Preclinical Studies on Irreversible Electroporation

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Abstract

Irreversible electroporation (IRE) employs a series of brief electric pulses to destabilize cell membranes by altering the transmembrane voltage to create nanoscale defects that induce cell death. Its nonthermal mechanism makes it an ideal treatment modality for treatment of tumors near critical structures and vasculature, which are contraindications for other thermal ablation modalities. Since its conception, IRE has been investigated for applications in several organs to determine its safety and efficacy. IRE ablation zones, thermal effects, potential for real-time imaging, and cell death mechanism have been thoroughly investigated in vivo, leading to its successful transition to the clinical environment. This chapter describes IRE findings in the preclinical setting, with a focus on the pancreas, kidney, liver, bone, brain, prostate, and lung, and general implications with respect to successful therapy outcomes. These include consistency of ablation zones with different treatment parameters, histology of ablation zones showing tissue and tumor destruction, in vivo safety during and after treatment, and efficacy in terms of tumor regression. In addition, the patency of critical structures such as blood vessels, nerves, and ductal systems is briefly discussed. Newer

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preclinical findings such as immune response activation, adjuvant therapies, and modulating pulse regimes to improve treatment outcome and ease treatment delivery are outlined.

Contents

Introduction

Electroporation is a process in which short, high voltage electric pulses cause an increase in the biological cell membrane permeability, due to the formation of nanoscale defects, referred to as "pores." For applications that involve cell drug delivery, the goal is to limit the pulse amplitude and number so that the pores are temporary, allowing transmission of small molecules across the membrane, and the cell remains viable (Overview and History of Electrochemotherapy). Alternatively, irreversible electroporation (IRE) utilizes a higher pulse amplitude and number to induce cell death as a tissue ablation modality. With appropriately designed pulse protocols, IRE creates lesions without causing significant thermal damage to the tissue (Davalos et al. 2005). The mechanism of IRE is generally characterized by irreversible structural defects, chemical imbalances due to the influx and efflux of ions, and subsequent cell death (Electropore Energy and Thermodynamics) (Lee et al. 1993).

The use of IRE as a therapy for ablating tumors was first demonstrated in an animal tumor model in 2007 (Al-Sakere et al. 2007). IREs clinical efficacy has since been demonstrated in numerous organs for soft tissue targets, particularly tumors. IREs ability to generate regions of dead cells while maintaining the critical extracellular architecture allows IRE to be used in regions containing sensitive structures that make such targets contraindicated for other focal therapies or surgical resection. In this chapter, the wide array of preclinical IRE studies in numerous animals (including animal tumor models) and organs are presented. These studies characterize IRE ablations in response to different treatment parameters and show its efficacy in relation to preservation of extracellular components.

Considerations for Reducing Thermal Damage

IRE employs brief (~100 μs) but intense square wave electric pulses (50–400) delivered via electrodes inserted into or adjacent to the targeted tissue. These pulses induce an electric field distribution in the target and surrounding tissue, depending on the electrode configuration, pulse amplitude, and tissue type. For a given tissue and set of pulse parameters (pulse length, number of pulses, delivery rate), an electric field threshold can be determined that induces cell death and forms a lesion. Numerical models are often employed to predict the induced electric field distribution and ablation zone, using a priori knowledge of the tissue electrical properties.

The electric pulses used in IRE treatment delivery induce collateral heating due to the resistive properties of biological tissue, known as Joule heating. In order to maintain IRE's distinction from thermally based therapies, pulse protocols must be designed to limit Joule heating below thresholds that cause structural thermal damage to the tissue and surrounding sensitive structures.

Numerous publications in the literature use numerical and experimental studies to address consideration of sufficient IRE without the clinically limiting thermal damage. These studies aim to delineate IRE cell death from the thermal damage that occurs when tissues are exposed to temperatures higher than their physiological norm for extended periods of time. While 50 °C is typically chosen as the threshold to begin introducing thermal damage at the cellular scale, and it has been noted that prolonged exposure to mildly elevated temperatures as low as 43 °C can lead to cell death, what is considered instantaneous thermal damage occurs as high as 83.6 °C (prostate) or 74.7 °C (liver) (Thomsen and Pearce 2010).

