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Pilot Study of Irreversible Electroporation for Intracranial Surgery

Paulo A. Garcia, John H. Rossmeisl, Jr., John Robertson, Thomas L. Ellis, and Rafael V. Davalos

Abstract— **Irreversible electroporation (IRE) is a new minimally invasive technique to treat cancer using intense but short electric pulses. This technique is unique because of its non-thermal mechanism of tissue ablation. Furthermore it can be predicted with numerical models and can be confirmed with ultrasound and MRI. We present some preliminary results on the safety of using irreversible electroporation for canine brain surgery. We also present the electric field (460 V/cm – 560 V/cm) necessary for focal ablation of canine brain tissue and provide some guidelines for treatment planning and execution. This preliminary study is the first step towards using irreversible electroporation as a brain cancer treatment.**

I. Introduction

NON-THERMAL irreversible electroporation (IRE) is a promising new technique for the ablation of tissue and tumors [1-7]. This minimally invasive procedure involves placing electrodes into or around a targeted area and delivering a series of short and intense electric pulses to induce the irrecoverable structural changes in cell membranes [4]. To achieve IRE, the electric field in the targeted region needs to be above a critical value, which is dependent on a variety of conditions such as tissue type and pulse parameters [4].

Davalos, Mir, and Rubinsky postulated and demonstrated that IRE can be isolated from traditional thermal damage and used to destroy substantial volumes of tissue *in vivo* [1]. Their finding that IRE can be used as an independent modality for tissue ablation was subsequently confirmed in small and large animal models in the liver [3, 7], the prostate [2], and on mouse sarcoma tumors [5]. Rat liver tissue was ablated with a single 20-ms long pulse at 1000 V/cm [7], swine liver was treated with 90 pulses (100 µs) with a voltage-to-distance ratio between 1000 V/cm and 1667 V/cm [3], canine prostate was treated with 80 pulses (100 µs) at 1500 V/cm [2], and implanted sarcoma tumors in athymic mice were treated with four sets of 20 pulses (100 µs) at 2500 V/cm [5].

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R.V. Davalos, Ph.D., is with the Virginia Tech-Wake Forest University School of Biomedical Engineering and Sciences, Blacksburg, VA 24061 USA (phone:540-231-1979, email: davalos@vt.edu).

P.A. Garcia is with the Virginia Tech-Wake Forest University School of Biomedical Engineering and Sciences, Blacksburg, VA 24061 USA (pgarcia@vt.edu).

J. H. Rossmeisl, Jr., D.V.M., M.S. is with the Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg VA 24061 USA (jrossmei@vt.edu).

J. Robertson, D.V.M. is with the Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg VA 24061 USA (drbob@vt.edu).

T. L. Ellis, M.D. is with the Department of Neurosurgery at Wake Forest University School of Medicine, Winston-Salem, NC 27157 USA (tlellis@wfubmc.edu).

The advantages of IRE over other focal ablation techniques lay within its ability to kill tissue through a non-thermal mechanism. The mechanism of cell death is not well-understood but it has been postulated that it is via necrosis presumably due to loss of homeostasis. This method preserves the extracellular matrix, major blood vessels, and other sensitive tissues, enhancing treatment outcome [2, 6]. Furthermore, the ablation area can be predicted using numerical modeling for accurate treatment planning, and application of the procedure can be monitored in real-time using ultrasound and confirmed with both ultrasound and MRI [3, 6, 8]. The ablation of the targeted areas exhibits rapid lesion creation and resolution, prompting the repopulation of the region with healthy cells [2, 6]. Though treatment success is not dependent upon the immune system, a tumor specific immune response capable of helping to destroy any residual micro-metastases occurs, decreasing the chances of recurrence [2, 6]. These aspects, in conjunction with short treatment times and the minimally invasive nature of treatment administration, show strong potential for using IRE as an effective brain cancer treatment.

