Biochemical and Biophysical Research Communications 435 (2013) 651–656

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Changes of apoptosis in tumor tissues with time after irreversible electroporation

Hong-Bae Kim [a], Chang-Kyu Sung [a,*], Ku Youn Baik [b], Kee-Wook Moon [c], Hyung-Sik Kim [d], Jeong-Han Yi [d], Jong-Hyun Jung [e], Min-Hoan Moon [a], Ohc-Kyung Choi [a]

[a] Department of Radiology and Seoul National University Cancer Research Institute, Seoul National University College of Medicine, SNU-SMG Boramae Medical Center, 39 Boramae-Gil, Dongjak-Gu, Seoul 156-707, Republic of Korea
[b] Department of Electrical and Biological Physics, Kwangwoon University, 26 Kwangwoon-gil, Nowon-gu, Seoul 139-701, Republic of Korea
[c] Solco-Biomedical Institute, Solco-Biomedical Co. Ltd., 34-6 Geumam-ri, Seotan-myeon, Pyeongtaek, Gyeonggi-do 451-852, Republic of Korea
[d] Department of Biomedical Engineering, Research Institute of Biomedical Engineering, College of Biomedical & Health Science, Konkuk University, 322 Danwol-dong, Chung-ju-si, Chungcheongbuk-do 380-701, Republic of Korea
[e] Computational Nano-Materials Physics Lab., Department of Physics and Astronomy, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-742, Republic of Korea

ARTICLE INFO

Article history:
Received 7 May 2013
Available online 17 May 2013

Keywords:
Irreversible electroporation
Apoptosis
Contrast-enhanced magnetic resonance image
Histological examination
Devascularization

ABSTRACT

Irreversible electroporation is a novel method of ablating living tissues through its non-thermal effects, unlike radiofrequency ablation which has a severe problem of heat sink. It is due to high-energy direct current which leads to permanent disruption of lipid bilayer integrity in terms of exchanges between intra- and extracellular components via nano-sized pores. That finally causes irreversible damage to cellular homeostasis. Irreversibly damaged cells may undergo apoptosis followed by necrosis with time after electroporation. This damage can make it possible to monitor the ablated area with time post-IRE through MR imaging and an ultrasound system. Most previous studies have investigated the immediate response of undesired tissue to IRE. In our study, we showed changes of tumor tissues with time post-IRE by histological analysis and MR imaging. Tissues under IRE ablation showed a peak apoptotic rate at 24 h after IRE ablation with viable tissues at the peripheral rim of treated tissues in histological analysis. This phenomenon was also observed with no enhancement on contrast-enhanced MR images due to devascularization of IRE ablated zones.

1. Introduction

Irreversible electroporation (IRE) has pervaded the clinical field since the report of application of IRE in ablating of pig's liver without harming surrounding tissues by the Rubinsky group [1]. IRE is a modality using microsecond electrical pulses across cells to induce permanent permeabilization of the cell membrane, after all, cell death if the applied power is high enough. IRE has been known to produce precisely delineated ablation zones with cell scale resolution between ablated and non-ablated areas, with preserved massive blood vessel congestion in the sinusoids of the treated region. IRE can be thus applied to ablate target tissues without affecting the surrounding normal tissue including blood vessels [2]. This localized ablation of IRE make it possible to immediately monitor the IRE treated region via histology, magnetic resonance image (MRI) [3,4] and an ultrasound system [5].

Previous studies have reported the immediate response of IRE treated tissues and have shown that IRE induced cell apoptosis and necrosis [3,4]. However, these changes should be followed by many other processes and it may last over one day. To monitor these changes with time *in vivo* is very important to assess IRE effects on tissues. Furthermore, previous studies have used normal animal organs or cancer cell lines, and few studies have used tumor tissues *in vivo*. Due to different properties of tissues, to apply IRE directly to tumor tissues is important for estimating the capacity of IRE for cancer therapy. In this pilot study, we assessed the changes within tumor tissues after IRE ablation with time by using histological analysis and MR imaging.

2. Materials and methods

2.1. Tumor cell line

The human prostate cancer line PC-3 was received from Korean Cell Line Bank and cultured in RPMI 1640 (ATCC) with L-glutamine (300 mg/L), 25 mM HEPES and 25 mM $NaHCO_3$ supplemented with 10% fetal bovine serum (Sigma–Aldrich). Cells were maintained in culture petri-dishes at 37 °C in a humidified atmosphere containing 5% CO_2. Before each implantation procedure, viability of the

* Corresponding author. Fax: +82 2 870 3539.
E-mail address: sckmd@naver.com (C.-K. Sung).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.05.039

cells was tested through trypan blue staining (confirming >90% cell viability for each tumor implantation procedure).

2.2. Tumor models

All studies were approved by our institutional animal care and use committee and were performed in accordance with institutional guidelines. *Female athymic nude mice (BALB-c-nu/nu, 5–7 weeks old, 15–20 g, n = 15; Japan SLC, Inc., Hamamatsu, Japan) were introduced.* These mice were underwent the process of medical inspection and were given a resting period of one week after introducing. All mice were cared under facility-specific measure (SPF). PC-3 human prostate cancer cells were each visually injected at 1.0×10^6 cells/ml into both flakes (one for control, the other for IRE). These tumors were retained to be 10 mm in diameter to be suitable for IRE treatment procedures. Following growing of the tumor, these mice were randomly allotted as following time: 5, 12, 24, 72 h after IRE including the control, having 3 mice each time.