From an experimental perspective, in vivo studies have measured temperature during pulse delivery to examine the potential for thermal damage. The increase in temperature during treatment is proportional to the applied electric field and is therefore dependent on the electrode configuration utilized in the study. Two configurations, namely, the monopolar and bipolar electrode configurations, are commonly employed to deliver IRE treatment. The monopolar electrode configuration contains the source and sink electrodes on two different needles and spacers need to be used between the needles to maintain electrode distance. On the other hand, the

bipolar electrode configuration has the source and sink on the same needle. This is accomplished using concentric cylinders inside the needle, with insulating material between the two cylinders. For monopolar and bipolar needle electrodes, the electric field is highest closest to the electrodes and decays away from the electrodes, and therefore, the temperature is always the highest near the electrodes. For flat plate electrodes, the electric field is uniform between the electrodes and so is the temperature distribution.

In a clinical canine case using IRE to treat a soft tissue sarcoma, the temperature immediately adjacent to the electrode was measured using a fiber optic temperature probe. This study employed monopolar or bipolar electrodes of spacing 8–15 mm to deliver 80 pulses of 70–100 μs width and 800–1250 V at a rate of 90 pulses/min or immediately following the R-wave peak on the electrocardiogram. The maximum temperature rise at this location during the IRE procedure was 2.4 °C (Neal et al. 2011) with the study showing successful tumor remission. Furthermore, an in vivo study in brain with thermal probes attached to the electrodes showed that it was possible to generate substantial volumes of IRE tissue while max tissue temperature rise was 1.15 °C (Garcia et al. 2010). An additional in vivo study on porcine kidneys employed a more aggressive IRE protocol of three to four monopolar electrodes with electrode spacing and exposure of 15 mm to deliver three sets of 70 pulses, each 90 μs long, at a rate of 90 pulses/min. The peak temperature recorded at the center of the three- and four-electrode configuration was 57 and 79 °C, respectively (Wagstaff et al. 2014). Consistent with the rapid decay in thermal effects with increasing distance from the electrodes, max temperatures of 40 and 42 °C were measured 1 cm outside the electrode geometries, which was still within the IRE ablation zone.

These studies demonstrate that while IRE ablation occurs primarily due to a nonthermal mechanism, careful attention must be paid to heating effects to ensure they remain below those to cause thermal damage to the critical structures and surrounding vasculature. In current IRE protocols, the temperature rise close to electrodes is inevitable and may lead to thermal damage near the electrodes (▶ Chap. 54, "Treatment Planning for Electrochemotherapy and Irreversible Electroporation of Deep-Seated Tumors"); however, majority of the ablation zone away from electrodes is nonthermal, caused by electroporation-induced effects. Several studies have suggested techniques to reduce temperature rise during IRE treatment, including shorter pulse durations, reducing the pulse delivery to allow convective and conductive cooling between pulses, as well as proactive interventions to cool the electrode and/or tissue.

Organ-Specific Ablations

IRE's potential for ablation has been studied in several organs, each showing different implications in regard to successful therapy outcomes. This section addresses some of the more common IRE therapeutic applications and the preclinical data regarding the use of IRE for these targets.

Pancreas

Locally advanced pancreatic cancers comprise approximately 40% of the pancreatic tumors. Although not metastasized, these tumors have innervated and surrounded critical structures such as the pancreatic duct and the superior mesenteric artery. Therefore, the tumors around this region cannot be safely resected without damage to these critical structures, thereby, risking patient morbidity and mortality. Furthermore, the use of thermally ablative therapies in such environments has largely been avoided due to the possibility of thermal injury-induced pancreatitis. This leaves a large number of pancreatic cancers inoperable by the time of diagnosis. IRE's ability to spare the major vasculature and potentially reduce risk of pancreatitis offers an approach to addressing the tumor in these regions. It could be used as either a stand-alone treatment modality, in conjunction with drugs, or to augment the treatment margin of surgical resection.