The study of how electrical fields can be applied to living tissue to induce irrecoverable damage to cell membranes without causing thermal damage is an emerging area of research, and it is critical to capitalize on all of the benefits of this new technique. To the best of our knowledge, this paper presents the first *in-vivo* experimental results supporting our hypothesis that IRE can be used to predictably treat brain cancer. We used shorter pulse durations (50 µs) than those used in the studies [2, 3, 5, 7] described previously in order to reduce the charge delivered to the tissue and decrease the heating generated by the procedure to protect the integrity of the brain. Our results suggest that IRE will be a promising technique for the ablation of brain tumors due to the non-thermal mechanism of cell death that allows for a quick, predictable, and minimally invasive treatment.

II. Materials And Methods

A. Clinical Procedure

The pilot study was approved by the Institutional Animal Care and Use Committee and performed in a Good Lab Practice (GLP) compliant facility. A female purpose bred beagle was used. The dog was systemically healthy and neurologically intact prior to entrance in the study based upon the findings of normal physical and clinical neurologic examinations. The results of complete blood counts and serum biochemical profiles were within normal reference ranges. In addition, no abnormalities were detected on scalp-

recorded electroencephalograms and baseline MRI examinations of the brain.

After induction of general anesthesia, a routine parietotemporal craniectomy defect was created to expose the right temporal lobe of the canine brain. A neuromuscular blocker (atracurium at 0.4 mg/kg IV) was administered to suppress patient motion prior to the IRE treatment. A superficial, focal ablative IRE lesion was created in the ectosylvian gyrus of the temporal lobe using the NanoKnife® (Angiodynamics, Queensbury, NY USA) blunt tip bipolar electrode that contains energized and grounded surfaces. After insertion of the electrode, we delivered nine sets of ten 50 µs pulses with a voltage-to-distance ratio of 2000 V/cm between the electrodes. We alternated the polarity of the electrodes between the sets to minimize charge build-up on the electrode surface. These parameters were determined from *ex-vivo* experiments on canine brain and they ensured that the charge delivered during the procedure was lower than the charge delivered to the human brain during electroconvulsive therapy, a treatment for major depression. The NanoKnife® was programmed to deliver each set of ten pulses at a frequency of 4 Hz. The electrode was inserted 2 cm parallel to the rostrocaudal length of the target gyrus, and 2 mm below the external surface (Fig. 4). The blunt tip bipolar electrode is similar in dimensions to those already used in deep brain stimulation, a therapy that alleviates the symptoms of otherwise treatment-resistant human disorders such as Parkinson's disease, tremor, and dystonia. In addition, the blunt tip probe has smaller dimensions than those used in ultrasound guided biopsy so there is minimal damage, as the healthy neural tissue was gently displaced during the probe insertion procedure.

B. Numerical Models

Numerical modeling can be used to simulate the irreversible electroporation treatment in tissue [4]. We modeled the electric field distribution in the canine brain tissue for the electrical parameters used in the pilot study. An open source image analysis software (OsiriX, Geneva, Switzerland) was also used to calculate the IRE focal ablation volumes from 3-D reconstructions of immediate post-operative MRI scans of the canine brain. The numerical models were used to correlate the reconstructed IRE lesion volumes with electric field distributions. In this manner we determined the electric field threshold needed to achieve IRE in canine brain tissue. The electric field threshold is critical for IRE treatment planning on future brain cancer canine patients in order to predict the complete treatment of the tumor volumes in the brain.

The methods for predicting IRE areas are similar to the ones described by Edd and Davalos [4]. The electric field distribution is given by solving the Laplace equation

$$\nabla \cdot (\sigma \nabla \varphi) = 0 \qquad (1)$$

where σ is the electrical conductivity of the tissue and φ is the electrical potential [4]. The electrical boundary condition along the tissue that is in contact with the energized electrode is $\varphi = V_o$. The electrical boundary condition at the interface of the other electrode is φ = 0. The boundaries where the analyzed domain is not in contact with an electrode are treated as electrically insulative.

