely used in molecular biology to transform bacteria, yeast, protoplasts and is performed using the electroporators. Currently, the process seems to be a real solution that enables a targeted drug to act with maximum efficiency on cells and tissues requiring treatment, resulting in obtaining a good therapeutic effect without major side effects. Therefore, pharmaceutical companies are trying to demonstrate through preclinical studies the potential efficacy of this technology, succeeding in recent years to achieve important steps in this direction.*

Keywords: *electroporation, therapy, tumor, medical application*

INTRODUCTION

Modern medicine is always looking for new technologies applicable to the diagnosis, prevention and treatment of diseases. Among these, the applications of molecular biology represent a direction of the future, particularly in the treatment of diseases currently considered incurable. Translational medicine applies the new discoveries, in molecular and genetic biology, as well as the techniques resulted from these, for therapeutics based on biology, which gradually replaces therapeutic based on chemistry. For example, by applying electric current at cell level, can be inserted into altered cells, the therapeutic molecules or transfection of genetic material can be achieved.

ELECTROPORATION

Electroporation is the electropermeabilization of cell membranes, which consists in increasing the electrical conductivity and permeability of the plasma membrane of cells, being caused by the application of an external electric field. This procedure is particularly useful in molecular biology in order to insert certain substances in a cell such as a molecular probe or a drug that can induce changes in cell functions or coding DNA [1].

Electroporation is a dynamic process that depends on local transmembrane voltage for each point at cell membrane level. For a certain type and duration of the

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pulse, there is a threshold of the transmembrane voltage in order to allow the expression of transmembrane electroporation phenomenon between 0.5 V and 1 V. This leads to defining threshold value of the magnitude of the electric field necessary to achieve the phenomenon of electroporation. Only the cells found in an electric field greater than the *threshold* value can be electroporated. If a second threshold is reached or exceeded, electroporation will compromise the viability of the cells, this phenomenon being called *irreversible electroporation* [2].

In molecular biology, the process of electroporation can be used to transform prokaryotic and eukaryotic cells. Bacteria that present a cell wall composed of peptidoglycans for protection, resistance and transport of substances are protected from environmental changes. Based on this characteristic, bringing the bacteria into contact with particular plasmids of interest may have the effect of transferring the plasmid to other bacterial cells, subsequent to an electroporation process by applying several hundred volts over a distance of a few millimeters. Subsequently, the cells must be handled carefully until they divide into new cells that contain new plasmids; this process is about ten times more efficient than chemical transformation [3].

This process is also highly efficient for the insertion of foreign genes in cell cultures and in particular in mammalian cell lines. For example, the process is used to produce *knock-out* mice (transgenic mice in which is inactivated a single target gene for the study of phenotypic characteristics encoded by this) as well as in tumor treatment, gene therapy, and cell-based therapy. The process for the insertion of foreign DNA molecules into eukaryotic cells is known as transfection. Electroporation is particularly effective for transfecting cells in suspension in a cultivation medium using an electroporation cuvette.

Electroporation has proven effective for use on *in vivo* tissues for *in utero* or *in ovo* transfection applications. The adherent cells to the substrate can also be transfected using electroporation, thereby providing an alternative to researchers for the trypsinization of the cells prior to transfection. Electroporation is performed using electroporators, purpose-built appliances which creates an electromagnetic field in the cell suspension, which is pipetted into a glass or plastic cuvette foreseen with two aluminum electrodes on its sides. For the electroporation of bacterial cells, a suspension of about 50 µl is used which, prior to electroporation, is brought into contact with the plasmid of interest. The mixture is pipetted in the cuvette, the voltage and the volume to be applied to the probe are set, and the cuvette is placed in the electroporator. Immediately after electroporation, 1 ml of liquid medium is added to the Eppendorf cuvette /tube and is incubated at a temperature which allows the multiplication of bacteria, to recover the cells and to express **antibiotic resistance**, followed by seeding on plates with nutrient agar.

For electroporation to be successful, the plasmid must be "*free of all salt*" because solutions with high concentration of sodium chloride can cause an electrical discharge (known as arcing), a phenomenon which often reduces cell viability.

For a more detailed investigation of the process, special attention should be given to the output impedance of the porator device and to the input impedance of the cell suspension (e.g. the salt content). Because the process requires direct electrical contact between the electrodes and the suspension, and it does not occur between isolated electrodes, it is obvious that the process requires the existence of electrolytic effects due to mild currents and electric fields [4].

Electroporators are special laboratory equipment that give the possibility to achieve electroporation for multiple samples at the same time, with sets of electrodes specific for cell cultures, such as X2HT Gemini system manufactured by BTX-Harvard. Electroporators can be set to different operating parameters, allowing researchers to optimize the electric field strength depending on the cell type and by the presence or absence of the cell wall [5].

Electroporators have been used on a wide range of cells, including *Escherichia coli* (for transformation) and mammalian cells (neurons, astrocytes, neuroglial, lymphocytes, monocytes, fibroblasts, epithelial and

endothelial cells) from humans, mice, rats, and monkeys (for transfection [6].