3. IRE procedures

3.1. IRE apparatus and dosing condition

An electropoator (Nano-porator; SolcoBiomedical Ltd., Co.) as an apparatus was used for IRE procedures with the following specifications: 1500 V output and 50 ampere at 100 μs pulse width maximum respectively, ranging from 100 μs to 100 ms for pulse duration, in accordance with the Korean Food and Drug Administration (KFDA) regulations. Two monopolar-electrodes were also used for all mice IRE procedures. Electrode array was constructed using two needles made of tungsten (each 50 mm in length with a diameter of 0.6 mm) whose surface was coated with pure-platinum through unbalanced magnetic field sputter of 10 μm in thickness without any toxicity. These electrodes were designed to be exposed only 4 mm in length and other parts were enveloped with a plastic blocker for insulation. They were inserted 4 mm into tumor tissues with 5 mm spacing between electrodes. Following previous studies [6],we chose 1.2 kVsquare wave pulses with 100 μs duration and 100 μs pulse interval to reduce thermal damage.

3.2. IRE procedure

Before IRE procedures, mice were anesthetized with Zoletil (Virbac, Carros, France) solution 10 mg/kg i.v. The two-parallel-electrode was put in one of the two tumors, being aligned along the axis of the largest tumor dimension on the site of mouse flake so that the electrodes can be positioned to thoroughly exert on the tumor. The electrodes were then wired to the electroporator and pulses were applied in pulse train mode. Following electrical application, the electrodes were pulled out and the tumor was inspected for bleeding, and care was taken if bleeding was present. Then the mice were moved in the SPF and bred until MRI.

3.3. MR images

Magnetic resonance imaging (MRI) was performed to evaluate the effects of IRE for some mice prior and at 12 and 72 h after the IRE procedures, using a 1.5-T clinical scanner (Magnetom Avanto; Siemens Medical Solutions, Erlangen, Germany). All MR images were taken without electrodes in the tumor tissues because the magnetic fields could be affected by the metallic electrodes. Thus, we did not identify localization of electrodes within tumor tissues for further imaging analysis. MRI scanning of the nude mice was performed in coronal plane. MR sequences included T1-weighted turbo spin-echo (TSE) images (repetition time [TR] ms/echo time [TE] ms, 300/8) and T2-weighted TSE images (TR/TE, 3500/60). The thickness was 2.0 mm, the space was 0.5 mm, the matrix was 256 × 256, and FOV was 150 mm. Contrast enhanced T1-weighted TSE images were also obtained after application of contrast agent via tail vein.

3.4. Histology

Following completion of MRI, the mice were anesthetized following the same protocol as above and the tumors were harvested from the mouse and stored in the solution of NBF (composed with formaldehyde, distilled water, NaH_2PO_4, and NaH_2PO_4). The mice were then finally euthanized. After fixing, the tumors were embedded in paraffin for histological examination. Tumor tissues were sliced in 4-μm-thick sections across the lesion including the control. These sections were stained with hematoxylin and eosin (H&E) staining and Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), which is a method for detecting DNA fragmentation by labeling the terminal end of nucleic acids for apoptotic pathology. Histological slides were digitized with an image acquisition system (Olympus BX-51 with digital imaging system (Image-Pro plus 4.5)). Software (Matlab) for image analysis was employed to automatically qualify the region of interest containing areas of cellular necrosis on each image.

3.5. Simulation

For simulation of the ablation zone, we calculated the electric field by using a homemade C++ code. Following previous studies [7], we solved the equation $\nabla \cdot (\varepsilon \nabla \varphi) = \rho = 0$ using the relaxation method (Jackson, Classical Electrodynamics, 3rd ed.). The geometry of the problem was a 3 dimensional ellipsoid with two penetrating electrodes parallel to the z axis. The electric potential of the electrodes was ±600 V, and the potential of the surrounding environment was 0 V. The presented result (Fig. 1A) is the electric field over the cross section of the ellipsoid, cutting the ellipsoid into two equal parts in the x–y plane. We assumed the dielectric constant to be constant over the model organ. The dimension of the ellipsoid was 25 × 15 × 10 mm. We set a cubic lattice with a lattice constant of 0.2 mm. To present the result with higher accuracy, the cross section was interpolated using the gnuplot v. 4.4.

4. Results

All animals were alive during the experimental procedures, and showed no postoperative complications. The electrodes were inserted into the tumors along the long axis so that the applied electric fields could cover the entire tumors.

4.1. Apoptotic evaluation with post-IRE time

The IRE ablation area was in advance anticipated based on our electric field simulations for assessment of IRE ablation to cancerous tissues (Fig. 1A). Electric field is sharply localized between and beside electrodes. Based on such simulations, macroscopic evaluation of IRE-ablated lesions was performed using H&E staining and TUNEL assay at 5, 12, 24, and 72 h after IRE including the control. H&E staining and TUNEL assay showed clear aggressive progression post-IRE treatment (Fig. 1B) at all times. H&E images showed the irregular morphology of IRE treated tissues to the control. Necrosis can be distinguished between the electrode insertion sites. The TUNEL assay showed evidence of apoptosis by IRE, having brown color. Fig. 1C exhibits the result of apoptotic rate quantified the brown colored area by using Matlab. The peak apoptotic