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REVIEW

Electroporation for therapeutic DNA vaccination in patients

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Abstract DNA vaccination has historically failed to raise strong immune responses in humans. Recent delivery techniques such as the gene gun and in vivo electroporation (EP)/electrotransfer (ET) have completely changed the efficiency of DNA vaccines in humans. In vivo EP exerts multiple effects that contribute to its efficiency. The two central factors are most likely the increased DNA uptake due to the transient membrane destabilization, and the local tissue damage acting as an adjuvant. To date, several studies in humans have used in vivo EP/ET to deliver DNA. Some of these results have been quite promising with strong T cell responses and/or transient effects on the viral replication. This suggests that improved strategies of in vivo EP/ET can be a future way to deliver DNA in humans.

Keywords Therapeutic vaccine · HBV · DNA vaccine · Electroporation · T cell

DNA vaccines

DNA vaccines were first described in the early 1990s. It was shown that by simply injecting a plasmid, expressing a viral antigen under the control of a eukaryotic promoter an immune response to the viral antigen could be achieved [1]. Basically, the plasmid should be taken up by the cells, transported to the nucleus, start transcription and translation of the viral antigen. The antigen is then presented in both the major histocompatibility complex class I- and II-pathways, presumably by the transport of the antigen, and/or transfected cell, by dendritic cells to a nearby lymph node. The lymph node is most likely the key site for the priming of T cells to the vaccine antigen, albeit the role for the transfected cell in antigen presentation has not been fully elucidated. The DNA vaccine technology was advanced to human testing where it became painfully obvious that DNA vaccines did not work as well in humans as they did in small animals [2–4]. Why was that? One factor is certainly the poor uptake of the DNA when simply injected in the muscle or in the skin. This started an era of trying to improve the delivery of DNA vaccines to larger animals.

In vivo electroporation (EP) or electrotransfer (ET)

The concept of applying a current over cell membranes has been used since the 1970s for introducing various compounds and substances to cells [5, 6]. The strong but transient electrical pulses either introduce transient pores in the cell membrane or destabilize the cell membrane whereby extra-cellular compounds can enter the cell. Thus, one can argue whether EP or ET is the best term to use.

The technology was later transferred to the in vivo situation when it was noted that anticancer drugs were poorly taken up by cells in vivo [7]. Hence, by either injecting the anticancer drug into the tumor or administering the anticancer drug systemically, and then applying transient electrical

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pulses over the tumor resulted in cellular uptake of the drug [8–10]. This technology has been applied in numerous studies and is now in clinical practice in the European Community.

The first studies with applying in vivo EP/ET to gene transfer and DNA vaccination were performed in the late 1990s ([11, 12]). These studies nicely showed that the uptake of DNA was improved by in vivo EP/ET (Fig. 1). Later studies also showed that in vivo EP/ET also improved the activation of both humoral and cellular immune responses [13, 14].

Histological analysis of the site of injection revealed that the transfection efficiency was greatly improved and the variability between animals was reduced (Fig. 2; [13]). Importantly, it was also shown that in vivo EP/ET induced a rather impressive local tissue destruction followed by inflammation (Figs. 1 and 2). It is highly likely that this inflammation acts as an adjuvant that participates in the generation of the improved immune responses (Fig. 3). Exactly how in vivo EP improves immune responses or which cellular signal pathways are involved is not yet completely defined. Danger signals that arise upon in vivo EP/ET such as heat-shock proteins, necrotic/apoptotic cell debris and prokaryotic DNA are known to stimulate the innate myeloid cells through TLR/NOD pathways and are potent adjuvants that can enhance the efficiency of direct presentation and cross-presentation of transfected cells to promote priming of antigen-specific cellular responses [15, 16]. These pathways could contribute to the beneficial effect of in vivo EP/ET to render the vaccination more effective, and therefore, lower doses of DNA can be used to achieve the same effect.

