Opening doors for exogenous agents

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The efficient delivery of therapeutic agents *in vivo* is a *sine qua non* of successful medical treatment. Often, though, practice falls short of the ideal. Gene therapy, in particular, has been shackled by the poor efficiency of viral and liposomal vectors¹. In this issue, Rols et al.² examine the ability of an alternative approach (electroporation) to potentiate delivery of a gene and/or the protein it encodes to murine melanoma. While wide application of electroporation may be limited by tissue accessibility, the enhanced efficiency of gene and protein transfer offers some significant advantages.

Several systems are currently under development for introducing a therapeutic gene/protein into a specific target tissue. Retroviruses have been used *ex vivo* to transfer genetic information to cells, which can then be introduced back into the body. Adenoviruses and DNA-liposomes have also been used to transfer genes directly *in vivo*. In addition, physical means of gene delivery have been investigated, including ballistic techniques that inject DNA directly³, together with stress-wave⁴ and electrical fields that induce cellular and tissue uptake of genetic material.

Over the past 40 years, it has been shown that the application of an electric field is a very efficient means to permeabilize the cell membrane. The site of action is the lipid portion of the plasma membrane, which plays a role in amplifying the applied electric field. Experimental and theoretical investigations carried out by several groups in the 1970s and early 1980s showed that the electrically induced increase of membrane permeability is a result of pore formation—a process called electroporation⁵.

Interest has grown rapidly in electroporation because of its potential applications in cell biology, biotechnology, and medicine. To date, the most frequent applications of this technique have included loading cells with molecules for drug delivery or research purposes⁶, transporting molecules across tissue for therapeutic purposes (e.g., transdermal drug delivery^{7,8}), and the electroinjection of macromolecules (DNA, plasmids, etc.²).

Although gene therapy has been widely studied *in vitro* and with animal models, there is little clinical evidence of its efficacy in humans. One possible explanation is the poor efficiency of gene transfer *in vivo*. For example, although liposomal delivery of a gene encoding histocompatibility complex protein (HLA-B7) to melanoma tumors of HLA-B7-negative patients resulted in tumor specific expression of plasmid DNA and HLA-B7, immunofluorescent staining revealed that only approximately 1% of the cells contained the protein⁹. Moreover, only one of five subjects showed tumor regression. Thus, both uptake and clinical efficacy were poor.

Electroporation holds the promise of more efficient gene transfer. To date, most

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applications of the technique involve cell transfection: Since the pioneering studies of Neumann and his colleagues on L-cells¹⁰, lymphocytes, neuronal cells, endocrine cells, and hematopoietic stem cells have all been shown to be amenable to transfection. Electroporation has also been applied to unicellular organisms, plant protoplasts, and bacteria. The advantage of cellular electroporation is the high frequency of permanent transfectants and high efficiency of transient gene expression. In many cases, electroporation is the only method that works.

As with viral and liposomal systems, there are two strategies in electroporation-induced gene delivery: The first is *in vitro* transfection of cells taken out of an organism and subsequently reintroduced¹¹; the second is direct *in vivo* delivery^{12,13}. The direct method has been used to transform skin cells in mice¹² and to transfer genes to murine liver¹³. In addition, impressive human clinical results have been obtained by treating superficial melanomas with direct application of electroporation to the tumor following the intravenous administration of bleomycin^{13,14}.

In the present study, Rols et al. evaluate electroporation as a means of direct transfer of both a gene and its protein *in vivo*. They directly injected the marker protein β -galactosidase, or a plasmid expressing this protein, into murine melanomas, followed by the transcutaneous application of electropora-

tion to the tumor. After 72 hours, tissue analysis showed that the protein and gene encoding the protein were present in 20% and 4% of the cells, respectively.

Although it is not possible to relate these numbers to human clinical efficacy, the success of localized bleomycin treatment via electroporation^{13,14} suggests that the technique holds promise for the treatment of superficial tumors. Moreover, the results suggest that the direct transfer of a therapeutic protein via electroporation may result in greater clinical efficacy than transfer of a gene encoding for the same protein.

This aside, gene and protein transfer to tumors via electroporation is not likely to replace viral- or liposomal-based technologies any time soon. All these methods appear to have similar *in vivo* efficiencies of a few percent. However, electroporation has the key limitation of being primarily limited to superficial tumors accessible to direct application of an electric field—at least in the absence of surgical intervention. For less accessible and/or widely disseminated tumors, viral-based and/or liposomal-based methods have a clear advantage. Moreover, recent studies with cationic liposomes, demonstrating 1000-fold greater expression in murine lung than in the liver, suggest that targeted, tissue-specific uptake is possible with these vectors¹⁵.

Rols et al.² have effectively demonstrated gene and protein transfer *in vivo* using electroporation. Given the earlier clinical success with electroporation-induced uptake of bleomycin in melanomas^{13,14}, it will be interesting to see the clinical impact of this technique for the treatment of skin cancer.

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