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Electroporation-Mediated Transcatheter Arterial Chemoembolization (E-TACE) in the Rabbit VX2 Liver Tumor Model

Yang Guo, MD¹, Yue Zhang, BS^{1,2}, Ning Jin, PhD^{1,4,5}, Rachel Klein, BS¹, Jodi Nicolai, MS¹, Robert J. Lewandowski, MD^{1,3}, Robert K. Ryu, MD¹, Reed A. Omary, MD, MS^{1,4,6}, and Andrew C. Larson, PhD^{1,3,4,6}

¹Department of Radiology, Northwestern University, Chicago, IL, USA

²Department of Bioengineering, University of Illinois at Chicago, Chicago, IL, USA

³Department of Radiation Oncology, Northwestern University, Chicago, IL, USA

⁴Department of Biomedical Engineering, Northwestern University, Chicago, IL, USA

⁵Cardiovascular MR R&D, Siemens Healthcare, Chicago, Illinois, USA

⁶Robert H. Lurie Comprehensive Cancer Center, Chicago, IL, USA

Abstract

Rationale and Objectives—Electroporation involves the application of electrical pulses to increase cell membrane permeability. The purpose of our study was to demonstrate the potential to use electroporation-mediated transcatheter arterial chemoembolization (E-TACE) approaches to increase liver tumor drug uptake while using magnetic resonance imaging (MRI) for intra-procedural optimization of these procedures.

Methods—14 VX2 tumors were grown in the left hepatic lobes of 8 rabbits. Two tumors were grown in each of 6 rabbits (one tumor serving as E-TACE treated tumor and other as non-electroporated control) and solitary larger tumors were grown in 2 rabbits (half of the tumor treated with E-TACE, remaining half serving as control). Each rabbit was selectively catheterized under digital subtraction angiography (DSA) guidance. Baseline MRI was performed to generate tumor contrast enhancement curves following catheter-directed infusion of gadopentetate dimeglumine to estimate the proper time delay between subsequent bolus infusion of cisplatin and application of electrical pulses (electrodes were used to deliver 8 100µs 1300V pulses at the selected delay interval post-infusion). 3 hours after E-TACE, rabbits were euthanized and tumors sectioned for inductively coupled plasma mass spectroscopy (ICP-MS) measurements of platinum concentration (serving as reference standard of cisplatin uptake levels).

Results—ICP-MS results demonstrated significantly increased cisplatin uptake in E-TACE treated tumor tissues, increases of 6.0 ± 3.3 fold compared to transcatheter infusion alone ($p=0.017$).

Corresponding Author: Andrew C. Larson, Ph.D. Associate Professor Departments of Radiology and Biomedical Engineering Northwestern University Feinberg School of Medicine 737 N. Michigan Ave, 16th Floor, Chicago, IL 60611 phone: (312)926-3499 fax: (312)926-5991 a-larson@northwestern.edu.

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Conclusions—Our findings suggest that our E-TACE approach may significantly increase liver tumor drug uptake following targeted transcatheter infusion. MRI measurements permitted intra-procedural guidance during these catheter-directed E-TACE procedures.

Keywords

Liver Cancer; Transcatheter Arterial Chemoembolization (TACE); Electroporation; MRI; Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)

Introduction

Transcatheter tumor therapy involves the intravascular delivery of therapeutic agents via selective catheter placement with imaging guidance¹. Various agents such as chemotherapeutics, embolic particles, or radioactive materials can be injected via the feeding vessels of the tumors resulting in tumor ischemia and necrosis, and transcatheter arterial chemoembolization (TACE) is one such commonly used approach^{1,2}. Despite several recent advances and technical refinements, the long-term survival outcomes with this approach are not fully satisfactory, mainly as a result of the high rates of tumor recurrence. Widely accepted criticisms of TACE include: a) permanent embolic effects leading to hypoxia prompting angiogenesis and potentially accelerating tumor progression, b) insufficient internalization of poorly permeant chemotherapeutic agents, and c) poor retention of the chemotherapeutic agents within the vascular bed of the targeted tumor³⁻⁵. Modifications of the TACE procedure are needed to avoid potential embolic agent related side effects and achieve increased chemotherapeutic agents uptake in the targeted tumors to enhance treatment response⁶.