Preclinical data in four female swine showed IRE can be safely administered in the pancreas (Charpentier et al. 2010). Treatment parameters involved the use of monopolar electrodes at a spacing of 9–15 mm; 90 pulses of 1350–2250 V were delivered to the healthy pancreas. All animals survived their designated times of 2 h, 2 days, and 2 weeks with no treatment-related complications. The tissues in and around the ablation zones were sliced into sections of thickness 5 mm, perpendicular to the electrode placement. The 5 mm section in the center of the ablation cavity had heights ranging from 10 mm to 21 mm and widths ranging from 10 mm to 16 mm. Pancreatic tissue at 2 weeks after IRE showed scarring in the ablation zone with preservation of pancreatic ducts. This study was also the first to show that triphenyl-tetrazolium chloride (TTC) can predict the IRE zone of ablation within 2 h of treatment. TTC stains the live mitochondrial activity in cells. While IRE ablation zones are detectable visually without staining, TTC staining enhances the contrast between the ablated and non-ablated areas.

Another study in swine pancreas was performed with two electrode configurations – monopolar (19 gauge, i.e., diameter 0.9 mm) and bipolar (16 gauge, i.e., diameter 1.2 mm) – and the animals were euthanized after 3, 7, and 14 days (Bower et al. 2011). The monopolar electrode configuration consists of two needle electrodes, with source on one needle and sink on the other. Alternately, the bipolar probe contains both the source and sink on the same probe at a fixed spacing of 8 mm. Treatment parameters included electrode spacing from 8 to 20 mm, pulse numbers of 50–100, and applied voltage ranging from 2300 to 3000 V. All animals survived the study, with transient increases in amylase and lipase that normalized on third day. Amylase and lipase are key digestive enzymes, produced by the pancreas, that help break down starch and digest fats. High levels of these enzymes are indicative of pancreatitis. Gross analysis of samples revealed ablation zones of median size of height 3 cm and width 2.8 cm. Histological analysis revealed significant destruction of the pancreatic tissue with patent vascular structures and no significant difference between the two types of probes. Additionally, terminal deoxynucleotidyl transferase dUTP nick end

labeling (TUNEL) assays' staining identified apoptotic markers in the IRE ablation zone.

Furthermore, IRE was shown to be effective in treating pancreatic ductal adenocarcinoma, an aggressive form of pancreatic cancer, in orthotopic mouse models (José et al. 2012). IRE treatment exhibited significant antitumor effects and extensive tumor necrosis, reduced tumor cell proliferation, and disruption of microvessels. Data in this study indicated that IRE leads to an increase in survival (from 42 days in untreated mice to 88 days in the IRE-treated group) with 25% of mice showing complete tumor regression.

Kidney

The increase of high-resolution diagnostic imaging is partially responsible for a rapid rise in the number of diagnoses of asymptomatic small renal masses, with over half of new cases detected incidentally. Frequent detection of low-risk renal tumors has encouraged care toward less aggressive, nephron-sparing approaches. Preservation of renal function in the affected kidney is vital for patients with solitary kidneys, comorbid conditions, multiple tumor sites, and genetic predisposition for recurrent bilateral renal tumors (von Hippel-Lindau disease). Partial nephrectomy still requires considerable invasiveness, while sensitivity of the ureter and high blood perfusion rate mitigate the efficacy of thermal therapies. These factors provide considerable value for IRE treatment of small renal masses.

Preclinical investigations into the ablation of kidneys investigating IRE effects on macro- and microscopic healthy renal tissue show complete cell death of glomerular and tubule cortex structures while sparing major vasculature. In an IRE study on eight female Yorkshire pigs, laparoscopic ablations were performed with either monopolar or bipolar electrodes (Tracy et al. 2011). The pigs were euthanized between 10 min and 14 days after IRE, and the kidneys were harvested for gross and histological analysis for cellular viability. Monopolar ablations were performed at 2300 V and 90 pulses at a pulse length of 100 μs and 1.5 cm spacing, while bipolar ablation was performed at 2700 V and 90 pulses at a pulse length of 70 μs. The spacing between electrodes was fixed at 0.8 cm. Overall lesion volume was smaller when the bipolar probe was used than when the monopolar was used. In the bipolar group, the mean gross lesion size, immediately and an hour after death, showed a volume of 1756 mm^3 (assuming ablation shape of a prolate spheroid). In the monopolar group, the mean volume was 4440 mm^3. By 7 days, the ablations in both groups remained similar to their immediately post-IRE size (10 min). However, by 14 days, there was substantial lesion contraction, regardless of the modality applied. There was heterogeneous and unpredictable involvement of the urothelium, ranging from no damage to complete ulceration and necrosis. However, it was more common with the bipolar probe, which was placed to traverse the collecting system as opposed to the monopolar probe technique where the ablation zone traversed the collecting system without direct puncture. The urothelium was partially spared in 60% of the cases, with further evidence of repair/regrowth by 14 days despite direct involvement during ablation.