For calculating the heating of the tissue resulting from each procedure one can use the Pennes Bioheat equation with the additional joule heating term, $\sigma|\nabla\Phi|^2$ [4]. The modified equation is then

$$\nabla(k\nabla T) + w_b c_b (T_a - T) + q''' + \sigma|\nabla\varphi|^2 = \rho c_p \frac{\partial T}{\partial t} \qquad (2)$$

where k is the thermal conductivity of the tissue, T is the temperature, w_b is the blood perfusion, c_b is the heat capacity of the blood, T_a is the arterial temperature, q''' is the metabolic heat generation, ρ is the tissue density, and c_p is the heat capacity of the tissue [9]. The mathematical models were solved using a commercial finite element package (Comsol Multiphysics, v.3.5a, Stockholm, Sweden). To solve (2), it is assumed that the entire tissue is initially at the physiologic temperature: T(x,y,z,0) = 37°C. The outer surface of the analyzed domain and the surfaces of the electrodes are taken to be adiabatic to produce an upper limit to the calculated temperature distribution in the tissue. We assumed the blood perfusion to be zero to achieve an upper limit of the temperature distribution even though the brain has significant vascularization that dissipates heat. In addition, the metabolic heat generation can be neglected due to the short duration of the treatment.

TABLE I
PHYSICAL PROPERTIES USED IN THE STUDY

Material	Quantity	Units	Value	Ref.
Brain	σ, electrical conductivity	S m^{-1}	0.285	[10]
	k, thermal conductivity	W m^{-1} K^{-1}	0.565	[9]
	c_p , heat capacity	J kg^{-1} K^{-1}	3680	[9]
	ρ , density	kg m^{-3}	1039	[9]
Insulation	σ, electrical conductivity	S m^{-1}	0.0	[11]
	k, thermal conductivity	W m^{-1} K^{-1}	0.01	[11]
	c_p , heat capacity	J kg^{-1} K^{-1}	3400	[11]
	ρ , density	kg m^{-3}	800	[11]
Stainless Steel	σ, electrical conductivity	S m^{-1}	2.22E6	[5]
	k, thermal conductivity	W m^{-1} K^{-1}	15	[11]
	c_p , heat capacity	J kg^{-1} K^{-1}	500	[11]
	ρ , Density	kg m^{-3}	7900	[11]

III. Results

A. Clinical Procedure

Grossly visible brain edema and surface blanching of the gyrus overlying the ablation site was apparent within 2 minutes of completion of IRE procedure. This edema resolved completely following intravenous administration of 1.0 g/kg of 20 % mannitol.

Neurosonography performed intra-operatively, at 1hr and 24hr post-operatively demonstrated clearly demarcated ablation zone and visible electrode tract within the targeted brain parenchyma. MRI examination performed immediate post-operatively demonstrated that IRE ablation zones were sharply demarcated T1 iso-to hypointense, T2 hyperintense and mild and peripherally contrast enhancing following intravenous administration of gadolinium. The images are

consistent with fluid accumulation within ablation sites and a focal disruption of the blood-brain-barrier. Fig. 1 shows an axial slice of the MRI sequence in which the IRE lesion is seen as the bright 1.1cm x 1.3cm area.

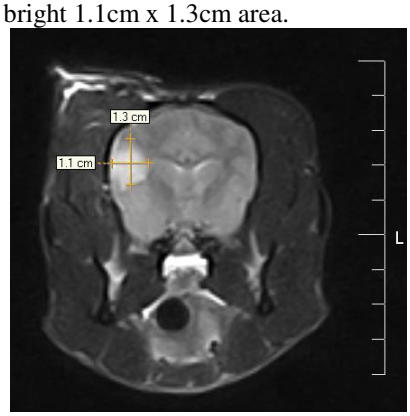

Fig. 1. T2 weighted MRI of superficial irreversible electroporation ablation site in the canine brain; the dog's left is normally projected on the right.

