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Electroporation based gene therapy – from the bench to the bedside

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***Abstract*— A critical aspect of gene transfer is effective delivery of the transgene to the appropriate target. Electrically mediated delivery (electroporation) of plasmid DNA has been accepted as a viable approach to achieve effective delivery. One promising area is delivering plasmid DNA to skin. Gene transfer to the skin with electroporation is currently being evaluated for its potential for inducing angiogenesis for wound healing and for delivering DNA vaccines to the skin. Experiments utilizing a plasmid encoding for vascular endothelial growth factor has demonstrated how wound healing could be accelerated. In another study, delivery of a plasmid encoding Hepatitis B surface antigen have demonstrated that high antibody titers can be induced after two applications (prime/boost). Our laboratory has also examined the use of electroporation to delivery plasmid DNA encoding various cytokines as a potential therapy for melanoma. The plasmid is injected directly into the tumor followed by the administration of electroporation. Extensive preclinical work provided the rationale for a Phase I proof of concept first in human trial in patients with accessible cutaneous melanoma metastases. Biopsies of treated lesions showed significant necrosis of melanoma cells within the tumor as well as IL-12 expression. Lymphocytic infiltrate was observed in biopsies from patients in several cohorts. Clinical evidence of responses in untreated lesions suggested there was a systemic response following therapy was observed. Since this trial several other clinical studies utilizing electroporation to deliver plasmid DNA have been initiated. It is clear that this delivery approach has tremendous potential to facilitate the translation of gene transfer protocols from the bench to the bedside.**

I. Introduction

GENE therapy has held great promise for its potential to provide a means for effective treatment as well as the potential for effective vaccines. Successful gene therapy is dependent on effective delivery of the desired gene to the appropriate target and achieving the appropriate expression to achieve the desired clinical response. While both viral and non-viral delivery methods have uses in gene therapy, plasmid DNA based gene transfer is an attractive approach because it removes the need for a biological vector. This has inherent advantages such as improved safety, reduced immunogenicity, reduced potential for integration into the genome, and reduced potential for environmental spread. However, plasmid DNA-based gene delivery is handicapped by the lack of efficient delivery across cell membranes and low levels of expression in many tissues [1-3].

To enhance the efficiency of non-viral delivery, several physical methods have been tested including lipid, polymer, or nanoparticle conjugation, hydrodynamic delivery, ultrasound and *in vivo* electroporation [4-6]. Electroporation of plasmid DNA (electrogenetransfer) is quickly being accepted as a viable approach to achieve effective delivery. The first observations of this concept were in skin, liver, muscle, and orthotopic brain tumors [7-10]. Since then, this technique has been applied to many other tissues and animal models including skin, kidney, liver, testes, brain, cartilage, arteries, prostate, cornea, skeletal muscle and many tumor types in rodents, guinea pigs, dogs, rabbits, and non-human primates [11-13].

Our laboratory has been evaluating several potential therapeutic or prophylactic applications utilizing electrogenetransfer. One promising area is the delivery of plasmid DNA to the skin. This approach has been tested in several animal models with varying skin thickness resulting in significantly increased expression levels. Another very promising area is delivery to solid tumors. Several studies have been initiated including the first in human trail to deliver plasmid DNA utilizing electroporation.

II. Materials and Methods

A. Plasmids

Plasmid encoding Hepatitis B surface antigen was obtained from Aldevron. The human $VEGF_{165}$ plasmid was cloned by sub-cloning the hEF1-HTLV-hVEGF165 sequence from pBLAST49-hVEGF Invivogen) into the promoterless backbone of pVAX1. For *in vivo* use, all plasmids were commercially prepared (Aldevron). Endotoxin levels were <0.1 EU/µg plasmid. The plasmid for the clinical trial, pUMVC3-hIL-12 -NGVL3, was produced under Good Manufacturing Practices under a grant from the National Gene Vector Laboratory, Indiana University Medical Center, Indianapolis, IN, at the recombinant DNA production facility at the City of Hope Center for Biomedicine and Genetics, Duarte, CA.

