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A new local ablation for unresectable primary liver tumor: effect of electrothermal and electrochemical therapy on rat liver

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Abstract: Background: Electrochemical therapy (ECT) has been used to treat unresectable hepatic tumor. In order to improve its efficacy, we combined ECT with hyperthermia induced by electrothermal needle (ETN) (ETECT). The aim of this study is to investigate the destructive effect of ETECT on normal rat liver. Methods: Twenty rats were randomized into 4 treatment groups (n=5 in each group): control, ECT alone, hyperthermia alone and ETECT. Following the treatment, sections of the livers were histologically examined by light microscopy and the destructive volumes were measured with micrometer. Results: We found that the destructive volumes in ETECT group were the largest (P<0.01). In ETECT group coagulative necrosis was found in both anode and cathode areas, around which transition zones existed. The transition zones can only be seen when coulomb was increased in ECT group. Conclusion: ETECT was demonstrated to enhance the destructive effect of ECT. This study provides theoretical and experimental basis for a new local ablative treatment for unresectable primary liver tumor.

Key words: Hyperthermia, Electrothermal needle, Electrochemical therapy, Liver, Rat
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INTRODUCTION

There is a high incidence of primary hepatocellular carcinoma (PHCC) in China. China accounts for about 43% of all the patients dying of PHCC worldwide each year (Nie, 2004). Although surgical resection remains the "golden standard" for PHCC, unfortunately only 20% of these patients are suitable for liver resection. This is due to multiple factors such as early-stage dissemination and metastasis, decompensated cirrhosis and multifocal genesis (Ye, 2002; 2004). Therefore a wide range of local ablative techniques has been developed as an adjunct to surgical therapy of PHCC. They include cryotherapy (Larson *et al*., 2000), radiofrequency ablation (Gillams and Lees, 2004), laser interstitial thermotherapy (Gillams and Lees, 2000), microwave coagulation therapy (Dong *et al*., 2003), and high-intensity focused ultrasound (Daum *et al*., 1999).

Electrochemical therapy (ECT) is also an additional method to treat local tumor. It involves using destructive electrolysis induced by low-level direct electric current passing through a pair of electrodes inserted in the tumor. This creates a large pH change which leads to cell death. In China more than 10000 patients with various malignant tumors have been treated with ECT over the past ten years (Nilsson *et al*., 2000; Xin, 1994). These reports highlight the potential of electrolysis as an ablative technique in patients with unresectable PHCC.

However, ECT is a slow process, and the typical treatment time is 3 to 4 h (Wemyss-Holden *et al*., 2004). In order to enhance the efficacy of ECT, we develop a new technique called electrothermal and

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electrochemical therapy (ETECT), using ECT and hyperthermia in combination. We also developed an electrothermal and electrochemical device (ETECD) based on the model of electrothermal needle (ETN) which was devised by Sun Cai-jun and his colleagues to induce interstitial hyperthermia (Sun and Chen, 1986; 1992). There is no report on the destructive effect of the combination of ECT and hyperthermia.

The aim of this study is to investigate the destructive effect of ETECT on the normal rat liver and to discuss its potential mechanisms. Hopefully this will provide theoretical and experimental basis for a new local ablative therapy for unresectable PHCC.

MATERIALS AND METHODS

Materials

The patented ETECD was designed by Sun Cai-jun together with his colleagues (Sun, 2004). There are 4 electrodes (1 anode and 3 cathodes) in this instrument. One ETN is inserted in each cathode, so the cathode is also used to induce hyperthermia (Fig.1). The digital thermometer (wny-150) was purchased from Shanghai Tong Yong Voltmeter Factory. The double-headed microscope with micrometer discs was provided by the Department of Pathology of Sir Run Run Shaw Hospital, Zhejiang University. Twenty percent urethane and pH papers were provided by the Department of Physiology, School of Medicine, Zhejiang University.