Physical mechanisms of electroporation

Electroporation allows inserting into the cell DNA molecules, which do not have the ability to passively cross the cell membrane (the lipid hydrophobic bilayer). There are several ways in which changes can be caused to the electric field at the cell membrane level with very different mechanisms and effects. The electroporation advantage lies in the fact that lipid molecules are not altered in terms of chemical and the membrane has the ability to form a pore that will serve as a conductive path through the lipid bilayer [4].

Electroporation is a process with many stages and distinct phases [7]. Initially, a brief electrical pulse of 300-400 mV must be applied for less than 1 ms, which causes changes at membrane level related to the migration of ions in the surrounding solution. Once the critical stage is reached, a local rearrangement on the morphology of the lipids rapidly occurs and the resulting structure is considered to be a "pre-pore" because it is not a good conductor, but quickly leads to the formation of a conductive pore. Most evidence in support of this idea, come from the "flickering" process that occurs at the level of the pre-pores with the formation of hydrophobic regions of approximately 3Å [8].

If this theory is correct, the transition to the conductive pore may be explained by a rearrangement at the edge of the pores in which the lipid ends create a hydrophilic interface.

Finally, the conductive pores may suffer processes of reorganization, expansion or rupture, which can be influenced by various local factors such as the place and the moment in which the change occurs, the mechanical stress and the energy of the lipid bilayer existing at the time of trial [9].

MEDICAL APPLICATIONS

The optimization of electroporation is difficult to achieve, but it has been demonstrated that high voltage electroporation can almost irreversibly destroy the target cells, neighboring cells remaining unaffected. Thus, electroporation can form a new therapeutic approach in the treatment of cancer, cardiac disease and other pathological conditions which require the excision of tissue [10].

A recent technique called **non-thermal irreversible electroporation** has proved successful in the treatment of various types of tumors. This procedure involves the use of small electrodes (about 1 mm in diameter), placed inside or around the target tissue for applying short electrical pulses, repeating at a predetermined frequency and voltage, which increases resting transmembrane potential. As a result, when the applied electricity is higher than the threshold value for the target tissue, forming nanopores in the plasma membrane can become permanent and the cell repair mechanisms of "immortal" cancer cells become ineffective and the cells die.

The procedure is unique, due to the fact that compared to other tumor ablation techniques it does not affect the healthy tissue surrounding the tumor. In contrast, **reversible electroporation** occurs when electricity is applied through the electrodes below the threshold level of the electric field of the target tissue. Applying an electrical energy below the threshold allows the cells to rebuild the phospholipid bilayer and to resume normal operation. Reversible electroporation is carried out in the normal way for the administration of treatments which involve the insertion of some therapeutic molecules (drugs, genes, etc.) into cells. Since not all tissues have the same threshold of the electric field, precise calculations are needed before starting treatment, to ensure operating efficiency.

A major advantage of using non-thermal irreversible electroporation is that when done correctly, as a result of careful calculations made, it affects only the target tissue, while the proteins, the extracellular matrix and some critical structures such as blood vessels and nerves remain unaffected by this treatment. This allows a faster recovery and enables a faster replacement of the necrotic tumor cells with healthy cells. The first successful treatment of malignant skin tumors in mice was conducted in 2007 by a group of scientists (Garcia, Paulo A. & al.), who conducted the

complete ablation of the tumor in 12 of 13 mice. They accomplished this by sending 80 pulses of 100 µsec to 0.3 Hz with an amplitude of the electric field of 2500 V / cm, to treat skin tumors. Prior to performing the procedure, the therapist must carefully calculate exactly what should be done and to customize the therapy to the patient needs using imaging (CT and MRI) to create an accurate three-dimensional image of the tumor. This information is very important because one can estimate the tumor volume and decide on the type of therapy, place and angle of insertion of electrodes and applied voltage, sometimes even during the procedure, especially when electroporation is used to treat brain tumors.

The whole procedure is quick (about 5 minutes); the success rate is high and therefore very promising for future treatment extrapolation to humans. A disadvantage would be that the released electricity can stimulate muscle cells causing their contraction, which in some cases can have serious or even fatal consequences. Therefore, a muscle relaxant agent (curare-like compounds) should be used while performing the procedure. The researches conducted so far have been successful, but the taken action must be subject to the existence of a risk when general anesthesia is being used. A recent technique called **irreversible high-frequency electroporation**, uses electrodes to apply bipolar electrical pulses at a high frequency, as opposed to low frequency monopolar electrical pulses. This type of procedure also leads to tumor ablation with the difference that it does not produce muscle contraction and therefore does not need a muscle relaxant agent [11].

Researchers have had success in treating the test animals with multiple tumor types. Even if you are not prepared to routinely apply this technique in the treatment of tumors in humans, non-thermal irreversible electroporation is used in practice for treating cutaneous and subcutaneous tumors. There have also been attempts to treat human cancer of the prostate, lung, kidney and liver. There are still many things to be set in place, on how to damage the animals and especially the man. Kenneth Thompson et al. tested the safety of applying this technique on humans who had tumors in their lungs, kidneys and liver. The treatment was found effective in 71% of cases and, respectively, in 49 patients out of the 69 cases of patients treated was achieved complete ablation of the tumor [12]. The highest rate of success was recorded in liver tumors. The result of this study thus provides encouraging evidence related to the future use of this technology as an alternative method of treating cancer in humans.