Electroporation, or electroporabilization, is the phenomenon associated with an increase of cell membrane permeability when microsecond to millisecond electrical pulses are applied across the cell. This permeabilization can be either reversible (temporary) or irreversible (permanent) depending on the electrical field magnitude, the pulse duration and the number of pulses applied⁷. Reversible electroporation has become an important tool in drug delivery, gene transfer and cell infusion which increases local cellular uptake of therapeutic agents due to the transient increases in cell membrane permeability. With reversible electroporation and sufficiently rapid resealing of the cell membrane, this approach has been recently approved in clinical practice and described as electrochemotherapy (ECT) for the treatment of both cutaneous and sub-cutaneous tumors⁸. In addition to transfection-enabling cell permeabilization, ECT pulses can cause transient decreases in blood flow in the electroporation zones described as a ‘vascular-lock’ effect; these lead to a transient (reversible) embolic effect and drug retention in the targeted region⁹. The salient combination of electroporation and TACE approaches (E-TACE) may improve uptake of chemoagents (e.g. Cisplatin) in targeted tumors while avoiding permanent embolization-related side effects.

Transcatheter intra-arterial perfusion - magnetic resonance imaging (TRIP-MRI) has been demonstrated effective for intra-procedural monitoring of TACE procedures¹⁰⁻¹². Recently, both T1-weighted (T1W) and T2-weighted (T2W) MRI has been used to monitor tissue response during intra-hepatic electroporation procedures^{13,14}. The purpose of our study was to demonstrate the potential to use E-TACE approaches to increase liver tumor drug uptake while using MRI measurements for intra-procedural guidance of these image-guided catheter-directed procedures.

Materials and Methods

Animal Model

All experiments were approved by our institutional animal care and use committee. Eight New Zealand white rabbits (Covance, Denver, PA) weighing 3.5 - 4.5kg initially were used in these experiments. VX2 cells were first grown in the hind limb of a donor rabbit. Two viable tumor portions approximately 8mm³ in volume were harvested from the donor rabbit and prepared for liver VX2 implantation in each rabbit. After sterile preparation of the subxiphoid abdominal area, a mini-laparotomy procedure was performed to expose the left medial hepatic lobe. First, one tumor portion was implanted into the liver using a fine tip forceps; the area was covered with a small piece of hemostatic gauze to arrest bleeding and avoid migration of tumor. Next, the second portion was implanted in the same lobe, roughly 3cm from the first site. One tumor was targeted for E-TACE treatment while the other served as an internal control. The abdomen was closed using a three layers technique.

Conventional Digital Subtraction Angiography (DSA)

Each rabbit was successfully catheterized under DSA guidance. Conventional DSA was performed using a C-arm unit (PowerMobil; Siemens Medical Solutions, Erlangen, Germany). Anesthesia was first induced by using a mixture of intramuscular Ketamine (44 mg/kg) and Xylazine (5 mg/kg) in each rabbit and maintained with intermittent intravenous injections of Nembutal (10-20mg/kg) given to effect. The femoral artery was catheterized through a cut-down using a 3-F vascular sheath (Cook, Bloomington, IN). A 2-F catheter (JB-1; Cook, Bloomington, IN) was advanced over a 0.014-inch-diameter guidewire and the celiac artery was selectively catheterized. The catheter was advanced into the left hepatic artery of each rabbit using manual injection of 2mL iodinated contrast agent (Omnipaque 350, Amersham Health, Princeton, NJ). After contrast agent injection through the catheter, DSA results confirmed superselective placement of catheter in the left hepatic artery with both tumors enhanced on DSA images (Fig. 1).

MR Imaging

MR imaging was performed using a 1.5T clinical MR imaging scanner (Espree; Siemens Medical Solutions, Erlangen, Germany). Rabbits were imaged in the supine position using body matrix array coil and spinal array. T2W turbo spin-echo scans were acquired to optimize imaging positions for subsequent TRIP-MRI measurements with the following parameters: TR/TE = 3640/56ms, slice thickness = 4mm, averages = 3, voxel size = 1.6×1.6×8.0mm, matrix size = 192×120 and bandwidth = 205 Hz/pixel.

After localization, baseline TRIP-MRI was performed to generate tumor enhancement curves with a manual intra-arterial (i.a.) injection of 3.0ml of 10% gadopentetate dimeglumine (Magnevist; Berlex Laboratories, Wayne, NJ) contrast injection over 5 seconds. For TRIP-MRI, a two-dimensional multi-section saturation recovery spoiled gradient-echo (GRE) pulse sequence was used with the following parameters: TR/TE/TI=300/1.26/110msec, slice thickness = 8mm, flip angle = 15°, FOV = 200×125mm, matrix = 128×80, bandwidth = 601Hz/pixel, 1-3slices, 0.3sec/slice/measurement, 150-300 measurements for each slice, mean total imaging time = 90sec. These contrast enhancement curves were generated to estimate time delay between IA bolus infusion and the period of maximum tumor uptake (for planning purposes to optimize delay interval, Td, between later IA cisplatin infusion and application of electrical pulses).

