

Biomedical application of electroporation: electrochemotherapy and electrogene therapy

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Abstract — Electroporation refers to exposure of cells to external electric field that results in transiently or permanently increased permeability of cell membranes. Cancer treatment, where local application of electroporation to tumor nodules is combined with chemotherapeutic drugs bleomycin or cisplatin is called electrochemotherapy. The antitumor effectiveness of electrochemotherapy is primarily based on direct killing of tumor cells due to the increased chemotherapeutic drug uptake, but other mechanisms, such as vascular disrupting action was also demonstrated. Our group is involved in elucidating the underlying mechanism of vascular disrupting action by studying the changes in cytoskeletal proteins after electrochemotherapy in endothelial cells by immunocytochemistry. In addition, metastatic potential and global changes in gene expression of melanoma cells that survived electrochemotherapy were also studied, demonstrating that metastatic potential of cells is not changed and that a very low percentage of genes are down or up regulated after electrochemotherapy, which further supports its safe use in the clinical setting. Another application of electroporation is delivery of plasmid DNA into the cells for gene therapy. Our group is involved in preparation of plasmid DNA with tissue specific promoters and without genes for antibiotic resistance, thus enabling safe use of gene therapy in line with USA Food and Drug Administration (FDA) and European medicines Agency (EMA) recommendations.

Index Terms — electrochemotherapy, electrogene therapy, electroporation, preparation of plasmids without genes for antibiotic resistance.

1 INTRODUCTION

Due to the physical nature of the method, all types of living cells (prokaryotic and eukaryotic) can be efficiently electroporated. Currently electroporation is used in food and biomass processing and as a cancer treatment. In food processing, the aim is to kill the microorganisms, while preserving the color, taste and the level of antioxidant in the processed food. Electroporation was successfully used for extraction of intracellular components from plant and has shown a potential in pretreatment of sludge and other substrates leading to increase in biogas production. Other applications, such as water treatment, extraction of oil from algae, sugar extraction from sugar beet are also being investigating. In cancer treatment, electroporation is currently used in combination with the chemotherapeutic drugs bleomycin and cisplatin for treatment of cutaneous and subcutaneous metastases of various histological types of tumors. Electroporation can also be used for introduction of genetic material - plasmid DNA - into the cells and therefore used for DNA vaccination and gene therapy of various diseases.

2 ELECTROCHEMOTHERAPY – EFFECTS ON ENDOTHELIAL CELLS

Electrochemotherapy consists of chemotherapy followed by local application of electric pulses to the tumor to increase drug delivery into cells. Drug uptake can be increased by electroporation only for those drugs whose transport through the plasma membrane is normally impeded. Among many drugs which have been tested so far, only bleomycin and cisplatin have transitioned from preclinical testing to clinical trials. In vitro studies demonstrated a several-fold increase in their cytotoxicity after electroporation of cells. In vivo, electroporation of tumors after local or systemic administration of either of the drugs, i.e. electrochemotherapy, proved to be an effective antitumor treatment. Several clinical studies were performed demonstrating that electrochemotherapy is effective in local tumor control of cutaneous and subcutaneous tumor nodules of different histology. So far, predominantly melanoma skin metastases have been treated, with ~70% long-lasting complete responses of the treated nodules [1]. Electrochemotherapy is also used in veterinary oncology, where it is used also for treatment of primary tumors and with similar results as in human medicine [2].

Besides membrane electroporation, which facilitates drug transport and its accumulation in the cell, other mechanisms that are involved in antitumor effectiveness of electrochemotherapy, have been described, potentiation of immune response, blood-flow modifying effect and vascular disrupting action. The application of electric pulses to tissues induces a transient but reversible reduction in blood flow. In vitro studies have shown that application of electric pulses to a monolayer of endothelial cells results in a profound disruption of microfilament and microtubule cytoskeletal networks, loss of contractility, and loss of cadherin-formed cell-to-cell junctions in the vascular endothelial lining immediately after electroporation, which recovered within 60 minutes after electroporation, without any significant loss of cell viability. The cytoskeletal effects of electroporation were paralleled by a rapid increase

in endothelial monolayer permeability, giving an indication of putative mechanisms responsible for the observed increase in permeability and cessation of blood flow in vivo[3]. When electroporation is combined with chemotherapeutic drugs the effects on vasculature are even more pronounced and resulted in electrochemotherapy with bleomycin in complete abrogation of blood flow in vivo. Currently, we are exploring the underlying mechanisms of this effect at the cellular level, by studying the effects of electrochemotherapy on adherent microvascular endothelial cells. We found that nuclear morphology changes as well as changes in microtubules and actin filaments network are more profound and long lasting after electrochemotherapy with bleomycin compared to changes induced by electroporation or bleomycin alone (Fig. 1). In addition, endothelial monolayer intactness was severely compromised after electrochemotherapy.