Another swine study evaluated the effect of IRE on the renal urine-collecting system using intravenous urology and urinary cytology, which are routine urological examinations, magnetic resonance imaging (MRI), and histology (Wendler et al. 2012). Each IRE procedure consisted of nine sets of ten IRE pulses, pulse length 70–100 μs, voltage 2300–2700 V. All animals were euthanized after a 28-day period. MRI scans did not show substantial lesion immediately after IRE; however, the sizes increased to an average diameter size of 16.5 mm at day 7 and shrunk to ~8 mm at day 28. These sizes showed good correlation with the ones from gross macroscopic analysis at day 28. Initial urothelial injury was noted, but the urinary system was completely regenerated 28 days post-IRE with the preservation of urothelial basement membranes and no urinoma evidence.

Similarly, other IRE studies showed that renal ablations showed a demarcation between necrotic and normal tissue, 24 and 36 h after IRE treatment with evidence of tubule degeneration (Deodhar et al. 2011). Ablations showed cortical fibrosis, regenerating renal pelvic epithelium, and intact pelvic extracellular matrix. No thermal injury, renal pelvis, or blood vessel injury was seen. Two to four weeks later, there was evidence of regenerating tubules and pelvic epithelium with intact extracellular matrix and ablations turning to contracted scars. The urine-collecting system was essentially preserved with regenerated urothelial tissue. This study therefore demonstrated the nonthermal and connective tissue-sparing mechanism of action of IRE.

Liver

The size of the liver relative to typical tumors, coupled with its regenerative capacity, makes the liver a common target for focal ablation of tumors. IRE has been clinically applied in this environment, particularly for targeted regions near the inferior vena cava, gallbladder, and external sensitive organs such as the bowel, pancreas, heart, and spleen. Further, the relative homogeneity and isotropy of hepatic organization make the liver a suitable model for investigation of various aspects for IRE therapy.

The greatest collection of preclinical investigations regarding the effects from IRE therapy exists for liver ablations. Many of these studies are generalized to effects of IRE as a whole, such as monitoring IRE ablation zones using different imaging techniques, as well as studies into different pulse parameters and electrode designs.

In a study conducted on 16 pigs, IRE ablations were imaged with ultrasound (US), magnetic resonance (MR), and computed tomography (CT) (Lee et al. 2010). Treatments were delivered with two electrode configurations – a single 16-gauge bipolar probe and two to three 18-gauge monopolar probes. Treatment parameters include inter-electrode spacing between 1.5 and 3.0 cm and 90 IRE pulses, each lasting 100 μsec at 2000–3000 V. At gross section examination, the mean diameter of the ablation zones was 33.5 ± 3.0 mm which was achieved in 6.9 min (mean total procedure time per ablation), with a mean difference of 2.5 ± 3.6 mm between US and gross section measurements. No complications were seen in any of the 16 animals. IRE ablation zones were well characterized with US, CT, and MR imaging,

and real-time monitoring was feasible with US. Bile ducts and vessels were completely preserved. Areas of complete cell death were stained positive for apoptotic markers, suggesting involvement of the apoptotic process in the pathophysiology of cell death caused by IRE.

Additional studies on the liver analyzed the effects of different treatment parameters on IRE ablation sizes (Ben-David et al. 2012). An IRE study performed in 25 pig livers using two 18-gauge (diameter 1.2 mm) monopolar electrodes evaluated the effect of five treatment parameters including pulse number (20–90), pulse length (20–100 µs), voltage (2250–3000 V), inter-electrode spacing (1.5–2.5 cm), and length of active electrode exposure (1.0–3.0 cm). For all combinations of pulse parameters, the study reported average ablation widths between 2.6 and 3.9 cm and depths between 1.3 and 5.0 cm. In the range tested, the study found that increasing voltage amplitude had a statistically significant increase in ablation size. However, increasing pulse numbers or pulse duration did not have a significant increase. Therefore, IRE is not strictly energy dose dependent, i.e., increasing energy through pulse number or duration does not have the same effect as increasing energy through voltage amplitude.

