

Advances in Therapeutic Electroporation to Mitigate Muscle Contractions

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Abstract

There is a growing effort among researchers to develop unique ways to apply pulsed electric fields for therapeutic applications that reduce the intensity or extent of muscle contractions in order to eliminate the use of muscle relaxants in clinical practice.

Keywords: Irreversible Electroporation; Focal ablation; Reversible electroporation; Electro chemotherapy; Pulsed Electric Field; Bipolar pulses; Muscle contractions; Nerve stimulation

Abbreviations: PEF: Pulsed Electric Field; nsPEF: nanosecond Pulsed Electric Field; IRE: Irreversible Electroporation; EBT: Electroporation-Based Therapy; ECT: Electrochemotherapy

Introduction

Electroporation is a phenomenon resulting from the application of an electric field across cell membranes, where in structural defects, commonly referred to as nanopores [1], are formed in response to an elevated transmembrane potential. The external electric field is conventionally unipolar, and its parameters can be regulated to create reversible defects, which enhance the permeability of the plasma membrane to molecules without compromising viability, or irreversible defects that ultimately lead to cell death. Currently, both modalities are being implemented as a therapeutic means to combat cancer. In applications of reversible electroporation, favorable clinical results have been obtained when low dosages of chemotherapeutic agents [2] or plasmid DNA [3] are used in combination with pulsed electric fields (PEFs). Alternatively, irreversible electroporation (IRE), performed with a comparatively higher field strength, duration, or pulse number, has been recognized as a non-thermal tissue ablation modality [4] capable of treating clinical tumors without adjuvant molecules [5].

Electroporation-based therapies (EBTs) are gaining interest as viable alternatives to surgical resection, chemotherapy, radiation therapy and thermal ablation techniques such as radiofrequency ablation, cryoablation or high-intensity focused ultrasound. As opposed to highly-toxic chemotherapy and radiation therapy regimes, all EBTs are well-tolerated by patients due to a lack of post-procedural complications [5,6]. Because the mechanism of cell death does not rely on thermal processes, outcomes are not subject to heat sink effects from blood perfusion, which can protect tumors from thermal therapies. Additionally, the treatment volume is predictable based on the electric field distribution in the tissue [7,8]. Specific to IRE, there is a distinct demarcation between ablated and non-ablated tissue that is visible in real-time on multiple imaging platforms [9,10]. Within the IRE ablation zone, it has been shown that extracellular matrix components are spared when parameters are chosen to avoid thermal damage. This permits treatment of surgically inoperable tumors in close proximity to major blood vessels and nerves [9,11,12] and the rapid repopulation of healthy cells post-IRE [10].

A challenge with EBTs is that the delivery of unipolar electric pulses characteristic of electroporation causes muscle contractions. To reduce movement, muscle relaxants may be administered to patients prior to treatment [10,13-15]. This poses additional concerns

for the anesthesiologist, as the dosage of muscle relaxants must be continually monitored to ensure an adequate neuromuscular blockade and proper respiratory function [16]. Electrically induced movement, while not ideal, has not prevented EBTs from being established as safe and effective treatment options for cancer [2,3,5,17,18] and other non-cancerous pathologies [19]. However, there has been a growing emphasis in the literature on developing novel techniques for performing electroporation that reduce the intensity or extent of muscle contractions [20-24].

Current State of the Art

Perhaps the first attempt to reduce muscle contractions during EBTs was made by Daskalov et al. [21]. Stemming from their work in the field of electrical stimulation [25], the authors designed a bipolar pulse generator to perform reversible electroporation with chemotherapeutic agents, or electrochemotherapy (ECT). The bipolar pulses consisted of a rectangular positive phase (50 μ s duration) followed immediately by a negative phase of equivalent duration. The system was tested at electric field strengths up to 1250 V/cm on patients with basal cell and spin cell carcinoma by delivering eight individual pulses at 1 s intervals or a single burst of eight pulses with 1 ms spacing. For comparison to conventional electroporation protocols, eight unipolar rectangular pulses (100 μ s duration) were also delivered at 1 s intervals in some tumor locations. Only a local anesthetic was used in the procedure without any muscle relaxant. All patients responded positively and completely to treatment, regardless of the implemented pulsing protocol. While the authors make no comment on the severity or extent of muscle contractions, they do note that bipolar pulses were better tolerated by patients. Additionally, for the application of eight pulses as a single burst, the patients experienced only a single electrical sensation, as opposed to series of eight [21]. The technique of raising the pulse repetition rate above the frequency of tetanic contraction was later confirmed to be an effective option for unipolar pulsing as well [18,26], assuming optimal drug dosing [27].

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Received March 13, 2012; Accepted March 15, 2012; Published March 20, 2012

Citation: Arena CB, Davalos RV (2012) Advances in Therapeutic Electroporation to Mitigate Muscle Contractions. J Membra Sci Technol 2:e102. doi:10.4172/2155-9589.1000e102

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While applying bursts of microsecond-long unipolar pulses can reduce the total number of muscle contractions per treatment, the force of contraction is similar to that generated during an individual unipolar pulse [26]. However, as alluded to by Daskalov et al. [21], altering pulse polarity can be employed to reduce the intensity of muscle contractions. According to classic literature on electrical stimulation, a bipolar pulse has a higher current threshold for action potential excitation as compared to a unipolar pulse of equivalent phase duration [28]. This effect is enhanced as pulse duration is reduced. When a microsecond order pulse is applied, there is a latency period between the offset of the pulse and the rising phase of the action potential. A rapid reversal of polarity falling within this latency period can accelerate passive repolarization and inhibit action potential generation [29]. Therefore, with proper tuning of bipolar pulse parameters, it is possible to achieve a drop in muscle force, which can be attributed to the cessation of action potentials in part of the motor unit population.

