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Plasmid IL-12 electroporation in melanoma

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Abbreviations: IL-12, interleukin-12; pIL-12, plasmid interleukin-12

Intratumoral gene electroporation uses electric charges to facilitate entry of plasmid DNA into cells in a reproducible and highly efficient manner, especially to accessible sites such as cutaneous and subcutaneous melanomas. Effective for locally treated disease, electroporation of plasmid DNA encoding interleukin-12 can also induce responses in untreated distant disease, suggesting that adaptive immune responses are being elicited that can target melanoma-associated antigens. In vivo electroporation with immunomodulatory cytokine DNA is a promising approach that can trigger systemic anti-tumor immune responses without the systemic toxicity associated with intravenous cytokine delivery and potentially offer complete long-term tumor regression.

Introduction

Gene therapy seeks to add, repair or replace genes by introducing wild-type or functional genes into specific tissues. The promise of therapy, however, has not yet been fully realized due to the difficulties in targeting abnormal tissues, transporting genes efficiently across cell membranes and achieving consistent expression. Carrier viruses are often employed for gene transfers but are hampered by their intrinsic immunogenicity that can reduce their efficiency or provoke systemic immune-related toxicities involving cytokine release, and for retroviral vectors, the potential for integration of viral components into the host genome. Plasmid DNA-based methods are less likely to trigger host immune responses, but they have a much lower efficiency in transmembrane delivery, intrinsically have no targeting properties to tumors or non-transformed host cells, and are thus handicapped by inconsistent and inadequate transgene expression levels.

In vivo electroporation utilizes high-intensity electric fields that transiently increase plasma membrane permeability to facilitate delivery of nucleotides, cytotoxic agents or other macromolecules into the cell. Since the first demonstration of electroporation of DNA into cells by Neuman and colleagues in 1982[1] many advances in technique and understanding of electroporative gene transfer have occurred in the last 3 decades.[2,35] This technique was adapted to deliver DNA into liver,[3] skin,[4] heart muscle,[5] skeletal muscle[6-8] and brain tissue.[9]

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Electroporation can be used safely in humans and many trials using electric fields to deliver chemotherapy have been done over the past 20 years.[10-14]

Electroporation In-Vivo

Several parameters affect transfer efficiency in in-vivo electroporation.[15] These include electric field strength, pulse width, shape, number and frequency and electrode design, number, shape and type and the vehicle used and target tissue.[16] Basically, a series of electric pulses are used to drive macromolecules across the plasma membrane which is the greatest barrier to entry.[3] There is a large literature that describes the optimization of parameters for appropriate transgene expression. An important consideration is the correct mating of target tissue and desired expression to electrode and electric properties. Typically, high field strength is combined with short pulse widths and low field strength with long pulse width so tissue integrity is maintained. Electrode design (penetrating or non penetrating) is also very important. For example in muscle, Mir et al.[6] showed that 200 V/cm resulted in optimal expression with non-penetrating electrodes. With penetrating electrodes in the same tissue 100 V/cm resulted in optimal expression.[17] Tissue also makes a big difference; in muscle low field strength and long pulses result in higher expression[8] while in hepatocellular carcinoma, short high strength pulses result in higher transfer efficiency.[18] An alternative approach involves the use of constant current which appears to be effective in muscle[19] and may avoid tissue damage. Since voltage equals current X resistance, and since tissue impedance decreases during electroporation, using constant voltage could lead to increased current which in turn could cause tissue damage. However, tissue damage can occur with constant current as well.[20]

Despite extensive experimental data and theoretical analysis it is still unclear exactly how electroporation works. It is thought that electrotransfer involves both electroporation (across membranes) and electrophoresis where molecules such as DNA are ferried across permeabilized membranes. Therefore some delivery methods use short high intensity electroporative pulses for electroporation with long low intensity pulses for electrophoresis.[21]

Multiple electrode designs are currently in use from commercial sources and custom made ones can be fabricated easily. At least 6 current electrode designs are in use; suppliers include IGEA (Capri, Italy), BTX Molecular Delivery Systems (Holliston, MN), Cyto Pulse Sciences (San Diego, CA), NEPA

Gene (Ichikawa, Japan), Tokiwa Science (Fukuoka, Japan), Oncosec Inc., (San Diego, CA) and Ichor Medical System (San Diego, CA).

Intratumoral Gene Therapy with Immune Modulators

Many studies in experimental tumor models have shown regression of tumors. These studies have used immune modulators, cell cycle regulators, suicide genes, antiangiogenic genes and fusion proteins incorporating toxins.[22] Many of these constructs have shown significant tumor regression. However, complete long-term tumor regression after plasmid electroporation has been demonstrated in a relatively small number of studies; these include delivery of IL-12 in several tumor types[18,23] and interferon in squamous cell cancer.[24] IL-12 appears to be especially effective in combination with Herpes Simplex Virus thymidine kinase,[25] or with Bacille Calmette Guerin (BCG)[26] for bladder cancer. Another successful strategy has been to use B7.1 with GM-CSF; indeed this appears very effective in a fibrosarcoma model.[27] In this model, both local and distant tumors responded.