In addition, preclinical studies have shown that IRE can be used to effectively treat liver tumors in small animal models (Guo et al. 2010). In this study, hepatomas were established in an N1-S1 rodent model. Thirty rats were divided into treatment and control groups. For treatment groups, IRE electrodes were inserted and eight 100 µs and 2500 V pulses were applied to ablate the targeted tumor tissues. Magnetic resonance images showed statistically significant tumor size reductions ($p \leq 0.05$) compared to the untreated tumors within 15 days post-therapy, with reduction in tumor diameters of $32\pm31\%$. Pathology correlation studies documented extensive tumor necrosis and full regression in nine of ten treated rats in 7–15 days after treatment.

Bone

While IRE has primarily been evaluated for implications in soft tissue ablation, several studies have examined its effects on regions of the bone. In a study performing IRE in porcine lumbar vertebrae, it was shown that IRE produces localized regions of well-demarcated necrosis with no detectable change in bone texture and limited neural toxicity (Tam et al. 2014). This study was performed in the lumbar vertebrae of ten pigs using bipolar electrodes. Treatment parameters utilized 20–90, 2700 V IRE pulses of pulse width 70 µs. Well-delineated areas of necrosis of the bone, bone marrow, and skeletal muscle adjacent to the vertebral body were found with gross ablation average widths of 9 mm and lengths of 33 mm. This study showed IRE to be a safe and feasible technique to be utilized in the spine, with preservation of nerves.

Brain

IRE utility in brain has been characterized with preclinical and clinical canine models. The high vascularization and extreme sensitivity of adjacent neural tissue

require precise treatment plans and blunt-tip electrodes to deliver the IRE pulses. Preclinical studies in canine patients have shown IRE to safely ablate pathologically heterogeneous brain tissue while preserving vascular integrity and patient neurological functions.

In a preliminary study on five dogs, focal IRE ablations were made in the ectosylvian gyrus (Ellis et al. 2011). Three dogs were treated with recommended IRE parameters, one dog was a control, and the other was treated with a higher voltage to determine the upper safety limits of the procedure. For the three dogs that were noncontrols, treatment parameters included electrode separations of 5–8 mm, electrode exposures 5–7 mm, voltages from 500 to 1600 V, 50 μs pulse duration, and nine sets of ten pulses. These resulted in average ablation volumes between 0.258 and 1.655 cm^3, with smaller volumes for lower voltage. Histological analysis showed submillimeter boundary between the necrotic and normal brain. The animals tolerated the procedure with no apparent complications except for the animal that was treated at the upper voltage limit.

Canine malignant gliomas exhibit similar clinical, biologic, pathologic, molecular, and genetic properties as their human counterparts providing a good translational model for clinical investigations. In an early case study, IRE attained a 75% reduction in tumor volume within the first week post-IRE (Garcia et al. 2011). Treatment parameters utilized 40–80, 500 V IRE pulses of pulse width 50 μs. The electrode spacing and exposure was 5 mm. Following adjuvant fractionated radiotherapy, the tumor was determined to be in complete remission prior to the suggestive onset of early-delayed radiation encephalopathy, though recurrent glioma could not be excluded. In a recent medium-term examination of the treatment cohort, it was found that for the patients that survived and were discharged after the procedure, Karnofsky performance scores were improved in 6/6 patients over pretreatment values, while seizure control improved in 5/6 (Rossmeisl et al. 2015).

Prostate

Optimal treatment strategies remain to be determined for low- to medium-risk organ-confined prostate cancer. While radical prostatectomy offers strong efficacy to tumor control of tumors that have not metastasized, it carries high rates of morbidity in regard to incontinence and impotence post-prostatectomy, including with robot-assisted procedures. This morbidity results from damage to the urethra and sensitive neurovascular bundles at the perimeter of the prostate. Thermal and other focal therapies also risk such adverse effects, and efforts to mitigate these risks may jeopardize the efficacy of the treatment. Where it remains to be well defined which prostate tumors require intervention, IRE may serve as an ideal focal or regional ablation approach to address identified tumor regions with significantly lower risk to potency and continence.