Our group invented a technique that utilizes bipolar pulses with a phase duration an order of magnitude shorter than that used by Daskalov et al. [21], to induce IRE without causing muscle contractions [20]. In this study, a pulse generator was developed that could deliver rectangular pulses with phase durations as short as 1 μ s. It is important to note that multiple cycles of bipolar pulses were applied in succession to generate a 200 μ s burst that was repeated once per second. The pulses were applied through electrodes inserted into the sensorimotor cortex of rats in order to study both direct stimulation of surrounding neck musculature through leak currents and induction of limb movement through stimulation of efferent neuronal pathways [12]. Qualitative assessment of the treated regions viewed histologically and under MRI indicated that bipolar pulses could non-thermally ablate tissue, albeit at a higher electric field strength when compared to unipolar pulses. However, even at higher field strengths (up to 4000 V/cm), there was no visual or tactile evidence of muscle contractions when bipolar pulses with phase durations of 1 to 2 μ s were tested. Conversely, all IRE protocols tested with 200 μ s long unipolar pulses produced detectable muscle contractions at field strengths as low as 500 V/cm [20]. In future work, additional experiments should be performed over the skin and in muscle for direct comparison to [21,29].

When pulse duration is reduced even further into the nanosecond range and stronger electric fields (10 – 100 kV/cm) are applied, it becomes possible to permeabilize both the plasma membrane intracellular structures [30]. These nanosecond pulsed electric fields (nsPEFs) have also shown great promise for treating cancer [31,32] by promoting calcium bursts [33], translocation of phosphatidylserine [33], and DNA damage [34], all of which are capable of triggering an apoptotic cascade. Typically, the pulse generator is based on a spark gap switched transmission line, and the resulting output closely resembles a unipolar pulse [35,36]. For unipolar pulses of this nature, cell death can be induced with a significant drop in muscle contractions as compared to unipolar microsecond-long pulses [23]. Experimentally derived strength-duration curves indicate that a 100 ns pulse requires a voltage two orders of magnitude greater than a 10 μ s pulse to excite muscle [37].

Nerve conduction block induced by electrical currents, as opposed to chemical muscle relaxants, is another, largely unexplored, option for mitigating muscle contractions during EBTs. While the biophysics are not completely understood, scientists have shown that a reversible, fast-acting peripheral nerve block can be obtained with high-frequency bipolar pulses [38,39] or unipolar nsPEFs [40] without any accompanying thermal damage. It is thought that disrupting the transmembrane potential across a nerve at a localized point

downstream from excitation may inhibit action potential propagation. Practical application of electrically mediated nerve blocking would require the placement of additional electrodes adjacent to those used for EBTs. If performed correctly, this technique could offer a safer alternative to muscle relaxant administration.

Recently, an alternative technique for mitigating muscle contractions during IRE has been proposed by Goldberg and Rubinsky [22]. Utilizing the concept of a Faraday cage, the authors have found that a certain arrangement of needle electrodes limits the amount of tissue exposed to electric fields above the threshold for muscle contraction, while having a minimal impact on the extent of electroporation. The design consists of a central energized electrode surrounded by an array of grounded electrodes. Similar geometries have been used successfully for cardiac defibrillation [41] and ECT [42]. Interestingly, by having 16 or more grounded electrodes and by reducing the insertion depth of the central energized electrode relative to the grounded electrodes, the predicted amount of tissue experiencing muscle contractions falls dramatically [22]. Upon *in vivo* translation, it is expected that this concept will reduce direct stimulation of denervated muscle, as suggested here, and also reduce motor unit recruitment from nerve stimulation outside the targeted electroporated zone.

Conclusion

The reports presented above indicate that many feasible routes exist for mitigating muscle contractions during EBTs. Continued development of all of these routes will be beneficial, as there may be certain applications for which some options are better suited. For example, the electrode configuration proposed above [22] requires an increase in the number of electrodes and may be most valuable in situations where the targeted region can be treated non-invasively, such as applications over the skin. Additionally, while positive results have been obtained with bipolar pulsing protocols in terms of reducing or eliminating muscle contractions, more work needs to be done in optimizing pulse parameters for specific applications. In a more recent study on ECT with bipolar pulses [43], a reduction in phase duration below 50 μ s reduced electrical sensation, but also had a negative impact on treatment efficacy. However, we have shown that IRE can be performed with a phase duration of 1 μ s by increasing the number of pulses and electric field strength [20]. Along these same lines, it should also be theoretically possible to cause cell death with bipolar nsPEFs of sufficient field strength. This may present an added improvement over the reduction in muscle contractions already seen with unipolar nsPEFs [23]. In the future, it is likely that continued work on mitigating muscle contractions will lead to the commercialization of new pulse generators and electrode designs that eliminate any need for muscle relaxant administration. This will further facilitate the widespread use of EBTs in clinical practice.

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