E-TACE Procedures

A BTX Electroporator (ECM830; Harvard apparatus, Holliston, MA) function generator and a parallel two-needle electrode array (10 mm spacing) were used for all rabbit

electroporation procedures. The parameters we selected for reversible electroporation were based on previous studies: electrical voltage = 1300V/cm, 8 square wave electrical pulses, 100µs pulse length, 100ms pulse interval⁹. Prior to *in vivo* studies, we used a commercial finite element modeling (FEM) software package (COMSOL Multi-Physics, version 3.3) to simulate the anticipated electroporation pattern based on the above-described parameters.

Our simulation closely followed previous electroporation modeling studies solving the Laplace equation to calculate induced electrical field potentials based on anticipated electrode position and tissue conductivities^{15,16}. A simple three-dimensional model was created in the shape of a 40mm diameter symmetric cylinder with the COMSOL software. In this simulation a 5mm diameter tumor was approximated as a sphere located within the center of this cylinder for the calculation of a standard protocol (used for all subsequent *in vivo* studies). Within this model, the two parallel electrodes, 10mm spacing, were positioned to straddle the tumor. For simplicity, the conductivity of the normal liver tissues and tumor tissues were assumed homogenous with a value of 0.125 S/m¹⁷. An electrical field threshold of 362V/cm was considered sufficient to for reversible electroporation (Fig. 2)¹⁸.

A mini-laparotomy incision was made to expose and visually locate the VX2 tumors within the left hepatic lobe. For E-TACE treated tumors, the parallel electrodes were inserted straddling the targeted tumor and the electrical pulses were applied at the selected delay interval T_d after IA administration of the cisplatin ($\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$, Spectrum Chemical Corp., NJ) solution (1mg/kg dissolved in 3ml saline) through the catheter. For two animals that developed only a solitary larger tumor, the latter procedure was altered with electrodes inserted off-center to intentionally treat only one half of the tumor tissue with the electroporation pulses. Following E-TACE procedures, the abdomen was closed with a 2-layer technique and each animal was immediately transferred back to MR scanner for post-treatment imaging.

Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)

After allowing 3 hours for cisplatin wash out, rabbits were euthanized with an intravenous administration of 150–200 mg/kg sodium pentobarbital (Euthasol; Delmarva Laboratories, Midlothian, Virginia). E-TACE treated tumor tissues, non-electroporated tumor tissues (non-E), normal liver tissues in the same lobe with tumors (NORs) and normal liver tissues in different lobe from the tumors (NORd) were collected and digested. Next, ICP-MS measurements were performed (X Series 2 ICP-MS, Thermo Fisher Sci., USA) to estimate platinum concentrations in each sample (serving as our reference standard to assess tissue cisplatin concentrations).

Statistical Analysis

All statistics were performed using the SPSS statistical software package (SPSS, version 17, Chicago, IL). Tissue cisplatin uptake levels were compared by paired t-test between E-TACE treated tumors and non-electroporated tumors, between E-TACE treated tumors and normal tissues in the same tumor-bearing lobes and between E-TACE treated tumors and normal tissues in the other lobes. Also, paired t-tests were used to compare cisplatin uptake levels in non-electroporated tumors and normal liver tissues in the same tumor-bearing lobe as well as in non-electroporated tumor tissues and normal liver tissues in the other lobes. Test was considered statistically significant with a P value <0.05.

Results

In the eight rabbits, fourteen VX2 liver tumors were grown in 16–28 days. Two tumors were grown in 6 rabbits (0.6–1.2cm diameter tumors; one serving as E-TACE treated tumor and

other as non-treated control) and solitary larger tumors were grown in 2 rabbits (2.1 and 2.0 cm; half treated with E-TACE, remaining half serving as control). Minor bleeding was observed from the electrodes insertion sites when electrodes were withdrawn; bleeding was stopped with cotton applicator and hemostat. No intra-procedural or early post-procedural complications (e.g., respiratory or heart arrest, massive internal bleeding, sudden death etc.) were observed in any of the rabbits. Gross anatomy images pre and post E-TACE treatment from a VX2 liver tumor rabbit are shown in Fig. 3.