Post-operatively, the canine subject did not experience any adverse clinically apparent effects attributable to the IRE procedure. There was no significant deterioration in neurologic disability or coma scale scores from baseline evaluations, and the patient was able to assume normal activities, including eating, within 10 hours post-IRE.

The microscopic lesions from the histopathology correlated well with gross appearance and with the clinical progress of the dog following IRE treatment. The areas of treatment are represented by foci of malacia and dissociation of white and grey matter. Small perivascular hemorrhages are present and there is sparing of major blood vessels. Notable in multiple sections is a relatively sharp line of demarcation (approximately 20-30 μm) between areas of frank malacia and more normal, organized brain substance (Fig. 2).

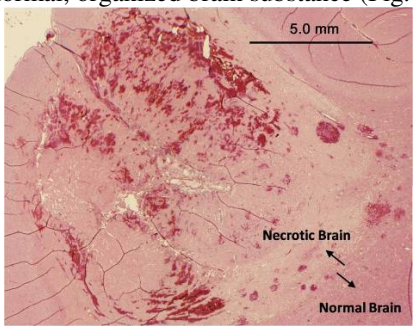

Fig. 2. Histopathology analysis showing the sharp delineation between the regions of normal and necrotic canine brain tissue after an IRE procedure.

B. Numerical Models

Fig. 3 shows the 3-D reconstructed lesion volume (1.655 ± 0.255 cm^3) corresponding to the IRE treatement of canine brain. The white rings that surround the reconstructed (green) lesion correspond to the margin between the lesioned and healthy brain as seen in the MRI. The blunt tip bipolar electrode runs along the major axis of the reconstructed lesion volume.

Fig. 4 shows the electric field distribution generated by the bipolar electrode used to generate the IRE lesions in the canine brain. We correlated the volume computed from the reconstructed lesion with the volume of the brain exposed to a minimum electric field between 460 V/cm and 560 V/cm.

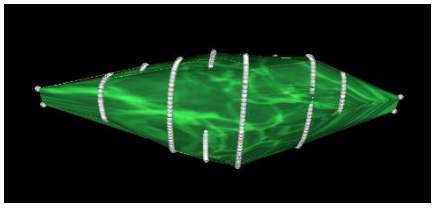

Fig. 3. Reconstructed 3-D lesion volume created by the IRE treatment in canine brain. The white rings correspond to the boundary between the lesioned and normal brain from the T2 weighted post-operative MRI.

The given electric field threshold range corresponds to the upper and lower computed volumes from the 3-D reconstructions respectively. Physicians and veterinarians should be aware of the electric field threshold in order to prevent unnecessary damage to surrounding healthy tissue or perform incomplete treatments.

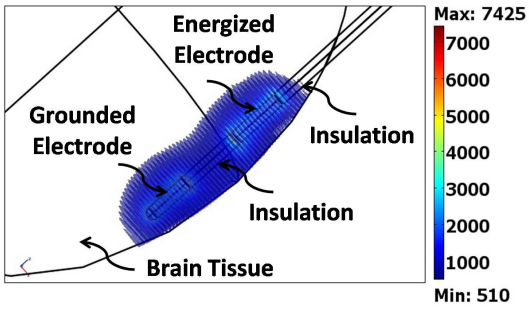

Fig. 4. Electric field distribution (V/cm) resulting from the single probe IRE pilot treatment in canine brain. The electric field threshold (460 V/cm - 560 V/cm) to achieve IRE was determined by correlating the 3-D reconstructed lesion volume with the resulting electric field distribution.

Fig. 5 shows the temperature distribution generated during the IRE treatment at the electrode-insulation interface, which is where the highest electric field is generated. From the plot, one can visualize that there is a mild temperature increase after each pulse which is virtually dissipated by the time the following pulse is delivered. Since thermal damage is a function of temperature and length of exposure, the negligible heating associated with our procedure is emphasized by the fact that an electroporation pulse is typically a very small fraction of a second long [4, 5]. Therefore, we confirm that the ablation of the canine brain tissue was a result of the irrecoverable cell membrane damage achieved by IRE rather than thermal damage.