Twenty female Sprague-Dawley rats (190~210 g) were purchased from the Experimental Animal Center of Zhejiang Academy of Medical Science. These rats were housed in a special pathogen-free animal facility in Sir Run Run Shaw Hospital, Zhejiang University.

In vivo treatment

The rats were randomized into 4 treatment groups (n=5 in each group): control, ECT alone, hyperthermia alone and ETECT. These rats were anesthetized by using intra-peritoneal injection of 20% urethane (6 ml/kg). Linear alba incision was then made and the liver was exposed. A group of electrodes (Fig.1) were inserted vertically into the left medial lobe at a depth of 2 mm. They were then given the following treatments: (1) Control group: No therapy was given; (2) ECT group: ECT was induced by one anode and three cathodes with direct electric current of 10 mA for 5 min. The dosage was 3 coulombs; (3) Hyperthermia group: Hyperthermia was induced by three cathodes (ETNs) with 520 mA for 5 min; we maintained a temperature of (56±1) °C at the cathode and (42±1) °C around the anode (the center of the tissue); (4) ETECT group: ECT was produced by one anode and three cathodes and hyperthermia was induced by three cathodes (ETNs) at the same time. The dosage given and the time applied were the same as above.

Preparation of specimens for histological examination

After the treatment, the rat was euthanized and its liver was immersed in 10% formol saline. The tissues were embedded in paraffin. Five micrometers sections perpendicular to the axis of electrodes were cut and stained with haematoxylin-eosin. The destructive area of the liver was measured with micrometer discs (Wang *et al*., 1994).

The total destructive volume=the summation of the destructive areas of every electrode×the depth of inserted electrode (2 mm).

The study protocol was approved by the Committee on Animal Research of Health Bureau of Zhejiang Province, People's Republic of China. All procedures described complied with "Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Marketing and Education".

Statistical method

All variables in four groups were analyzed by repeated two-way ANOVA to determine whether there was a factor-by-factor interaction between hyperthermia and ECT, which was considered statistically significant if P<0.01.

RESULTS

Data analysis

As shown in Table 1, the treatment with hyperthermia or ECT alone led to destruction of rat's normal liver. However the destructive volume induced by ETECT treatment ((57.48±0.336) mm^3) was substantially greater (P<0.01) indicating that there was a synergistic interaction between hyperthermia and ECT.

Table 1 The destructive volumes of rat livers in four groups (mm^3)

Group	Number	Destructive volume (mm^3, $x \pm s$)	P^*
Control	5	0	
Hyperthermia	5	16.56±1.126	
ECT	5	29.32±1.627	
ETECT (interaction)	5	57.48±0.336	<0.01

* Repeated two-way ANOVA. The destructive volume in ETECT group (57.48 mm^3) was larger than the sum of the destructive volumes of hyperthermia and ECT groups (45.88 mm^3). Therefore when ECT was combined with hyperthermia, the synergistic interaction between them was prominent

Morphological observation

In ECT, gas bubble was produced around the anode and the cathodes. There were erythematous and edematous changes around the cathode (pH 12~13). The tissue around the anode (pH 2~3) on the other hand appeared pale and shrunken. In ETECT, the same phenomenon occurred. However, the reaction was faster and more intense. While in hyperthermia, the central necrotic region became pale and the rest of the liver tissue appeared dully purple.

Microscopic manifestation

In ETECT group, coagulative necrosis was approximately a circle at both anode and cathode regions, where the hepatic architecture disappeared and the hepatocytes were poorly defined with lysed and fragmented nuclei (Fig.2). Transition zones were found around the necrotic regions of anode and cathodes (Fig.3). Outside the transition zones, there were normal hepatic architecture and hepatocytes. However the vascular sinusoids were dilated and filled with excess erythrocytes (Fig.4).

In ECT group, the manifestations at the anode and cathode regions were similar to those in ETECT group, however, the destructive areas were smaller and no transition zone was found. In addition, dilated vascular sinusoids with excess erythrocytes were not observed either in normal liver tissue.

