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TRANSFER OF MOLECULES INTO CELLS AND TISSUES BY ELECTRIC FIELD: WHAT DO WE KNOW?

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The focus of this lecture is to describe the basics of electrotransfer, its main clinical achievements and the different aspects of what is known of the mechanism of membrane permeabilization and associated gene transfer. Our main and new data will be presented and the actual limits of the DNA delivery into cells will be discussed.

Cell membranes can be transiently permeabilized under application of electric pulses. This treatment allows hydrophilic therapeutic molecules, such as anticancer drugs and DNA, to enter into cells and tissues. This process, called electropermeabilization or electroporation, has been rapidly developed over the last decade to deliver genes to tissues and organs, but there is a general agreement that very little is known about what is really occurring during membrane electropermeabilization. It is well accepted that the entry of small molecules, such as anticancer drugs, occurs mostly through simple diffusion after the pulse while the entry of macromolecules, such as DNA, occurs through a multistep mechanism involving the electrophoretically driven interaction of the DNA molecule with the destabilized membrane during the pulse and then its passage across the membrane. Therefore, successful DNA electrotransfer into cells depends not only on cell permeabilization but also on the way plasmid DNA interacts with the cells.

Until now, the dynamics of this process has been poorly understood because direct observations have been limited to time scales that exceed several seconds. We studied experimentally the transport of two types of molecules into cells (plasmid DNA, propidium iodide) which are relevant for gene therapy and chemotherapy with a temporal resolution of 2 ms allowing the visualization of the DNA/membrane interaction process during pulse application. DNA molecules interact with the membrane during the application of the pulse. At the beginning of the pulse application plasmid complexes or aggregates appear at sites on the cell membrane. The formation of plasmid complexes at fixed sites suggests that membrane domains may be responsible for DNA uptake and their lack of mobility could be due to their interaction with the actin cytoskeleton. New lines of research are now necessary to characterize the membranes domains observed during electrotransfer and the involvement of cytoskeleton. For that purpose, we use giant unilamellar vesicles to study the effect of permeabilizing electric fields in simple membrane models. Experiments showed a decrease in vesicle radius which is interpreted as being due to lipid loss during the permeabilization process. Three possible mechanisms responsible for lipid loss were directly observed and will be presented: pore formation, vesicle formation and tubule formation.

In the last part of the lecture, our new data for the understanding of the DNA electrotransfer process in tissues, obtained on multicellular tumor spheroids as an *ex vivo* model of tumor, will be presented. We used confocal microscopy to visualize the repartition of permeabilized cells in spheroids submitted to electric pulses. Our results reveal that if small molecules can be efficiently transferred into cells, including the ones present inside the spheroids, gene expression is limited to the external layers of cells. A key challenge for electro-mediated gene therapy is to pinpoint the rate limiting steps in this complex process and to find strategies to overcome these obstacles.

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