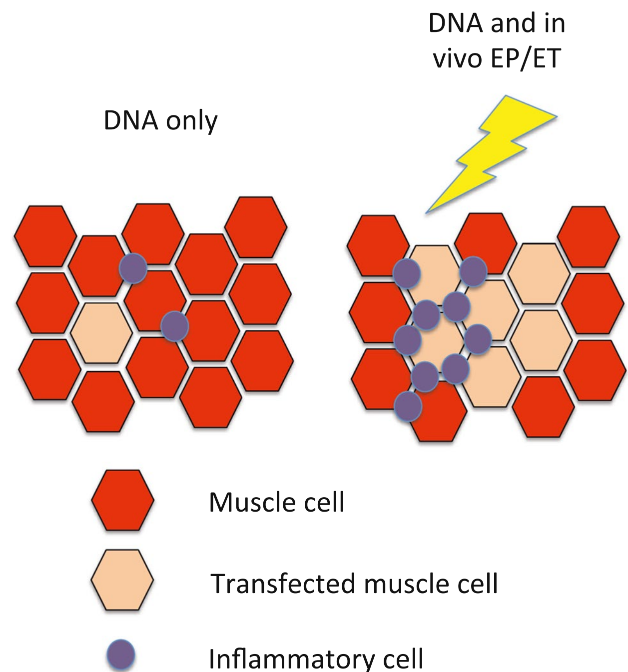


Fig. 1 Cartoon describing the histological scene when using in vivo EP/ET. After a standard intramuscular injection of plasmid DNA, only a few muscle fibers become transfected and a limited inflammatory response is present (*left*). In contrast, when adding in vivo EP/ET to the DNA injection, more fibers become transfected and a more massive inflammatory infiltrate is seen. These two events taken together are most likely key to the effectiveness of in vivo EP

In vivo EP/ET for DNA vaccines in humans

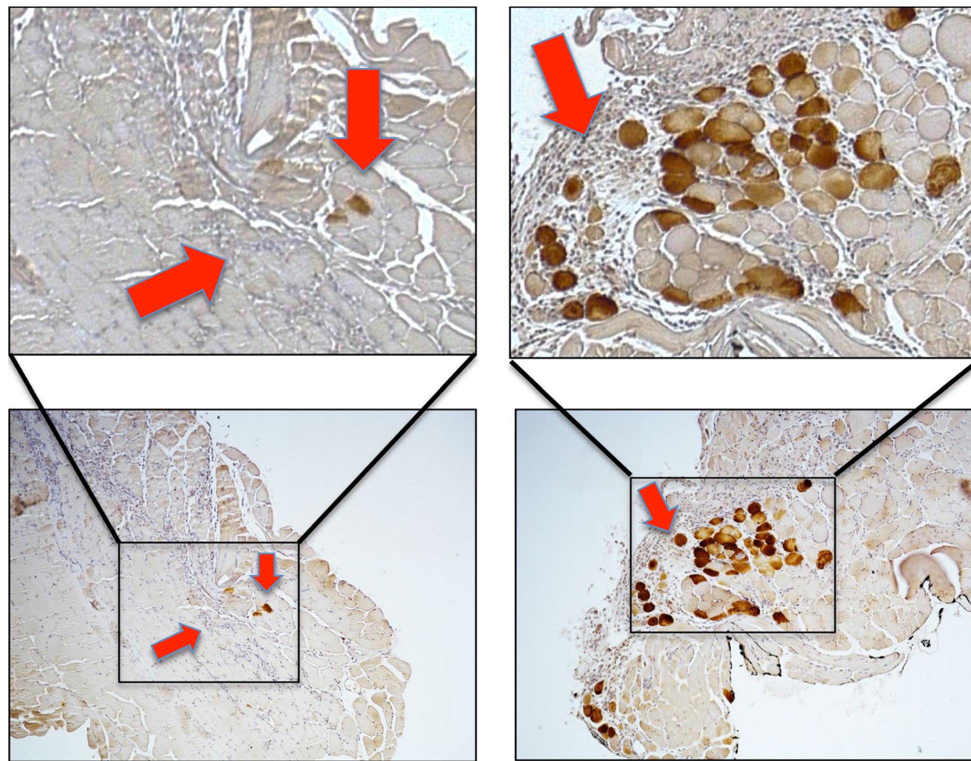
Several studies have now used in vivo EP to improve administration of DNA in humans. These studies have shown that immune responses can be raised in humans with DNA vaccines. The vaccination when given intra-muscularly has been shown to be rather painful with most devices used [17, 18]. The key factor for the pain is that the skin may be involved and that a rather high voltage (>200 V) is used. It is not known whether the transient pain or the muscle contraction at the time of treatment affects efficiency. There are approaches to make the treatment more tolerable, and these include shorter distance between electrodes and insulation of the electrodes as they pass the skin [19].

