Antitumor Efficiency of ElectroChemoTherapy by High and Low Frequency and Repetitive Therapy in Treatment of Invasive Ductall Carcinoma in Balb/c Mice

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Abstract: - The important unpleasant sensation of electrochemotherapy is muscle contraction that one causes of this sensation is electrochemotherapy by low frequency (1Hz). Therefore by increasing the repetition frequency above the tetanic frequency we can reduce this painful sensation. The aim of present study was to comparing the treatment efficiency of low and high frequency electrochemotherapy and also estimated the effect of repetition ECT treatment session. Electrochemotherapy performed by 8 pulse, 1000 v/cm, 100 µs duration at 1 Hz and 5 kHz repetition frequency and intratumoral injected belomycin on treatment of invasive ductal carcinoma in female Balb/c mice. In other part we use this ECT protocol for second session therapy three days after tumors regrowing and treatment effect measured by calculating the tumor volumes for 24 days after treatment. 1 Hz and 5 kHz pulse repetition frequency increas es inhibited tumors growth significantly but after the first treatment tumors start growing. Repetitive ECT session can increase curability of tumors to 40% in treatment group by 1Hz frequency and 60% in ECT group by 5 kHz frequency. Our results demonstrate that the effect of 1 Hz and 5 kHz pulse repetition frequency is comparable for inhibited tumor growth and with repetitive treatment can improve effectiveness of ECT.

Key-words: Electrochemotherapy, 1 Hz and 5 kHz Repetition Pulse Frequency, Repetitive Treatment

1 Introduction
Electroporation is a new method that increases the cells permeability. This method with sufficient electric pulses, improves the uptake of macromolecules, ions into the cells. When electroporation combines with chemotherapy, this technique defines as electrochemotherapy (ECT) [1-7]. ECT improves the delivery of chemotherapy drugs, such as cisplation and bleomycin, into the tumor cells. This new approach increases the toxicity of this drug locally therefore decreases the chemotherapy doses and side effects.

ECT protocol predicts that a train of 8 pulses with 1Hz frequency, high voltage amplitude (1000-1300V/cm) and 100 µs duration needs for treatment. Despite of successful result, this treatment induces unpleasant senses to the patient [11-13]. The majority of this senses back to the muscle contraction that induce with low frequency and high amplitude. Low frequency and high amplitude cause to muscle contraction for each pulses (8 muscle contraction for 8 pulses) [11-12]. But by increasing the repetition frequency of electric pulses above the tetanic frequency, it would be possible to decrease the number of contraction with increasing the uptake of nonpermanent molecules. In the previous study the same electrochemotherapy efficiency with high repetition frequency and 1Hz was observed [12].

The aim of this study is evaluating the efficiency of electrochemotherapy with low
frequency (1 Hz) and high frequency (5 kHz) in invasive ductal carcinoma tumors and also estimating the effect of repetition ECT treatment.

2 Method and Material

2.1 Chemotherapy drug
Chemotherapy was performed by injecting bleomycin directly into the tumors. Bleomycin diluted in normal saline (1.5mg/ml) and 0.016 ml/go of this solution injected into the tumors. 2 minutes after the intratumoral injection of bleomycin, electric pulses delivered.

2.2 Anesthesia
All the experimental measurements were conducted on anesthetized mice to minimize discomfort and movements. The mice were anesthetized by intravascular administration of 0.01 ml/g of mice body weights. The anesthetic mixture included 0.5 ml of Ketamine (1000 mg/mol, Virbac, Carros, France), 0.5 ml of xylazine (2%, Bayer HealthCare, Leverkusen, Germany) and 4ml NaCl.

2.3 Electrochemotherapy protocol
Electric pulses were applied to the tumors by (using) EC-SBDC at 2 min after bleomycin or PBS injection. Eight 100µs square- wave electric pulse of 1000 V/cm amplitude with repetition frequency of 1 Hz and 5 kHz were delivered via two parallel stainless - steel electrodes inserted subcutaneously on opposed sides of tumor.