One interesting phenomenon observed with intratumoral electroporation is the lack of serum spillover of cytokine as compared with intramuscular electroporation with the same cytokine.[17] Cytokines, when systemically administered, are significantly toxic; for instance, recombinant IL-12 (rIL-12) in patients resulted in multiple toxicities including temporary immune suppression and death.[28,29] Intratumoral delivery of IL-12 was associated with a potent "vaccine" effect in that treated mice appeared to be resistant to tail vein inoculation of B16 melanoma cells.[30] This distant effect was not seen in nude mice.

Electroporation of Plasmid IL-12

One of the notable advances was the demonstration that intratumoral delivery of cytokine DNA could robustly stimulate the immune system in immune-competent mice and eliminate tumors in syngeneic tumor models such as the B16 melanoma model.[22] The most effective cytokine in these experiments was IL-12. IL-12 is capable of promoting the development of T-helper 1 response, inducing the production of IFN and increasing the proliferation and cytotoxicity of NK an d T cells.[31-33] Studies have shown that IL-12 also acts to upregulate the expression of HLA class I and II and ICAM-1 on human melanoma cells, which may increase their immunogenicity.[34] Other cytokines such as interferon, GM-CSF or IL-2 (11) were also effective but with lesser complete responses than IL-12 in the B16 melanoma model. Using this model, Heller and coworkers showed that mice in whom intra-tumoral gene delivery resulted in tumor elimination also were protected against tumor re-introduction via tail vein[23] suggesting that immunological memory responses were formed. Other NK cell activating cytokines including IL-15 are active also when delivered in this way.[23]

Based on these results, we performed a detailed toxicological study of IL-12 plasmid electroporation in mice carrying subcutaneous B16 tumors.[22] We found that mice developed no significant toxicity associated with the electrically mediated delivery of a plasmid encoding IL-12. The only abnormality specific to animals receiving both plasmid and electroporation was inflammation associated with the kidney at the late time point. In fact electroporated mice had lesser histologic abnormalities in 12 organs examined and were in the best health compared with control mice that were sham electroporated or electroporated with vector only.

Given the extensive animal data reported by many investigators, we undertook a phase I clinical trial in patients with advanced melanoma. This trial closely paralleled mouse IL-12 experiments in the schedule and in electroporation parameters. Patients were eligible if they had metastatic melanoma with at least two subcutaneous or cutaneous lesions accessible for electroporation. Patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, adequate organ function, and normal blood and laboratory tests. The plasmid used was a human IL-12 construct, UMVC3-hIL-12-NGVL, which was manufactured for us by the City of Hope vector laboratory. The plasmid was injected with a 25-gauge needle into the tumor and immediately following the injection, a sterile six needle electrode array was inserted into the tumor and six pulses at field strength of 1,300 V/cm and pulse duration of 100 μs were delivered using a Medpulser DNA EPT System Generator (Inovio Biomedical Inc., San Diego, CA). Treatments were performed on days 1, 5 and 8 and no repetition of treatments was allowed.

Plasmid doses were tested at concentrations of 0.1, 0.25, 0.5, 1.0 and 1.6 mg/mL. For cohorts 1 through 5, the plasmid injection volume was determined by calculating the estimated tumor volume divided by 4. Patients in cohorts 6 and 7 were treated with a total dose of 3.8 or 5.8 mg dispensed among up to four tumors selected for electroporation on a treatment day. Tumor responses were assessed by a modification of Response Evaluation Criteria in Solid Tumors (RECIST).[36] Patients were considered to have a complete response (CR) if distant (non-electroporated) sites of disease at the start of treatment and all sites of disease treated or untreated regressed completely.

Seven dose cohorts with a total of 24 patients were enrolled and treated in this study between December 2004 and February 2007. Patient characteristics are reported in **Table 1**.[37] Most patients in this trial had advanced metastatic melanoma and had progressed despite multiple therapies including surgery, radiation, immunotherapy and other treatments. Nineteen of these patients had distant lesions that were not treated. All patients received a single cycle of treatment.

Clinical Responses with pIL-12 Electroporation

Local control with pIL-12 electroporation was established in the majority of treated lesions. Most electroporated lesions (76%) demonstrated tumor necrosis (> 20%) by day 11 at the time of follow-up biopsy or excision after the last injection. Of the 19 patients who had untreated distant disease, 7 patients had established systemic responses that prevented further tumor progression over intervals ranging from 4 to 20 mo. Another 3 patients, who had distant disease with disseminated progressive cutaneous lesions, had evidence of complete responses. Of these patients,