Preclinical studies, primarily in vivo canine prostate studies, have shown the ability to safely create ablation lesions while preserving integrity and regular system function. In an early study, IRE was shown to produce significant ablations in prostate while preserving the urethra and neurovascular bundle (Onik et al. 2007).

A further study investigated the implications of metallic seed implants within the prostate, identical to those used for brachytherapy (Scheffer et al. 2003). This study found that the presence of the seeds did not significantly affect the ablation zone or thermal effects from the electric pulses, though it does not consider tissue changes in response to prior radiotherapy, such as fibrosis.

Lung

Lung tumors have been treated with thermal ablation; however, their application is limited due to thermal sinks from blood vessels, air spaces, and potential thermal injury to critical tissues, including the heart, making it less safe and efficacious. IRE's nonthermal mechanism of cell death and sparing of critical structures make it a potential candidate to treat lung tumors. A preliminary study on swine lung evaluated the safety of IRE in the lung (Dupuy et al. 2011). This study performed percutaneous IRE on nine domestic swine using bipolar as well as monopolar electrodes delivering 90 and 70 µs pulses of 1700–3000 V with electrocardiogram synchronization. The results showed that all swine successfully completed IRE treatment without any cardiac arrhythmias. CT showed focal areas of spiculated high density ranging from 1.1 to 2.2 cm. Histological analysis revealed focal areas of diffuse alveolar damage with fibrosis and inflammatory infiltration with sharp boundaries of the interlobular septa. The bronchioles and blood vessels within the areas of IRE were intact and did not show signs of tissue injury. However, optimization of IRE procedures for pulmonary targets remains to be performed before this organ can be successfully targeted for IRE treatments, due to the unique electrical and thermal characteristics caused by dynamic air spaces within the lung and its unique parenchymal structure.

Preserved Patency of Sensitive Structures

There are numerous relevant critical structures that may be implicated within or around targeted volumes for IRE therapy. The relative importance of each will vary with the targeted organ and cancer variety.

Blood Vessels

Extensive work has examined the effects of IRE on different organ blood vessels, some of which have been mentioned above. While IRE is shown to kill the endothelial cells and disrupt capillary-level vasculature, preservation of the collagen framework facilitates continued blood flow in large blood vessels.

In a pilot study, the long-term effects of IRE on a large blood vessel were studied (Maor et al. 2007). Treatment parameters included a sequence of ten IRE

pulses of 3800 V/cm voltage-to-distance ratio, 100 μs each, applied at a frequency of ten pulses per second directly to the carotid artery in six rats. All the animals survived the procedure and showed no side effects. Histology performed 28 days after the procedure showed that the connective matrix of the blood vessels remained intact, and the number of vascular smooth muscle cells (VSMC) in the arterial wall decreased with no evidence of aneurysm, thrombus formation, or necrosis.

In another IRE study (Appelbaum et al. 2012), Doppler ultrasound showed continued blood perfusion through a major vessel contained within a tissue region immediately after IRE treatment. Further, longitudinal studies have demonstrated the regeneration of endothelial cells within the affected regions of the blood vessels within 7 days, permitting full recovery and long-term function of the vessel.

Nerves

In an early in vivo study, it was shown that canine prostate tissue could be ablated while preserving the neurovascular bundles necessary for potency and continence (Onik et al. 2007). Following this study, the possibility of preserved neural function has since been explicitly investigated.

In addition to the peripheral nerve effects from the vertebral bone IRE studies, a long-term in vivo IRE study was performed on a rat sciatic nerve (Li et al. 2011). A sequence of ten pulses with voltage-to-distance ratio of 3800 V/cm, each 100 μs long, was only applied directly on the sciatic nerve to produce a treated length of about 10 mm. Electrophysiological, histological, and functional studies performed immediately and up to 10 weeks following surgery showed that, despite an initial decrease in functionality, the nerve attained full recovery approximately 2 months later.

Ductal Systems

In addition to vascularity and neurological implications that may be present for many organs targeted for IRE ablation, there are also many organ-specific ductal systems which also seem to have preserved function and patency while being contained within or adjacent to ablation zones. These include bile duct preservation in pancreatic and liver ablations (Bower et al. 2011), collecting system and ureter in renal ablations (Deodhar et al. 2011), and urethra in prostate ablations (Onik et al. 2007).

