Therapeutic Effects of in vivo Electroporation: Facilitating Drug and Gene Delivery but not Only…

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Abstract

The membrane of cultured cells exposed *in vitro* to electric pulses of adequate field amplitude and pulse duration becomes permeable to otherwise "non permeant" molecules [1]. Indeed the delivery of these electric pulses results in changes in the structure of the cell membrane. This phenomenon, which affects the membranes of all live cells, is known as cell electroporation or cell electropermeabilization. It allows the introduction into the cells in culture of various types of molecules such as drugs, dyes, peptides, oligonucleotides, proteins, and large nucleic acids such as plasmids, mRNAs, minicircles, etc…

In vivo as well, adequate electric pulses facilitate the uptake of molecules by the cells of a large number of tissues (tumors, muscle, skin, liver, etc. [2]). Nowadays, two applications are well developed:

- The electrotransfer of cytotoxic drugs into tumor cells: the combination of electroporating electric pulses and drugs such as bleomycin, termed electrochemotherapy, results in very clear beneficial effects for the patients, with the complete destruction of about 80% of the treated nodules after one single treatment, with almost no side effect. After several years of development (technological improvements of the pulse generators, preclinical trials as well as clinical trials, preparation of Standard Operating Procedures [3, 4]), electrochemotherapy is now used in more than 140 cancer centres in Europe to treat skin tumors and cutaneous and subcutaneous metastasis of any origin. Electrochemotherapy is already reimbursed in 7 EU countries. The electrochemotherapy Users 2nd International Meeting, hold in 2013 in Italy, gathered 350 physicians using this antitumor approach, and more than 3 000 patients were treated in the EU in 2013.
- The electrotransfer of genes into various kinds of target cells (tumor cells, muscle cells, skin cells, hepatocytes, …): described at the end of the last century, the mechanism of DNA electrotranfer *in vivo*, which includes cells permeabilization and DNA electrophoresis inside the tissue, was analysed in several papers from 2000 to 2008 [5]. The first molecular description of the crossing of a lipid bilayer by a siRNA under ultra-short (10 nanoseconds) and very intense (6 MV/m) electric pulses, by means of numerical simulations which were experimentally validated, was reported recently [6]. Several clinical trials are presently exploring the feasibility in humans of this method of gene transfer for non-viral gene therapy and DNA vaccination.

Actually, *in vivo*, the electric pulses have consequences other than the increase of exogenous molecules uptake by the target cells as described here above.

- The occurrence of a vascular lock has been extensively described. It affects all the tissues exposed to the electric pulses, but its intensity is very different on normal versus tumor tissues. In normal tissues, under the electric conditions corresponding to the applications mentioned here above, the vascular lock does not last for more than a couple of minutes [7]. On the contrary, in tumors, the blood flow recovers only after several hours [8]. This vascular lock, unpredictable from the *in vitro* experiments, has very positive therapeutic consequences. Indeed, it results in the absence of bleeding after the application of penetrating electrodes (like bundles of needles) rendering the electrochemotherapy procedures very easy for the professionals and

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very bearable by the patients. Moreover, in some cases (intratumor injection of drugs like the cisplatin [9] or just of a concentrated calcium solution [10]), it blocks the active agent in the tumor volume allowing a prolonged (and thus more efficient) antitumor effect.

- The permeabilization of the membranes also allows the release of intracellular substances in the extracellular space. In the recent years, the immunogenic aspects of the cell death have been extensively revisited. It has been shown that in immunogenic cell death types, one of the DAMPs (Danger-Associated Molecular Patterns) is the ATP. Indeed, the ATP that can be released by the dying cell is a strong immunogenic signal because ATP plays a role of chemoattractant ("find me" signal) for the dendritic cells and their precursors [11] and favors their differentiation and maturation into dendritic cells with antigen-presenting capacity [12]. Thus it plays a pivotal role in raising an effective immune response. We have recently demonstrated that there is a very large release of ATP from the transiently electroporated cells that could add a new and important role for electroporation-based DNA vaccination [13].

Therefore, *in vivo*, cells electroporation is not merely a way to increase the uptake of drugs or nucleic acids by the cells. Electroporation-dependent vascular lock is certainly beneficial in electrochemotherapy protocols, and electroporation could be also a crucial adjuvant in vaccination protocols.

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