There is evidence that in vivo EP/ET improves the immune responses to DNA vaccinations also in humans. In a study involving healthy volunteers, the addition of in vivo EP/ET improved the response rates from zero percent to 88 % at the same 4.0 mg DNA dose [20]. Thus, there is today little doubt that in vivo EP/ET has beneficial effects on DNA transfection, expression, adjuvant effects, and in the end on the primed immune responses.

With respect to human studies, it is absolutely clear that humoral and cellular responses can be effectively primed by DNA delivered by in vivo EP/ET ([17, 18, 21, 22]; Table 1). In hepatitis C, it is possible that the T cells activated by that therapeutic DNA vaccination had transient effects on the viral replication [18]. However, this would need to be confirmed in controlled studies.

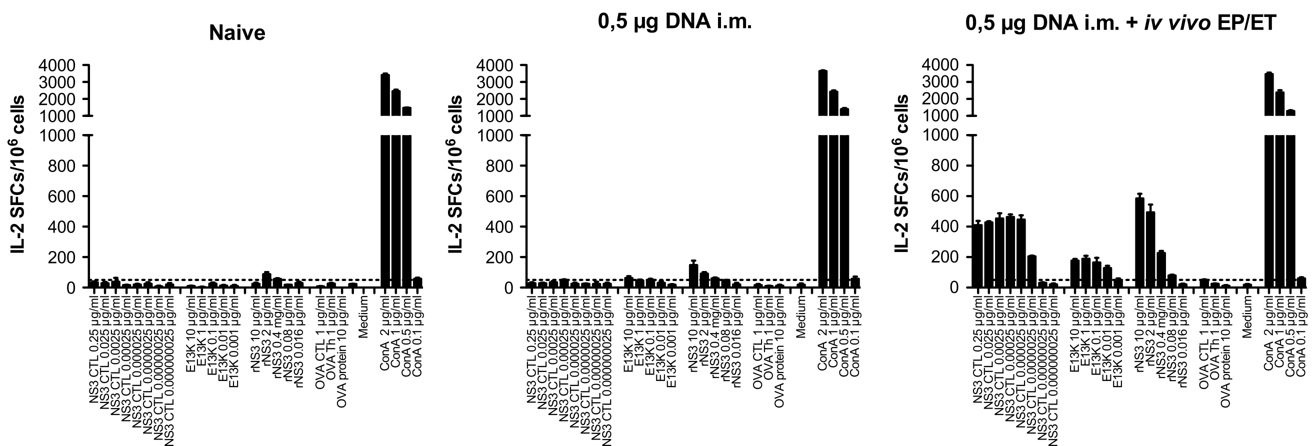
Factors affecting efficiency of in vivo EP/ET

A key issue is that the EP/ET treatment should involve the area where the DNA has been injected. This suggests that a two-step delivery procedure with a first injection of the DNA then followed by an insertion of the electrodes is suboptimal [18]. This type of early procedure allows for multiple operator-induced errors such as not treating the volume containing the DNA, or too long time between the DNA injection and the in vivo EP/ET. Several different devices have been designed that take these possible sources of errors into account. As an example, the Ichor Medical Systems



HCV NS3 expression and inflammation

Fig. 2 Effects of in vivo EP/ET in mice on transfection of muscle fibers (brown cells; *lower panel*) and inflammation (small infiltrating cells; *lower panel*). Mouse tibialis anterior muscles were injected with 5 µg HCV NS3/4A encoding DNA without (*left*) or with in vivo EP/ET (*right*). The muscles were removed 72 h after injection and were stained by Hematoxylin and Eosin and by an anti-NS3 antibody (brown muscle fibers). Please note that a larger area of the muscle becomes transfected when using EP/ET (*lower right*)