The action behind this preservation likely relates to the structural organization of many ductal systems, which are composed of relatively low-permeability connective tissue innervated with endothelial and epithelial cellularity. Although IRE will initially kill the cells within the ductal system architecture, preservation of the extracellular constituents permits continued function of these tissues and supports the recellularization of the systems over time.

Advances in Technology

The main focus of the early preclinical studies described here is targeted toward describing IRE's ability to destroy a targeted volume of tissue, while preserving critical structures in the vicinity, and describing the variation of observed effects in numerous tissue varieties. Following the successful translation of IRE to the clinic, newer advances to the technology are being explored at the benchtop and preclinical settings. These studies explore secondary effects of IRE that may further expand the treatment zone, improve selectivity, as well as improve ease of application. Some preclinical investigations into a selected number of these effects are presented below.

Immune Response and Activation

Where IRE is typically implemented by delivering electric pulses through electrodes inserted directly into the targeted tissue to induce the cell death, the affected tissue remains in situ following treatment. This permits the release of tumor-specific and tumor-associated antigens, as well as the various signals of cell distress into the treated and peripheral volumes. These molecules may have implications in the promotion of local and systemic immune responses, similar to those described from other ablation modalities.

One study explored the immunologic response to tumor ablation with IRE using a subcutaneous xenotransplanted osteosarcoma rat model (Li et al. 2012). The animals were randomized into four groups: the control, sham operation, surgical resection, and IRE group. Another set of rats without tumor cell implantation served as the normal non-tumor-bearing group. In each of the groups, anticoagulated venous whole blood samples were acquired before and days after the operation to monitor for T lymphocyte activity, the major source of cellular antitumor immunity in cancer patients. Specifically, the $CD3^+$ T lymphocytes that represent the major lymphocyte subset in peripheral blood, the $CD4^+$ (T helper cells) and the ratio of $CD4^+/CD8^+$ (T suppressor/cytotoxic cells), are generally used as an indicator of antitumor immunity. Additionally, rats were killed independently in each treatment group, and splenocytes were assayed for IFN-γ and IL-4 production using intracellular cytokine staining. T-cells exert their effector functions partly by producing and releasing cytokines and are characterized by their distinct cytokine expression patterns. Th1 cells secrete IFN-γ and IL-2, whereas Th2 cells produce IL-4, IL-5, and IL-10. The results of the study showed that the application of 1500 V/cm voltage-to-distance ratio in nine trains of ten direct current IRE pulses, each 100 μs long, could produce complete osteosarcoma cell ablation, while also providing a substantial immune response. Seven days after treatment, the IRE group had a significant increase in $CD3^+$ and $CD4^+$ cells and increased ratio of $CD4^+/CD8^+$. Additionally, IRE group showed an increased percentage of IFN-γ splenocytes.

A comparative in vivo murine study was conducted on immunocompetent (IC) versus immunodeficient (ID) mice implanted with RENCA murine kidney

cancer cells to gauge if IRE invoked a systemic immune response in the tumor environment (Neal et al. 2013). Two flat plate electrodes were utilized to apply IRE treatment on the subcutaneous murine tumors with a highly conductive gel facilitating improved current delivery into the tumor. Treatment parameters included voltage-to-distance ratio of 1500 V/cm, each 100 µs long, delivered at a rate of 1 pulse per second. A total of 100 pulses were delivered, reversing polarity after the first 50. Following pulse delivery, the electrodes were reoriented 90°, and the pulsing process was repeated, delivering a total of 200 pulses to the tumor. This protocol was selected due to its ability to produce an observable treatment response relative, but not strong enough to cause complete regressions in both strains of mice, which would make it difficult to discriminate any differences in treatment outcome. The treated IC group responded significantly better than the treated ID group, despite no inherent difference in initial tumor susceptibility. Similar susceptibility was indicated by tumors reaching a treatable size for both groups within the same number of days and similar tumor growth response to sham treatments. This suggests that although an immune response is not required for complete tumor regression, therapeutic response in immunocompetent patients may be better than predictions based on experimental studies using ID cancer models.

