Comparison of irreversible electroporation ablation in mice livers with or without a thermally controlled algorithm

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To the Editor: Irreversible electroporation (IRE) ablation mainly relies on the high-intensity pulsed electric energy instead of Joule heat.[1] Compared with thermal ablation, IRE is traditionally considered as a non-thermal modality. Recent *in vitro* and *ex vivo* studies have shown that IRE ablation may be associated with a certain thermal effect, and the ablation temperature mainly depends on the dynamic balance between the accumulated ablation energy and heat dissipation.[2,3]

Considering the risk of thermal injury during IRE ablation, researchers have proposed the temperature closed-loop feedback algorithm.[3] However, the security and effectiveness of the algorithm still needs to be further verified. As different from *ex vivo* experiments, the basal body temperature and heat dissipation state *in vivo* can affect the thermal change. Thus, this study performed *in vivo* IRE ablation using an appropriate set of electrical parameters with or without a thermally controlled algorithm in mice livers to compare their different effects.

A high-voltage pulse generator (HVPG), which can provide electric pulses with a width ranging from 40 μs to 2 ms, an output voltage of 500 to 1000 V, a maximum current of 60 A, and a fixed repetition frequency of 1 Hz, was used as the electric field generating unit and it can be triggered by an external signal. The electrodes consist of two 21-gage stainless steel monopolar electrodes with an exposure length of 5 mm. An epoxy resin coated thermistor (MEB05, 10 kΩ, B-value 3950, Lingee Corp., Shanghai, China) was used as the temperature sensor and placed close to the tip of one electrode. The sensor was connected to a microcontroller (Arduino UNO, Ivrea, Italy) and the collected analog signal was converted into a temperature signal using the Steinhart–Hart equation.

The feedback temperature in the experimental group with the thermally controlled algorithm was set to 42°C, while in the control group, the temperature was in an unrestricted open-loop state. Pulses were delivered at an initial repetition frequency of 1 Hz in both groups and it remained unchanged in the control group throughout the treatment process. But the repetition frequency changed in the experimental group when the algorithm detected that the temperature had reached the feedback threshold.

The *in vivo* study was approved by the Institutional Animal Care and Use Committee of Nanjing Medical University (Approval No. IACUC-2007039). A total of 18 Institute of Cancer Research mice aged 9 to 11 weeks old, both males and females, were randomly and equally divided into the experimental group and the control group. The electrodes were fixed at an edge-to-edge distance of 3.3 mm and then used to puncture the middle lobe of the liver to the cephalic side with a depth of 1 cm along the xiphoid process under general anesthesia. The success of puncture was verified by the computed tomography with a tube voltage of 80 kV, a tube current of 120 mA, and a reconstruction slice thickness of 0.625 mm. Finally, the electrodes were connected to the output terminal of the HVPG to deliver 20 pulses with a voltage of 600 V and a pulse width of 100 μs. The voltage and currents during the treatment were measured by an oscilloscope (ADS7102CA, ATTEN Corp., Shenzhen, China), a voltage probe (T3100B, OWON Corp., Zhangzhou, China), and a current probe (CC65, Hantek Corp., Qingdao, China). The treatment duration and temperature changes during the ablation of the two groups were recorded by a computer using the software SerialPlot (Hasan Yavuz Özderya, Turkey). The post-operative recovery and survival rate of the mice were observed.

All mice were sacrificed 24 h after the treatment, and gross liver specimens were collected for hematoxylin and eosin (H&E) staining and terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) Nick-End Labeling (TUNEL) staining. Slices were scanned by the platform ImageScope (Leica, Inc., Frankfurt, Germany) and the pathological results were evaluated by two independent pathologists using a double-blind method. Effective IRE ablation standards include complete ablation coverage between electrodes, cell apoptosis, and preservation of vascular structures in the ablation zone. Good completeness and uniformity of staining were regarded as satisfactory staining. The ablation area was delineated for the quantitative evaluation.