Effect on HCV NS3-specific IL-2 production

Fig. 3 The effect of in vivo EP/ET on the priming of splenic NS3-specific IL-2 production in mice. Groups of mice were left untreated (*left*), immunized with 0.5 µg of NS3/4A DNA (*center*), or immunized with 0.5 µg of NS3/4A DNA immediately followed by in vivo EP/ET (*right*). As can be seen, the addition of in vivo EP greatly improves the IL-2 production suggesting an improved immunogenicity with the addition of in vivo EP/ET

TRIGrid delivery apparatus has four electrodes placed in a rhomboid pattern and a needle with a motorized injection of the DNA in the center [19]. An additional feature of this apparatus is the insulation of the electrodes at the level of the skin, which probably increases tolerability [19]. This represents interesting ideas for further refining in vivo EP.

Table 1 Examples of DNA vaccines using in vivo EP/ET in clinical trials

Virus/type	CTL responses	Antigen genes	Effect on virus	References
HCV/genotype 1	Weak–moderate	NS3/4A	Transient	[18]
HIV/clade B	Weak–moderate	Env, Gag, pol	Healthy volunteer	[21]
HIV clades B/C	Weak–moderate	Env, Gag, pol, Nef, Tat	Healthy volunteer	[22]
HPV types 16/18	Weak to strong	E6/E6	NA	[17]

We have tested various pulse patterns and voltages for DNA delivery in mice. We have found that a short high-voltage pulse followed by a longer low-voltage pulse greatly improves transfection and immunogenicity in mice (Fig. 3). Importantly, not only IFNγ-producing cells, but also IL-2 producing cells increase in number (Fig. 3). A regular i.m. injection of a DNA into a mouse tibialis anterior muscle results in the following pattern: The inflammation and the transfected cells follow the needle track (indicated by arrows in Fig. 2, lower left). In contrast, the addition of in vivo EP/ET greatly improves both transfection efficiency and the inflammatory response. Neither the inflammation nor the transfected cells are located exclusively to the needle track, the treated area (volume, consider this in 3D) is much larger. Thus, it is safe to assume that the improvement seen by in vivo EP/ET is a result of the combination of the adjuvant effect of the tissue damage and the improved transfection efficiency.

Discussion

DNA vaccines have not been a great success when it comes to either protecting or treating humans from infections. This is largely explained by the fact that when DNA is simply administered without any type of improved/targeted delivery and/or adjuvant, the DNA vaccines simply do not work. And why do they not work? There are of course many reasons for this although the major issue must be the poor uptake of the DNA across the cell and nuclear membranes. The DNA must be efficiently delivered all the way into the nucleus to be transcribed. If not, the delivery failed. Here, RNA-based vaccines that act as an mRNA have a strict advantage, and they only have to pass the cell membrane to be translated. However, RNA is generally considered as being much more unstable than DNA, but new techniques may solve this issue.

Delivery techniques are central to making DNA vaccines effective. The Gene Gun where DNA is coated on gold particles and delivered under helium pressure into the skin is one way that has shown promise in humans [23]. Microneedles with multiple small needles coated with the DNA that delivers the DNA into the skin are also an attractive way [24]. Another is in vivo EP/ET. The advantage with in vivo EP/ET is that it does work in larger animals including humans. The disadvantage is the need for a rather complex apparatus and tolerability. However, new devices for in vivo EP/ET are constantly developed that takes these issues into consideration. Thus, when considering a DNA vaccine for viral hepatitis, in vivo EP/ET should be considered as one way to deliver the vaccine.

In the therapy for chronic hepatitis B, therapeutic DNA vaccines are likely to be well suited to activate the host immune responses to eliminate the need for life-long antiviral therapy. By vaccinating patients who have been on stable antiviral therapy for at least 6–12 months, it is possible that this can result in enough T cell activation to control the relapse of the viremia when the antiviral is removed. For this to work, the vaccine must be as immunogenic as possible with an optimal delivery and adjuvant effect. In conclusion, it is not unlikely that a good DNA vaccine delivered in the best possible way may achieve therapy responses in patients with chronic hepatitis B.

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Conflict of interest MS is a board member of Chrontech which holds the commercial rights to the NS3/4A-based DNA vaccine.

Ethical standard All studies discussed herein reported on clinical trials that had been approved by the appropriate ethical committees and government agencies.

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