Overall, the exploitation and encouragement of the immune response demonstrated from IRE treatment of tumors remains a promising yet relatively unexplored field for improving IRE therapy outcomes.

Adjuvant Augmentation of IRE Treatments

In addition to encouraging a more robust immune response to further encourage effective oncologic outcomes from IRE therapy, numerous other promising adjuvant approaches exist to increase ablation zone and oncologic outcome from a given pulse protocol for IRE therapy. The most obvious of these is the inclusion of targeted chemotherapeutics already shown for electrochemotherapy to selectively kill reversibly electroporated cells in the zone surrounding the irreversibly electroporated cells. In addition, the use of conventional therapies as adjuvants to IRE may also improve patient outcomes, such as the inclusion of standard chemotherapy regimens to kill any distant micro-metastases, while IRE kills the primary tumor, or the use of transarterial chemoembolization to further stress and promote death of tumor cells.

Several studies have examined potential IRE and reversible electroporation therapy augmentation by locally manipulating the properties of the cell membranes to increase their susceptibility to electroporation and IRE. One approach that has been suggested, with implications primarily likely for in vitro applications, is the addition of dimethyl sulfoxide (DMSO) in the cell membrane to alter the structural properties of the lipid bilayer (Jiang et al. 2014). This study along with the first IRE tumor study (Al-Sakere et al. 2007) also explored the potential of modifying the temporal mode of delivery of pulses on IRE outcome, where it was shown that adding delays between trains of pulses can decrease the IRE electric field ablation threshold.

An additional study examined the sensitization of cells to electroporation within a given electric field by altering the electrochemical environment of cells in tissue in vitro with cationic and anionic substances. This study found markedly promising results with the inclusion of cationic anesthetics in the cellular environment, with particular benefits exhibited from procaine and lidocaine, where a 50% reduction in the strength of the electric field is required to induce cell death with IRE. While it remains to be determined whether such a pronounced effect will translate to in vivo environments, it offers a valuable potential adjuvant to safely augment the treatment zone of IRE and other electroporation-based therapies (▶ Chap. 91, "Combining Electrolysis and Electroporation for Tissue Ablation").

High-Frequency Irreversible Electroporation

Apart from improving ablation sizes, preclinical studies have shown that the ease of application of IRE treatment can be improved by manipulating pulse parameters, such as pulse duration and polarity. Clinically, IRE requires the administration of paralytic agents to prevent muscle contractions during treatment that are associated with the delivery of unipolar pulses of longer durations (~>5 μs). By reducing the pulse duration and applying bipolar IRE-inducing pulses, the threshold for nerve stimulation is increased and the muscle contractions are eliminated, removing the need for paralytic agents.

The first preclinical study on high-frequency irreversible electroporation (H-FIRE) was conducted on rat brain (Arena et al. 2011), and muscle contractions were quantified via accelerometers placed at the cervicothoracic junction. MRI and histological evaluation was performed postoperatively to assess ablation characteristics. Treatment parameters consisted of bipolar pulses of 1–2 μs duration and 100–400 V amplitude delivered in 200 μs long bursts at a frequency of 1 burst per second. Blunt electrodes of 1 mm spacing and exposure were used for the treatment. Results showed that, even in the highest energy H-FIRE protocol, no detectable peaks in acceleration above the inherent noise of the system were observed. Histopathologic examination of brain sections from all treatments demonstrated clear areas of ablation and sharp delineation from adjacent normal brain. This study showed H-FIRE to be a feasible technique for nonthermal tissue ablation that eliminates muscle contractions.

Conclusions

IRE's unique nonthermal modality of ablation without denaturing extracellular proteins permits IRE treatment in sites containing critical structures and vasculature that contraindicate them for thermal ablation or surgical resection. The advantages of IRE have resulted in the treatment being performed in an array of applications and anatomical target regions. This chapter discusses the preclinical findings of IRE in different sites and its treatment outcome in terms of ablation size and preservation of

critical structures. Additionally, secondary effects that may foster exploitation for enhancement of IRE treatment, such as anti-immune response, adjuvant therapies, and advances to IRE pulse protocols, offer evidence suggesting improved clinical outcomes for the technology and its clinical implications as the base of knowledge continues to grow.

Cross-References

► Tissue Ablation by Irreversible Electroporation

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