Electroporation can also be used for the insertion of drugs or genes into the target cell through the use of short, intense electrical pulses to transiently permeabilize the cell membrane. This procedure is referred to as **electrochemotherapy** when the molecules to be transported are represented by a chemotherapeutic agent or **gene electrotransfer** when the molecule to be transported is the genetic material (DNA) [4].

PHARMACEUTICAL APPLICATIONS

Some pharmaceutical companies have performed surveys on the development of electroporation procedures with the scope of providing a viable alternative to cellular uptake of drugs. This approach is based on the fact that human cells do not allow the free entry of foreign matter through the cell membrane, which results in delay in the operational efficiency of a drug. Therefore, electroporation can be a real solution to allow a drug to act directly, quickly and with maximum efficiency on cells and tissue requiring treatment, resulting in obtaining a good therapeutic effect without major side effects.

Therefore, Inovio and OncoSec tested the effectiveness of this technology and managed in 2013 to achieve important steps in this direction. The results allowed them to also achieve substantial gains: the company Inovio Pharmaceuticals (NYSEMKT: INO had gains of almost 500% and the company OncoSec Medical had gains of 150%. Inovio performs studies using synthetic vaccines, while OncoSec is testing therapeutic agents that are already used during cancer treatment. The company Inovio Pharmaceuticals has developed about 12 "*electroporation pipes*", for the treatment of cancer and infectious diseases. Thus, the tests performed on the H1N1 influenza vaccine developed by the company's specialists have

highlighted the protective immune response comparable to conventional vaccines. Also, the SynCon DNA vaccine caused total protection against the infection with the viruses of Ebola and Marburg hemorrhagic fevers [13].

Preliminary studies for both types of vaccine, although in the initial stage, show that these exhibit a strong effect against the spread of these deadly viruses. On June 14th, 2014, Inovio announced that the H7N9 vaccine generated protective antibody HA with a percentage of 100% of the animals tested, and on July 10th, the results obtained from testing the HIV vaccine were published in the Journal of Infectious Diseases. Inovio found that the CELLECTRA system used for administering the Pennvax - B HIV vaccine improved the efficacy of the medicine in the first phase of testing. On July 18th, 2014 Inovio announced that electroporation significantly improves the outcomes of the DNA therapy in terms of stimulating the multiplication of blood elements. Administering with the CELLECTRA system the hTERT DNA vaccine used in cancer therapy and the anti-MERS vaccine has demonstrated that a good immune response has been generated during preclinical studies, improving the survival of patients [14]. OncoSec Medical conducted in preclinical testing centers studies for the treatment of metastatic melanoma, Merkel cell carcinoma (MCC) and cutaneous T cell lymphoma. In the study were included 15 patients with MCC, and so far the company's electroporation platform has been shown to be safe; in the last check, all patients showed an increase of IL-12 absorbing of at least 100 times, and in some cases up to 1,000 times.

This provides hope in regards to reducing the side effects that occur subsequent to immunotherapy. Also, studies have been conducted on a group of 25 melanoma patients. Were reported complete records for 21 of them and was observed that 38.1% had a favorable complete response lasting more than 6 months and in 61.1% of patients was observed a 30% regression of the tumor. All this led to an increase in the company's market share by 63%. It is considered that the near future will bring a series of novelties in this field, such as:

- the extension of the studies and obtaining positive results, for more than small populations of patients;

- reporting by Inovio of the data from the Phase 2 Study for VGX-3100 in the treatment of cervical dysplasia, which in Phase 1 has shown good immune response;

- reporting by OncoSec of the final data from the study of melanoma and for the Merkel cell carcinoma;

- presenting plans to launch a study for treating cutaneous lymphoma with T-cells, but also for the initiation of a new research for the treatment of solid tumors;

- OncoSec presenting the company's plans that expressly foresees combining the electroporation device with the anti-CTLA4, the anti-PD-1 and the anti-PD-L1. As is well known, the anti-PD-1s, particularly, was among the most frequently discussed cancer therapies last year, after Merck and Bristol-Myers have reported incredible data to the American Cancer Society (ASCO). All these led to the idea that electroporation increases the assimilation of some therapeutic agents and reduces side effects, thus making them even more efficient. Therefore, careful monitoring of therapy with anti-PD-1s and the OncoSec plans to expand the researches for the ImmunoPulse platform, finally pursue further researching for this therapeutic agent [15].

CONCLUSIONS

Therapeutic electroporation is a new treatment technology at cellular level. The method is based on the electroporation of the cell membrane which becomes selectively permeable to the therapeutic agents used. *In vitro* and *in silico* experiments are promising and have allowed the insertion of chemical or biological molecules in the target cells. The *in vivo* experiments on laboratory animals have given good therapeutic results, therefore, in the future may be applied to humans. Therapeutic electroporation can become in the near future, an important treatment for oncology, cardiac, infectious diseases etc., envisioning the predominant biological perspective of therapeutics in modern medicine.

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