Table 1. Clinical responses to plasmid IL-12 electroporation

Cohort	Patient	AJCC stage	IL-12 plasmid concentration (mg/mL)	Number of electroporated sites	Overall response	Duration (months)
1	1	IVA	0.1	3	PD	
	2	IVC	0.1	4	PD	
	3	IVC	0.1	2	PD	
2	4	IVC	0.25	4	PD	
	5	IVB	0.25	3	SD	4
	6	IVA	0.25	2	PD	
3	7	IIIC	0.5	4	CR*	> 18
	8	IIIC	0.5	4	PD	
	9	IVA	0.5	4	CR	> 20
4	10	IVA	1	3	SD	> 20
	11	IVC	1	3	PD	
	12	IIIC	1	3	PD	
5	13	IIIC	1.6	4	PD	
	14	IIIC	1.6	4	CR	> 16
	15	IIIC	1.6	4	PD	
6	16	IIIC	1.6	4	SD	4
	17	IIIB	1.6	3	SD	> 4
	18	IIIC	1.6	4	PD	
7	19	IIIC	1.6	2	PD	
	20	IIIB	1.6	4	SD	4
	21	IVA	1.6	4	SD	4
	22	IVA	1.6	2	PD	
	23	IVA	1.6	4	SD	> 6
	24	IVC	1.6	3	PD	

Abbreviations: AJCC, American Joint Committee on Cancer; CR, complete response; IL-12, interleukin-12; PD, progressive disease; SD, stable disease. Patient 7 was treated with dacarbazine after plasmid IL-12 treatment before developing a complete response.

2 patients did not receive any subsequent systemic therapy, while 1 patient had received dacarbazine following pIL-12 electroporation. All of these patients had only four lesions treated. Tumor regression was not evident until approximately 6 mo after electroporation and continued over a period of 18–20 mo.

While this was primarily a toxicity and dose finding study, an important readout for effective gene transfer was determining the level of IL-12 protein expression following treatment. We found that increased levels of IL-12 from locally treated lesions were observed at all dose concentrations between days 11 and 21 (Fig. 1 in ref. 37). However, IL-12 was not detected in peripheral blood (Data not shown). This data largely parallels the animal experiments. All patients reported momentary pain during the procedure, which was immediately resolved after treatment. No dose limiting toxicity was reported.

Immunologic Effects of pIL-12 Electroporation

Tumor effects in mouse models suggest that T cells were the predominant cell population responsible for antitumor activity after plasmid IL-12 electroporation. Exploratory studies were performed post hoc to assess whether similar immunologic effects were observed in these cohorts. Systemic T helper type 1 responses specific for melanoma antigens were observed by ELISPOT analyses, and of the 15 patients who had both baseline and post-treatment peripheral blood mononuclear cells, increases in interferon-gamma producing T cells were detected for MAGE-3 (4 patients) and MART-1 (3 patients) at 4 weeks after treatment.[38] Electroporation with plasmid IL-12 also modulated antibody responses to cancer-testis antigens. A number of novel host antigens were also classified by protein microarray profiling as having increased IgG and/or IgM specific-responses to treatment. Overall these results suggest that plasmid IL-12 electroporation can produce or modulate systemic cellular and humoral immune responses to mediate regression of distant melanoma lesions. Other approaches including an oncolytic herpesvirus expressing GM-CSF[39] and canarypox virus expressing B7.1[27,40] have also shown promise in melanoma with cutaneous metastasis, suggesting that this stage of melanoma may be uniquely susceptible to immune therapy.

Conclusion

Electroporation has been shown to be an effective method for treating a target lesion, but in clinical practice, these lesions are

limited to areas that are accessible to the electroporator device. Even with penetrating electrodes, current devices are best suited for superficial or subcutaneous lesions or for singular lesions intraoperatively. Disseminated disease remains a challenge. The systemic tumor responses seen with intralesional electroporation of plasmid IL-12 suggest that adaptive immune responses specific to melanoma can be elicited, thus offering promise in controlling distant untreated disease. Recent animal studies suggest that dendrite cells are recruited to electroporated sites and are responsible for antigen trafficking to lymphoid structures.[27,41] These results are also supported by the induction of IFN-gamma producing T cell response to melanoma-associated antigens, the modulation of systemic antibody responses to a number of cancer-testis antigens, and clinically by the regression of distant tumors. Disseminated cutaneous disease occurs in approximately 10% of melanoma patients and occurs due to metastatic migration of melanoma within the lymphatic system. This would be an excellent indication where definitive surgical excision cannot be feasibly executed due to the rapid formation of in-transit melanoma, and plasmid IL-12 electroporation is currently being evaluated in a phase 2 trial in stage IIIB to stage IVA melanoma.

Whereas the induction of systemic antitumor responses is evident, the mechanism of how plasmid IL-12 electroporation initiates these responses requires further investigation. Necrosis of locally treated lesions observed in the phase I study appears important for tumor antigen release. Dendritic cells are likely to be involved in processing and presenting antigens to infiltrating cognate T cells. However, tumor antigens that are prevalent or are exposed to the host may be tolerized to the immune system. Another issue is that in the phase I study the most durable stable and complete responses were noted in patients with mainly disseminated cutaneous involvement. Although hampered by limited sample size, it may highlight potential differences with preclinical models. Strategies to improve upon this method may include modification of schedule, combination with other immunomodulatory transgenes, or priming with relevant tumor antigens that have not been tolerized to the host. Overall, electroporation of DNA encoding IL-12 is a safe and promising approach to treat in-transit melanoma and further investigation is underway to confirm its clinical activity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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