In hyperthermia group, hepatic architecture disturbance and hepatocytes with pyknotic nuclei and vacuolated cytoplasm were found at the cathode (ETN) region. There was no transition zone.

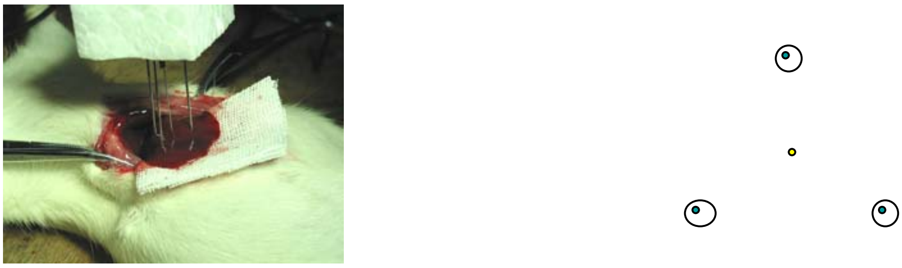
The hepatocytes and hepatic architecture were normal in control group.

DISCUSSION

As the current flows through the electrodes, negatively charged ions in the tissues are attracted to the anode and positively charged ions to the cathode, which cause destructive electrolysis. This leads to the release of chlorine, oxygen and hydrogen ions at the anode, and hydrogen gas and sodium hydroxide at the cathode (Nordenstrom, 1994). Due to these processes, the region around the anode becomes very acidic (pH 2), and the region around the cathode becomes strongly alkalotic (pH 12). The pH change is too great for cells to survive and hence there is cell death around each electrode. Under the extremely acidic condition around the anode, the tissue becomes dehydrated or even necrotic. The very alkalotic environment around the cathode causes swelling tissue with necrosis (Jaak and Daniel, 1994). In this study we found coagulative necrosis in both anode and cathode areas in the ECT group. We also observed dehydration around the anode and edema around the cathode. This was mainly caused by the migration of hydrated ion to the cathode. The reactions described above were much stronger in ETECT group.

In the research on effects of ECT on rat mammary and liver tissue, when the dosage of ECT was 5 coulombs, there was a narrow but distinct zone comprising of a few cells between necrosis and healthy tissues in the anode section of rat liver (von Euler *et al.*, 2001). Transition zones obviously existed around both anode and cathode regions in our ETECT group although the dosage was only 3 coulombs. This could be achieved only in the ECT group if the coulomb was increased. We consider this as the result of intense reaction between ECT and hyperthermia induced by ETN. The anode area was extremely shrunken because of dehydration, while the cathode area was excessively edematous. In addition, dilatation of hepatic sinusoids and extravasations of blood cells were found in normal liver among the electrodes in the ETECT group. In comparison, that only existed in the limited destructive region around the ETN (cathode) in hyperthermia group. We consider this as the result of enhanced reaction of hyperthermia induced by ETN with the help of ECT.

In this study we also found the destructive area of normal rat's liver with ETECT was significantly larger than either ECT or hyperthermia alone. We



(a) (b)

Fig.1 The arrangement of the group of electrodes. (a) The actual placement; (b) The draft of the electrode placement. The equilateral triangle was formed by connecting three points of three cathodes (white) with the side of 1 cm. The anode (yellow) was set in the middle of the triangle. One ETN (green) was inserted in each cathode

(a) (b)

Fig.2 The coagulative necroses at both cathode (a) and anode (b) regions in the ETECT group. The hepatocytes at the cathode region were swollen with poorly demarcated cytoplasm; whereas the hepatocytes at the anode region were shrunken with vacuole formation in the eosinophilic cytoplasm. HE×200

* The position of the inserted electrode

(a) (b)