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B. Animals

Female Hartley guinea pigs (250-300 g) or 200-250 gram male Sprague Dawley rats were anesthetized in an induction chamber charged with 3% isoflurane in O_2 then fitted with a standard rodent mask and kept under general anesthesia during treatment.

C. Patients

Eligible patients had pathologically documented metastatic melanoma, Stages III B/C or IV with at least 2 subcutaneous or cutaneous lesions accessible for electroporation. Patients had to have an Eastern Cooperative Oncology Group performance status of ≤ 2, be over 18 years of age, have adequate renal, hepatic and bone marrow function (creatinine < 1.5 x institutional upper limit of normal (ULN), bilirubin and SGOT (AST) within normal limits and an absolute neutrophil count >1500/mm^3). Patients with electronic pacemakers, defibrillators or a history of significant cardiac arrhythmia or seizure within the last 5 years were excluded from the study.

D. Random skin flap model

To create a rostral-based single pedicle RSF on the left lateral flank, rats were shaved and an 8-cm by 3-cm template was traced with a surgical marker. Full thickness incisions were made along the traced template lines. The skin and subcutaneous tissue were then elevated and sutured back to its bed using simple interrupted and running stitches with 4-0 non-absorbable sutures.

E. DNA vaccines

Plasmid encoding hepatitis B surface antigen (HBsAg) was delivered using electroporation to the skin of guinea pigs. Pulses parameters that have previously demonstrated "reasonable" and reproducible expression with a multielectrode array (300 V/cm, 150 ms) were used (14). The procedure was performed twice with a 2 week interval. Guinea pigs were bled through the jugular vein at various time points from Day 0 through Day 168. Blood was collected and serum isolated in serum separator tubes. Serum was diluted two-fold starting at 1:10 for all ELISAs. Anti-HBs were measured by ELISA over multiple time points.

F. Electroporation procedure

For all tissues injection of the plasmid at an appropriate concentration and volume is injected prior to application of electroporation pulses. For skin applications plasmid is injected intradermally and for cancer applications plasmid is injected directly into the tumor. For skin applications electric pulses are applied through the use of a nonpenetrating electrode array (14). For tumors, a penetrating 6 needle array was utilized (15).

III. RESULTS

A. Delivery of DNA vaccines

The induction of specific antibodies is an acceptable measure of the potential of a vaccine. Significant increases in antibody expression were found as early as one week after the second treatment and at all time points after (Figure 1). While peak expression for both injection only and electroporation groups occurred at week 7, their average titers varied greatly between the two groups. The fold increase over IO remained relatively constant at about 5 fold with the greatest fold increase over IO of 6.5 occurring at week 18.

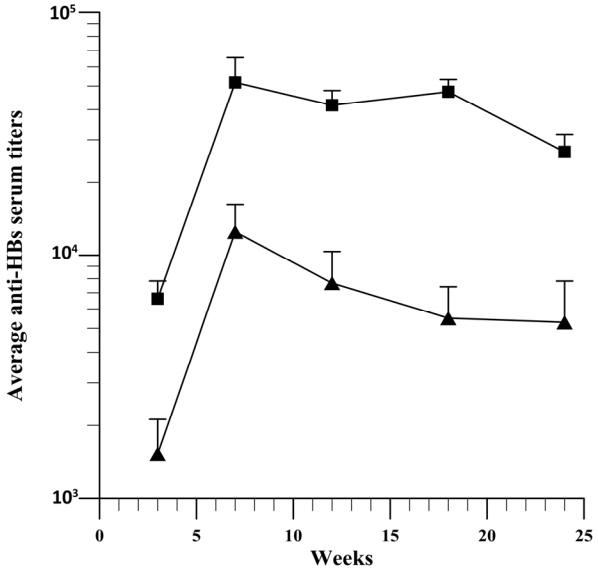

Figure 1. Evaluation of anti-HBs serum titer.
Guinea pigs were treated as described in text with pHBsAg. Serum was collected at multiple time points and an ELISA performed. End point titers were determined by the reciprocal of the dilution. Positive was considered greater than two standard deviations greater than the Day 0 average OD.