TRIP-MRI

Baseline TRIP-MRI images of the VX2 tumors acquired at peak enhancement demonstrated characteristic peripheral rim signal enhancement similar to that of the pre-treatment DSA images (Fig. 4a). Contrast enhancement time curves were generated (Fig. 4b) to estimate the time delay between IA bolus infusion and the period of maximum tracer uptake with the tumor (T_d). For these 8 rabbit studies $T_d = 17.7 \pm 5.6$ sec; for each rabbit, during subsequent EP procedures, after cisplatin bolus infusion the electroporation pulses were applied only after this rabbit-specific T_d delay time (estimated individually for each animal).

ICP-MS

In 6 out of 8 rabbits, ICP-MS results for platinum concentrations demonstrated significantly increased cisplatin uptake in E-TACE treated tumors, 6.0 ± 3.3 (mean $\pm$ SD) fold increases compared to transcatheter infusion alone ($p=0.017$). These measurements also demonstrated significantly increased cisplatin uptake in E-TACE treated tumors relative normal liver tissues in both the tumor-bearing lobe ($p=0.024$) and non-tumor bearing lobe ($p=0.024$). In the other 2 rabbits, ICP-MS results for platinum concentrations showed no increase in cisplatin uptake (Rabbit #5 and #8). A graph showing these platinum concentration measurements in the four different tissue regions from each rabbit is shown in Fig. 5. Platinum concentrations demonstrated no significant increase in cisplatin uptake between non-electroporated tumors and normal liver tissues in the same tumor-bearing lobe ($p=0.475$) and no significant difference between cisplatin uptake in non-electroporated tumors and normal liver tissues in the other lobes ($p=0.370$).

Discussion

All animals tolerated the E-TACE procedures. No distant effects of the electrical pulses or mortalities were observed during E-TACE procedures and follow up intervals. ICP-MS measurements of the cisplatin concentration in E-TACE treated tumor tissues showed significantly increased chemotherapeutic uptake, an increase of roughly 6.0 fold compared to transarterial infusion alone. E-TACE could potentially serve as a new alternative approach to conventional TACE methods for the treatment of liver tumors.

Previous ECT studies have shown that electroporation can temporarily increase cell membrane permeability to increase of cisplatin uptake within targeted tumors following intravenous drug infusion^{19,20}. Alternatively, for the current study we used a targeted intra-arterial chemo-infusion approach and observed that six out of eight VX2 tumor rabbits demonstrated a 2.71-11.0 fold increase in cisplatin uptake in E-TACE treated tumors compared to non-electroporated tumors (internal controls). Although we did not conduct longitudinal studies to assess tumoricidal efficacy, based on previous ECT efficacy studies in SA-1 tumors (increased response with 2-fold increases in cisplatin uptake detected by atomic absorption spectrometry)¹⁹, we anticipate that the 6-fold increases in cisplatin uptake within E-TACE treated tumors should lead to superior responses. However, further studies remain necessary to fully investigate the relationship between E-TACE mediated drug delivery and long-term therapeutic outcomes.

Two out of the eight VX2 rabbits showed no increase in cisplatin uptake within the E-TACE treated tumor tissues compared to the non-electroporated tumor tissue. In both of these cases, either tumor blush was present during selective DSA or tumors enhanced during contrast MRI; both of the latter factors suggesting that these were vascularized tumors. In one rabbit the cisplatin concentration in both electroporated and non-electroporated tumors was lower than that of the normal liver tissue; the other such rabbit showed a significantly higher concentration of cisplatin within both electroporated and non-electroporated tumors compared to the normal liver tissues. The source of these two inconsistencies is unclear; these may have resulted from either differential blood flow to the two tumors distal to the catheter-tip position, variant collateral supply to the tumors, or induced electrical field patterns that were inconsistent with those anticipated from our prior numerical simulations.