Fig.3 The transition zones (TZ) found around the necrotic regions (N) of anode (a) and cathode (b). There were dilated vascular sinusoids in the transition zone of the anode, in which there was a deposition of haemosiderin, and the hepatocytes shrank with pyknotic nuclei. On the other hand, in the transition zone of the cathode the vascular sinusoids disappeared because of swollen hepatocytes. These hepatocytes underwent fatty change and had pyknotic nuclei as well. HE×150

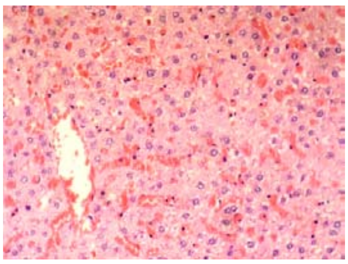


Fig.4 Outside the transition zones, the hepatic architecture and hepatocyes were normal, but the vascular sinusoids were dilated and filled with excess erythrocytes. HE×200

believe these findings are due to the synergistic effect of hyperthermia and ETN. Similar results were found in the study by Shou *et al*.(1998), where they studied the effects on growth inhibition of L78 human lung cancer cells with different treatments, including ECT, hyperthermia induced by water bath and ECT combined with hyperthermia. They demonstrated that there was no significant difference in L78 growth inhibition between control group and hyperthermia group, when the temperature was increased to 41 °C. However, when hyperthermia was combined with

ECT, the inhibition became prominent in comparison with other groups. They believed ECT could enhance the sensitivity of L78 to hyperthermia. Their results also supported the synergistic effect of the combination treatment. Our group has previously demonstrated that ETECT resulted in complete and rapid destruction of the S180 sarcoma implanted in mouse without any recurrence, which was not achieved by ECT or hyperthermia alone (Sun and Xie, 2003).

Potential mechanisms for strong destructive effect of ETECT are discussed below:

1. The movement of molecules in tissue will quicken when the temperature rises, which can decrease the resistance of tissue when electric current flows through. Therefore it can accelerate the electrolytic reaction induced by ECT. According to van't Hoff's rule: $K_{(t+10)}/K_t$=2~4, the rate of chemical reaction is increased by 2 to 4 fold for each 10 °C increment in temperature.

2. Hyperthermia possibly damages the stability of cell membranes and increases membrane permeability. It becomes easier to kill the tissue cells and inhibit sublethal cells repair because of reactive oxygen species, hypochlorous acid and chlorine gas produced by the anode and hydrogen produced by the cathode.

3. The thermosensitivity of the cells is elevated due to rapid changes of the intra-tissue pH induced by ECT around the anode and the cathode, which enhances the destructive effects of hyperthermia. Gerweck *et al*.(1974) showed that sensitizing effect occurred over a range of temperatures (41 °C to 44 °C) and increased with decreasing pH. The effect was particularly pronounced at 42 °C. Maintaining tissue culture cells at low pH after being heated was also found to increase the cytocidal effects of hyperthermia. They further found that when pH was decreased to as low as 6.7, the development of thermotolerance was inhibited in cultures exposed continuously to temperatures in the range of 42.0 °C to 42.5 °C. The degree of inhibition was directly related to the extracellular pH of the culture media (Gerweck *et al*., 1980). In Hahn and Shiu (1986)'s research, the thermosensitivity of the mammalian cells growing at pH 7.4 dramatically increased when the pH was decreased to 6.5 and the temperature was increased to 42 °C. The thermosensitivity of other cells cultured at pH 6.8 showed considerable change when exposed to similar conditions. This implies that the thermosensitivity of the tumors exposed to low pH environment long term can be produced only when there is a dramatic reduction of pH.

The destruction pattern in normal liver tissue exposed to electrolysis could also be achieved in tumor tissues (von Euler *et al*., 2001). We believe that actually tumors can have higher sensitivity to physical and chemical stimulations than normal tissues. Therefore the destructive effect caused by ETECT can also be achieved in PHCC.

In conclusion, ETECT was demonstrated to enhance the destructive effect of ECT. The findings from this research provide theoretical and experimental basis for a new local ablative technique on unresectable PHCC.

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