

GENE THERAPY BY *IN VIVO* ELECTRO DNA- TRANSFECTION

Bertil R.R. Persson

Executive Summary

The physical phenomenon of electroporation has been successfully exploited *in vitro* for the delivery of genes, drugs, and other molecules with increasing frequency over the past two decades. This type of electrically mediated delivery has been translated into an *in vivo* setting in more recent years with a focus on therapeutic molecules. One promising area is the delivery of genes as a therapy.

Advances in molecular medicine have produced a very large amount of information about genes that translate to therapeutic molecules when expressed in living cells. Current standard methods for transferring genes utilize viruses to deliver DNA into cells. These viral methods have not yielded optimal results in most cases. Therefore there is an increasing interest in non-viral methods for gene delivery *in vivo*. Electrically mediated gene delivery is such an attractive alternative because of the site-specific nature of delivery as well as the universal applicability of electroporation.

The studies reviewed below successfully delivered genes coding for luciferase, CAT, β -galactosidase, MCP-I, SV40 large T-antigen, and the E1A region of the adenovirus-2 to different tissues in animal models. Results from these studies indicate that *in vivo* electrically mediated gene delivery can be achieved. Although this technology is in its infancy, the results are highly encouraging for potentially developing gene-based therapies for the treatment of human disease.

There are several advantages to using *in vivo* electroporation to deliver DNA. First expression was noted only in cells that were electrically treated in the presence of plasmid DNA. This has been accomplished by pulsing a volume of tissue after perfusion with plasmid; expression has also been achieved by administering the DNA after electro pulsing. Thus, electroporation is a means of targeting expression to the cells of interest. Second, the use of plasmid DNA simplifies gene preparation, relative to using viruses, and also places few restrictions on the size of the gene for delivery. This, in turn, makes it much easier to test existing as well as new genes. An additional degree of specificity can be achieved by using tissue specific promoters on the delivered plasmid. Finally, the physical nature of electroporation makes this type of delivery applicable to all tissue types. This is demonstrated in part by the successful delivery of DNA to liver, brain tumours, coetaneous tumours, testis, and skin.

One interesting aspect of *in vivo* electro gene delivery is the differences in protocols that are used by different researchers. Some studies use needle electrodes and others utilize parallel plate electrodes. Pulses with durations ranging from 100 μ s to 50 ms have been used successfully. Both rectangular and exponentially decaying pulses have produced excellent results at field strengths ranging from about 70 to 1500 V/cm. Stimulation of muscles with low voltage alternating current has also been used though with some damage observed in the tissue.

Although optimal parameters probably exist for every particular delivery situation, a range of parameters has been used to successfully deliver genes in each presented study. This variation in the parameters used to deliver genes indicates that there is a great need for a method to verify the degree of electroporation achieved in tissue by applying various electric pulses. It is also important to control that the cells in the tissue is not killed by exposure to too high electric energy.

The future of gene delivery by electroporation is quite promising. The studies reviewed above provide a foundation that can be used for further development. Several different aspects of this technology must be examined in order to start the maturation process from animal studies to the clinic. Quantitative studies with controlled electropulsing aimed at optimising delivery and transformation efficiencies are needed to begin this maturation process. This will undoubtedly result in better understanding of the physical factors such as plasmid administration route and electrical treatment parameters that play an important role in electrically mediated gene delivery. Although reporter genes will probably be instrumental for optimisation purposes, the transformation efficiencies required to realize a therapeutic benefit are dependent on the disease and functional gene of interest.

Therefore, studies that clearly demonstrate efficacy using genes that code for functional molecules will also augment translation of this novel gene delivery technology toward clinical applications.

By applying controlled *in vivo* electro gene therapy to combinations of suppresser genes such as p53 and Stat3 β with cytokines such as interleukines (IL2, IL12) and interferon- γ a very efficient gene therapy modality for treatment of both local and generalized cancer could be developed.

Applications of controlled electro gene therapy for other diseases could be developed from the results of the rapidly emerging molecular medicine and there are already several interesting suggestions underway.

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