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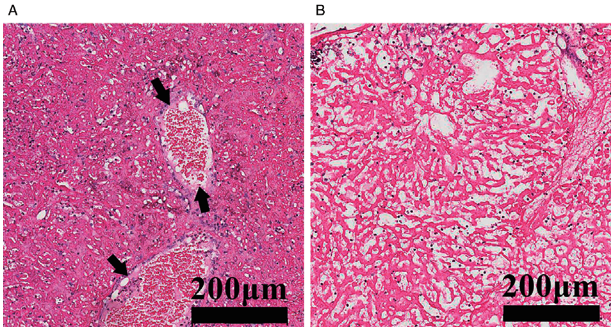

Figure 1: H&E staining of mice liver tissues after IRE. (A) Complete uniform staining in the experimental group and vascular structures (arrows) are well preserved. (B) Cytoplasmic de-staining of hepatocytes in the control group. H&E: Hematoxylin and eosin; IRE: Irreversible electroporation.

Quantitative data were expressed as mean ± standard deviation and the rates were expressed as frequency with a percentage. Comparison of quantitative data and rates were conducted by the Student's *t* test or chi-squared test. A $P < 0.05$ was considered as statistically significant. All statistical analyses were performed using the software SPSS 18.0 (SPSS Inc., USA).

As a result, all mice underwent successful percutaneous liver puncture and survived. The output pulsed voltage was stable at approximately 600 V in both groups. After 20 pulses were delivered, the treatment duration in the experimental group was significantly longer than that in the control group (41.5 ± 3.5 s *vs*. 20.0 ± 0.0 s, $P < 0.01$). The temperature and electric currents after treatment in the experimental group were significantly lower than that in the control group (42.3 ± 0.8°C *vs*. 55.6 ± 2.3°C, 5.28 ± 0.19 A *vs*. 8.84 ± 0.56 A, $P < 0.01$).

Typical IRE pathological manifestations were found in the ablation zone in all mice according to the H&E and TUNEL staining. However, in the experimental group, eight out of nine slices showed a complete and uniform staining of hepatocytes in the ablation zone [Figure 1A], with a staining satisfaction rate of 88.9%, while in the control group, eight out of nine were detected as incomplete staining or cytoplasmic de-staining [Figure 1B], with a staining satisfaction rate of 11.1%, which was significantly lower than that in the experimental group ($P < 0.01$). The effective ablation area in the experimental group was 15.2 ± 3.6 mm^2, which was significantly smaller than the 21.3 ± 5.2 mm^2 in the control group ($P < 0.01$).

In this comparative study, a temperature closed-loop thermally controlled algorithm was applied in the IRE ablation of an *in situ* percutaneous liver puncture mice model. The results demonstrated the safety of this treatment using a voltage of 600 V, an electrodes spacing of 3.3 mm, and a fixed repetition frequency of 1 Hz, or a variable frequency <1 Hz.

Since the heat is first generated around the electrodes, the algorithm waits for the tissue to dissipate heat by using a variable frequency to limit the rise in temperature. In the experimental group, limiting the temperature also limited the current that should have risen, indicating that the currents, conductivity, and temperature are interrelated.[4] Thus, suppressing currents or conductivity are also indirect methods for thermal control. Additional Joule heating may increase the coverage of the electric field and thus, this led to an increase in the ablation area in the control group, which is consistent with the results of the *in vitro* study.[3] Although the thermally controlled algorithm in this study caused the loss of ablation speed and ablation range, fortunately the overall effectiveness of ablation was not affected.

By magnifying the histopathological images of the two groups, we found that the thermally controlled algorithm not only changed the ablation speed and ablation area, but also altered the staining quality. The loss of cytoplasm and nucleus in the control group was significantly more than that in the experimental group. We speculated that this de-staining may be due to the coagulation necrosis caused by Joule heat. The energy received by the experimental group was more stable and thus, the cytoplasm was well fixed and could be well stained.

Our preliminary *in vivo* study also has some limitations, such as a small sample size and that only one good proven set of electric field parameters were used for comparison. Despite these limitations, our study has demonstrated that although the introduction of the thermally controlled algorithm affects the ablation process and outcome, it does suppress the temperature generated by the electric field without compromising the effectiveness of IRE, and provides a better histopathological staining quality.

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Conflicts of interest

None.

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