One difference between our E-TACE approach and most conventional TACE methods is that the cisplatin is not infused as an emulsification within ethiodized oil. For conventional TACE approaches, this oily emulsion is infused to simultaneously provide both a chemotherapeutic effect and an embolic effect by obstructing tumor feeding vessels to induce ischemia²; following transcatheter infusion of the ethiodized oil and chemotherapy drugs, typically an additional infusion of gel foam particles or embolic microspheres is used to slow flow even further. However, while conventional TACE methods are widely used in clinical settings, many are critical of permanent embolic approaches given the growing body of evidence that these may induce hypoxia effects that counterproductively stimulate angiogenesis and promote tumor growth, recurrence and metastasis²¹. Conversely, for E-TACE, the electrical pulses provoke transient vascular lock effects that should allow prolonged chemical retention (similar to a temporary embolization effect) but avoid the potential side effects of permanent embolization due to the transient nature of the flow obstruction⁹. Clearly, the requirement for electrode placement increases the complexity of E-TACE procedures relative to conventional TACE methods; however, image-guided percutaneous electrode placement is already standard clinical practice in interventional settings for intra-hepatic thermal and chemical ablation procedures and a commercially developed percutaneous device (NanoKnife™, AngioDynamics Inc.) has already received FDA approval for electroporation procedures in the liver. However, the E-TACE procedure (compared to irreversible electroporation ablation, IRE, methods) involves the placement of arterial infusion catheter for drug delivery in addition to the placement of electrodes; future studies are necessary to compare the efficacy of E-TACE and IRE methods along with the potential to combine these methods for improved outcomes.

TRIP-MRI methods were used to estimate the optimal delay time between subsequent cisplatin bolus infusion and electrical pulse application. We assumed: a.) given identical infusion parameters (injection rates and bolus sizes for both Gd contrast and cisplatin infusions) that the TRIP-MRI measurements would be predictive of the time period of maximum cisplatin content in the tumor post-infusion²² and b.) electroporation at this period would lead to the greatest increase in cisplatin retention within the targeted tumor. A limitation of this E-TACE feasibility study was that these latter assumptions, pertaining to MRI-based selection of timing parameters, were not rigorously validated. The primary focus of the study was a comparison of E-TACE to conventional transcatheter arterial infusion methods but future additional studies are clearly warranted to validate these plausible but nonetheless currently unproven assumptions (studies comparing relationship between TRIP-MRI measurements and cisplatin uptake for a broad range of different post-infusion electroporation delay intervals). Future studies should also rigorously compare longitudinal outcomes with follow-up tumor necrosis measurements in conventional TACE and E-TACE treated animals.

In conclusion, these studies in the rabbit VX2 liver tumor model demonstrated that the proposed E-TACE approach can significantly increase chemotherapeutic uptake following targeted transcatheter infusions. Intra-procedural MRI measurements offer the potential for patient specific optimization of these E-TACE methods. Long-term survival studies are now needed to evaluate *in vivo* anti-neoplastic therapeutic efficacy this approach with rigorous comparisons to conventional TACE methods.

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Figure 1. Coronal DSA image (a) and zoom in region of interest (b) in one representative rabbit confirming superselective catheter placement in left hepatic artery (arrow, blue) with peripheral enhancement of two adjacent VX2 liver tumors (arrowheads, red) after iodinated contrast agent injection through the catheter.

Figure 2.

Coronal (**a**) and transverse (**b**) depiction of 3D FEM model with 5 mm spherical tumor (circle, red) located between two parallel needle electrodes (10mm spacing, straddling the tumor (blue)). Coronal (**c**) and transverse (**d**) depiction of FEM-anticipated electroporation zone for selected parameters (E -field > 362 V/cm, color bar at right showing the E -field distribution above the 362V/cm threshold¹⁸).

Figure 3.
Gross anatomy images pre (**a**) and post E-TACE treatment (**b**) for VX2 liver tumor rabbit
(arrowheads indicate electrode insertion positions).

Figure 4. Baseline TRIP-MRI image showing peripheral enhancement of two adjacent rabbit VX2 liver tumors (**a**, red arrows); TRIP-MRI generated tumor enhancement curve and estimated time of peak enhancement, T_d (**b**).

Figure 5. Platinum concentrations in normal liver tissues from non-tumor bearing lobe (NORd, blue), normal liver tissues from tumor bearing lobe (NORs, red), non-electroporated tumor tissues (non-E, green) and E-TACE tumor tissues (E, purple).

Summary of VX2 tumor size, tumor-specific delay time (Td) and intra-tumoral cisplatin increase (%) in E-TACE treated tumor tissues relative to control tumor tissues.

Table 1

Rabbit No.	Td (second)	No. of VX2 Tumor	Tumor size (cm)		Tumor Cisplatin Increase (%)
			Control	E-TACE	
1	17	2	1.26	1.12	746
2	12	2	0.75	0.82	456
3	22	2	1.03	1.11	171
4	12	2	0.95	0.95	185
5	21	2	0.70	0.60	-59
6	12	1	2.03		408
7	25	1	2.13		1000
8	20	2	1.11	0.92	-1