B. Wound healing

One potential application of electrogenetransfer is to deliver therapeutic molecules that would accelerate the healing of large wounds. One means to accomplish this is to deliver a molecule such as vascular endothelial growth factor (VEGF) that can induce cell proliferation, promote cell migration and differentiation as well as induce angiogenesis and inhibit apoptosis. We have previously demonstrated that electrically mediated deliver of plasmid encoding VEGF can facilitate complete healing of a random skin flap on the flank (8 cm x 3 cm) of a Sprague Dawley rat (16). Recently we further evaluated this approach and found that similar results could be obtained by treating only 2 areas provided one was near the pedicle end and one was near the distal end. The treatment was administered two days postoperatively (formation of flap) to allow for peak VEGF

expression during the beginning of the proliferative phase of wound healing. All animals were observed for 14 days.

C. Cancer therapy

Several studies were performed to determine the preclinical efficacy and toxicity analysis [17-19] to obtain the necessary data to initate the first clinical trial using gene therapy delivered by *in vivo* electroporation (clinicaltrials.gov identifier NCT00323206). This study was performed in patients with metastatic melanoma [20]. The study was designed as a Phase I dose escalation trial (increasing dose of plasmid) for safety and tolerability following the electroporation mediated delivery of a plasmid encoding human IL-12. The treatment was performed three times over eight days. Treatment was well tolerated with only Grade 1 and Grade 2 toxicity were reported. No laboratory abnormality was noted following electroporation. Each patient reported transient pain and/or discomfort at the site of electroporation but all stated it was tolerable. Tumor necrosis was observed in most electroporated lesions by day 11 and IL-12 protein expression was detected at all electroporation sites including at the lowest dose tested (0.1 mg/ml). In addition to the localized response, two of the 19 patients with untreated lesions had a complete response in distant non treated metastases. Each of these patients had a large number of melanoma lesions. While only four of the lesions were treated, all of their lesions shrank and faded away over a 6-12 month period. A third patient who received chemotherapy with DTIC following the IL-12 gene therapy also had a complete response in distant non-treated metastases. These responses have persisted to date (>2 years in all cases). Seven patients had stable disease where tumor progression was halted but no shrinkage of existing tumors was seen. No new lesions at any site appeared in the complete responders or those with stable disease.

IV. Conclusion

The use of electroporation for the *in vivo* delivery of plasmid DNA has seen tremendous growth in the past decade. Published reports have demonstrated several potential therapeutic and prophylactic applications that this approach could be used for (11-13). With electrogene transfer, both localized and systemic effects can be elicited. Gene therapy is applicable to many diseases including metabolic diseases and cancers. Currently, there are many preclinical studies being performed using electrogene transfer with the intention of translating those findings to clinical trials. During the past five years, many clinical studies utilizing electrogenetranser have been initiated. Currently, there are 19 clinical trials listed on www.clinicaltrials.gov using *in vivo* electroporation to perform gene delivery to several tissues, including both therapeutic and vaccine approaches. As these studies conclude the utility of electrogenetransfer will be better understood. It is anticipated that as additional application and preclinical studies come to successful conclusions additional clinical trials will be initiated.

REFERENCES

[1] S.D. Li , L. Huang, "Gene therapy progress and prospects: non-viral gene therapy by systemic delivery," *Gene Ther.* , Vol 13 pp1313-1319, 2006.

[2] X. gao, K.S. Kim, D. Liu, "Nonviral gene delivery: what we know and what is next." *AAPS J* , Vol. 9 pp E92-104, 2007.

[3] S. Braun H. "Muscular gene transfer using nonviral vectors," *Curr Gene Ther, Vol.* 8, pp 391-405, 2008

[4] T. Niidome, L. Huang, "Gene therapy progress and prospects: nonviral vectors," *Gene Ther,* Vol . 9 pp 1647-1652, 2002.