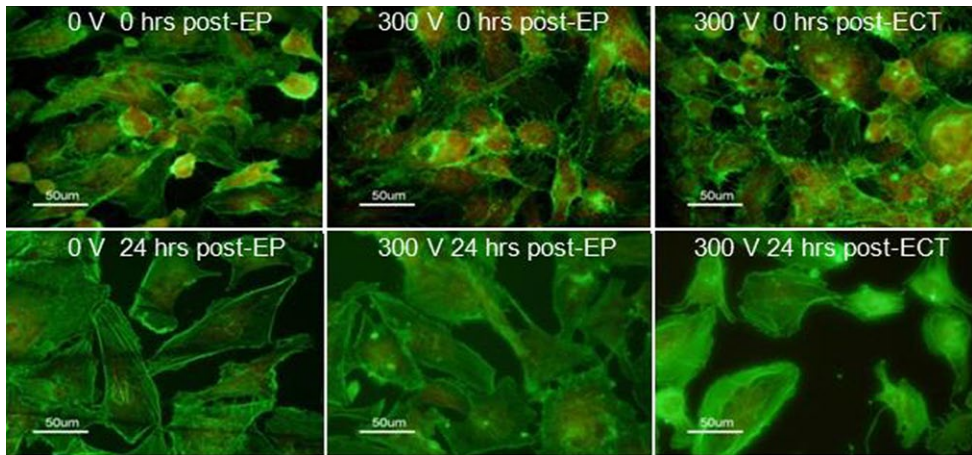


Fig. 1. Monolayers of Human Microvascular Endothelial Cells (HMEC-1) are differently affected by electroporation (EP) and electrochemotherapy (ECT). Monolayers stained for F-actin with FITC conjugated phalloidin. Note the honeycomblike appearance at 0 hrs for both 300V EP and ECT. Recovery is present at 24 hrs post-treatment, while in the presence of 300 nM bleomycin (ECT) cells start to die.

3 ELECTROCHEMOTHERAPY – EFFECTS ON METASTATIC POTENTIAL OF MELANOMA CELLS

Another topic of our research in the field of electrochemotherapy was to address the clinical relevant question i.e. if metastatic potential and global gene expression of melanoma cells that survive electrochemotherapy is changed. Namely, it is known that metastatic progression is a multi-step process that requires acquisition of many specific cell properties, each fulfilling a unique function in the metastatic cascade for successful establishment of metastases. Alterations in tumor cells and their microenvironment induced by therapy can play an important role in metastasis induction. We studied on viable cells 48 hours after electrochemotherapy

their ability to migrate and invade through Matrigel coated porous membrane. In addition, microarray analysis was used to detect changes in gene expression after electrochemotherapy. Cell migration and invasion were not changed in melanoma cells surviving electrochemotherapy. In addition, only a low number of tumorigenesis related genes was differentially expressed after electrochemotherapy (Fig. 2), demonstrating that the metastatic potential of melanoma cells was not affected by electrochemotherapy and confirming its safety in the clinics [4], [5].

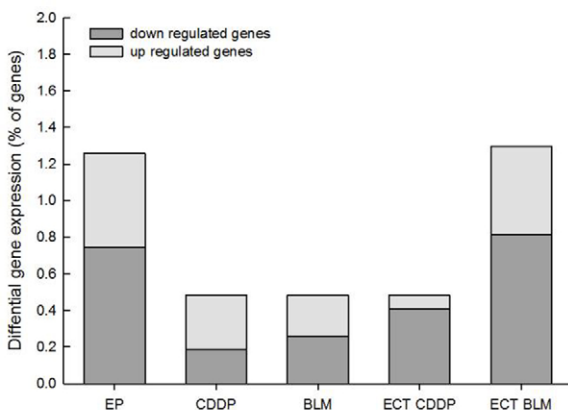


Fig. 2. Differentially expressed tumorigenesis related genes after electrochemotherapy. From 0,5% to 1,3% of all evaluated genes were differentially expressed after exposure to electroporation (EP), cisplatin (CDDP), bleomycin (BLM) or electrochemotherapy (ECT) with CDDP or BLM. Bars represent percentage of down regulated or up regulated genes of the total number of tested genes.

4 ELECTROGENE THERAPY – PREPARATION OF PLASMID DNA WITHOUT ANTIBIOTIC RESISTANCE GENE

Another application of electroporation is delivery of genetic material i.e. plasmid DNA into the cells. Due to the concerns connected to viral vectors, non-viral approaches for transfection are becoming very attractive [6]. One of the goals of researchers dealing with gene therapy or DNA vaccination is to develop safe and efficient systems for gene transfer into the target cells. Namely, this topic is still one the major hurdles in the progress of different approaches in gene therapy and is crucial factor for success of the therapy. Other factors that should be considered for development of gene therapy are the choice of appropriate therapeutic gene, regulation of its expression and administration route. Our group at the College of Health Care Izola in collaboration with Institute of Oncology Ljubljana, National Institute of Biology and University of Ljubljana Biotechnical faculty is planning to address two of these factors for the use in cancer gene therapy: safety of plasmid DNA by preparation of plasmid DNA without marker for antibiotic resistance and regulation of therapeutic gene expression by linking the therapeutic gene to cellular promoters to achieve either stress induced (p21 and radiation) or physiological regulation (tissues specific

promoters for endothelial and muscle cells and fibroblasts) of expression. We are planning to prepare plasmids encoding reporter or therapeutic genes under the control of tissue specific or inducible promoters using standard molecular biology techniques and plasmids without gene for antibiotic resistance according to the instructions of the producer of ORT® plasmid. The results of this on-going project, if successful, may have impact on design and execution of gene therapy trials.

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