IV. Discussion

This paper demonstrates the first example of irreversible electroporation for intracranial canine surgery. We created a superficial focal lesion in the right temporal lobe of a canine subject using a blunt tip bipolar electrode, and found a reconstructed lesion volume of 1.655 ± 0.255 cm^3 from the post-operative MRIs. The ablation was confirmed with the histopathology analysis, which showed a sharp boundary (20μm-30μm) between the necrotic and normal brain. The canine subject did not demonstrate any adverse clinical effects after the IRE procedure and was eating and able to assume normal activities within 10 hours of surgery.

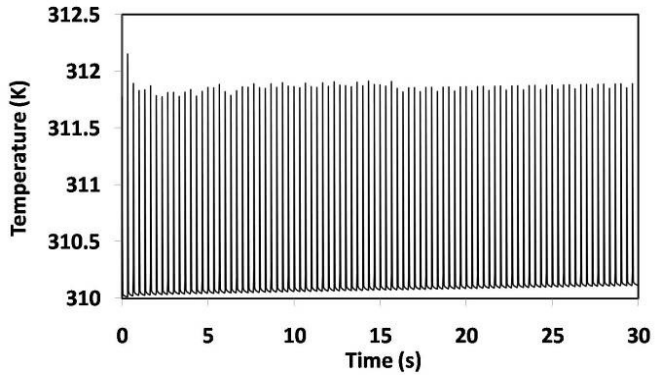

Fig. 5. Highest temperature distribution generated in the canine brain during an IRE treatment calculated at the electrode-insulation interface. The change in temperature is not sufficient to generate thermal damage which confirms the non-thermal aspect of irreversible electroporation [1].

The non-thermal mechanism of cell death in IRE allows for predictable lesions and we have correlated this volume range to the corresponding electric field range between 460 V/cm-560 V/cm using numerical models. We also assessed the increase in temperature generated by the electric pulses and confirmed that the energy generated by the procedure is not sufficient to achieve thermal damage, which emphasizes that irreversible electroporation is an independent tissue ablation modality. Future models will incorporate tissue heterogeneities and the effect of irreversible faradaic reactions during the pulses to understand their influence in the resulting electric field and temperature distributions.

Although these results are encouraging, there are possible complications that could arise from this procedure, including edema, seizures or bleeding, that require further investigation. Edema should be manageable medically with corticosteroids and a hyperosmolar agent such as mannitol to prevent increased intracranial pressure but may be severe enough to require a more invasive operation. Seizures in most cases should be preventable by giving prophylactic anticonvulsants. Bleeding due to electrode insertion is estimated to be in the 1-2% probability range based on brain tumor experience in the human population.

Irreversible electroporation offers advantages (minimally invasive device and small electrode sizes, adaptability to virtually any neuroanatomic location with existing stereotactic guidance systems) over open surgery to resect brain tumors. It also offers biologic advantages (minimal heat generation during treatment and sparing of major blood vessels) that may make it attractive as a complimentary technique to facilitate complete tumor resection. Finally, the use of IRE to facilitate delivery of molecularly targeted anti-neoplastic biologics within the reversibly electroporated area of brain offers another tremendous potential area for research and development.

It is important to note that the histological results showed that the lesions generated by the IRE procedure are 1.0mm-2.0mm larger in maximal 2-D diameter than the 1hr post-operative MRI. In our future studies we will incorporate the growth of the lesion into our treatment planning. Provisions for administration of an adequate dose of neuromuscular blocker are vital to prevent patient motion during the IRE treatment and non-uniform delivery of the electric pulses. We have shown that it is safe to use irreversible electroporation for canine intracranial surgery and further studies will be performed in order to reliably reproduce and confirm the preliminary results presented in this paper. These initial results are the first step towards using irreversible electroporation as a brain cancer treatment.

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