[5] M.D. Lavigne, D.C. Gorecki, "Emerging vectors and targeting methods for nonviral gene therapy," *Expert Opin Emerg Drugs,* Vol. 11, pp 541-557, 2006

[6] S.L. Hart, "Multifunctional nanocomplexes for gene transfer and gene therapy," *Cell Biol Toxicol*, Vol 26, pp 69-81, 2010.

[7] A.V. Titomirov, S. Sukharev, E. Kistanova, "In vivo electroporation and stable transformation of skin cells of newborn mice by plasmid DNA," *Biochim Biophys Acta*, Vol 1088, pp 131-134, 1991

[8] R. Heller, M.J. jaroszeski, A. Atkin, D. Moradpour, R. Gilbert, J. Wands, C. Nicolau, "In vivo gene electroinjection and expression in rat liver," *Febs Letters,* Vol 389, pp 225-228, 1996

[9] T. Nishi, K. Yoshizato, S. Yamashiro, H. Takeshima, K. Sato, K. Hamada, I. Kitamura, T. Yoshimura, H. Saya, J. Kuratsu, Y. Ushio, "High-efficiency in vivo gene transfer using intraarterial plasmid DNA injection following in vivo electroporation," *Cancer Res,* Vol 56, pp 1050-5, 1996

[10] H. Aihara, J. Miyazaki, "Gene transfer into muscle by electroporation in vivo," *Nat Biotechno,l* Vol. 16, pp 867-70, 1998.

[11] L.C. Heller, R. Heller, "In vivo electroporation for gene therapy," *Hum Gene Ther*, Vol. 17, pp 890-897, 2006

[12] C. Favard, D.S. Dean, M.P. Rols, "Electrotransfer as a non viral method of gene delivery," *Curr Gene Ther*, Vol. 7, pp 67-77, 2007.

[13] A.M. Bodles-Brakhop, R. Heller, R. Draghia-Akli, "Electroporation for the delivery of DNA-based vaccines and immunotherapeutics: current clinical developments," *Mol Ther*, Vol. 17, pp 585-592, 2009

[14] R. Heller, Y. Cruz, L.C. Heller, R.A. Gilbert, M.J. Jaroszeski, "Electrically mediated delivery of plasmid DNA to the skin, using a multielectrode array," *Hum Gene Ther*, Vol. 21, pp 357-362, 2010

[15] R. Gilbert, M.J. Jaroszeski, R. Heller, "Novel electrode designs for electrochemotherapy," Biochemica Biophysica Acta, Vol. 1334, pp 9-14, 1997.

[16] B. Ferraro, Y.L. Cruz, D. Coppola, R. Heller, "Intradermal delivery of plasmid VEGF(165) by electroporation promotes wound healing," *Mol Ther*, Vol. 17, pp 651-657, 2009.

[17] M.L. Lucas, L. Heller, D. Coppola, R. Heller, "IL-12 plasmid delivery by in vivo electroporation for the successful treatment of established subcutaneous B16.F10 melanoma," *Mol Ther*, Vol. 5, pp 668-675, 2002.

[18] M.L. Lucas, R. Heller, "IL-12 gene therapy using an electrically mediated nonviral approach reduces metastatic growth of melanoma," *DNA Cell Bio*, Vol. 22, pp 755-763, 2003.

[19] L. Heller, K. Merkler, J. Westover, Y.C. Cruz, D. Coppola, K. Benson, A. Daud, R. Heller, "Evaluation of toxicity following electrically mediated interleukin-12 gene delivery in a B16 mouse melanoma model," *Clin Cancer Res*, Vol. 12, pp 3177-3183.

[20] A.I. Daud, R.C. DeConti, S. Andrews, P. Urbas, A.I. Riker, V.K. Sondak, P.N. Munster, D.M. Sullivan, K.E. Ugen, J.L. Messina, R. Heller R, "Phase I Trial of Interleukin-12 Plasmid Electroporation in Patients With Metastatic Melanoma," *J Clin Oncol*, Vol. 26, pp 5896-5903, 2008.