2.4 Tumor Volumes Monitoring
Tumor volumes were followed by measuring the diameters along the two largest dimensions with digital caliper every 3 days (each diameter was measured three times). Tumor volumes were calculated by the formula \( V = \frac{\pi a b^2}{6} \) where \( a \) is the larger diameter and \( b \) the smaller diameter. Normalized volumes for each mice was calculated from \( V_n/V_0 \) that \( V_n \) is tumor volumes in \( n \) days after treatment and \( V_0 \), tumor volumes in the treatment day. Also tumor volume inhibition rate was calculated by using the equation: Inhibition rate = \((1-\text{tumor volume of test group/tumor volume of sham group}) \times 100\%\)

![Graph showing tumor volumes over time](image)

Fig. 1: Electrochemotherapy of tumors in mice with different frequency. The results are presented as mean ± S.E. of the mean. EP: electric pulses alone, ECT: electrochemotherapy (BLM+EP).
3 Result

3.1 Electrochemotherapy with 1 Hz and 5 kHz repetition frequency
In this part we estimated the effect of high and low frequency in electrochemotherapy. Therefore we compared two electrochemotherapy protocols (8 pulses, 1000 v/cm electric field strength, and 100 µs pulse duration with 1 Hz and 5 kHz pulse repetition frequency). Two protocols could inhibit tumors growth significantly with P<0.05(fig 1). Our result showed that 1 Hz and 5 kHz caused to tumor volumes reduce for 9 days after treatment but in treatment group with 5 kHz pulse repetition frequency, tumors start growing in 15th day after treatment. But for the 24 days after treatment, tumor volumes in two electrochemotherapy groups were comparable (P<0.05). Electric pulses alone had significantly effect on inhibit tumor growth. Two protocols had no significantly differences till 24th days after treatment (P<0.05) (fig 1).

3.2 The effect of repetition ECT treatment
Our result showed that the tumors in electrochemotherapy group start growing. In order to the sufficient effect, we repeated electrochemotherapy 6 days after that the tumors start growing. In groups with once electrochemotherapy, complete response was not observed but with repeated electrochemotherapy 1 in 1 Hz group one mice of 2 mice and in 5 kHz ECT group 3 mice of 5 mice responded completely to it, and also tumors with partial respond did not start growing (table 1).

4 Discussion
Preclinical and clinical studies have demonstrated good antitumor effectiveness of electrochemotherapy, resulting in high curability rate of the tumors. The advantages of this therapy are requiring of low drug concentration with good antitumor effectiveness and therefore reducing systemic toxicity. In addition to these advantages, the most unpleasant side effect of electrochemotherapy is the muscle contraction and this effect is related to sensations during pulse delivery [11-14]. Before, Miklovcic and co-workers have demonstrated that it would be possible to reduce these unpleasant sensations and painful effect by using pulse repetition frequencies higher than tetanic, as an alternative to the standard pulse frequency of 1 Hz [12], but important part of electrochemotherapy is made easier uptake of impermanent molecules to cells which obtained with 1Hz repetition frequency. In another study the uptake impermanent molecules at the highest repetition frequencies have been examined and showed in vitro, uptake stayed at similar levels as the uptake at 1 Hz, also has been reported, different frequencies in different voltages corresponded to the maximum value of the uptake [11]. In addition of increasing the permeability of cells, an electrical pulse has effect on tissues blood flow and causes reduction of it [14- 19]. By decreasing tumor blood flow, drugs trap in the tumors and providing longer time for drug to act. This modification in blood flow could be particularly beneficial for intratumoral drug administration, as this would decrease drug wash out from the tumor [17]. In previous study, we observed that electrochemotherapy with 5 kHz repetition frequency have effect in blood perfusion more than 1Hz pulse repetition frequency and at frequency of 5 kHz rapid reduction in tumor perfusion is higher than at a 1 Hz frequency and with slower restoration of blood flow in tumors. Therefore in present study we examined electrochemotherapy by two different frequencies (1Hz and 5 kHz) in treatment of invasive ductal carcinoma as an important breast tumor in Iranian women. Our result showed that these various conditions can inhibit tumors growth the same as each other for 24 days after treatment (figure 1). Our data in same electric amplitude predict different uptake and reduction.
Table 1
Antitumor efficiency of electrochemotherapy with different pulse repetition frequencies and repeated treatment

<table>
<thead>
<tr>
<th>therapy</th>
<th>sham</th>
<th>EP 1Hz</th>
<th>EP 5 kHz</th>
<th>ECT 1Hz (once)</th>
<th>ECT 5 kHz (once)</th>
<th>ECT 1Hz (twice)</th>
<th>ECT5 kHz (twice)</th>
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EP: electric pulses alone, ECT: electrochemotherapy (BLM+EP); N: number of animals in each experimental group; CR: number of complete responses, PR: number of partial of responses.

In blood perfusion causes to obtain same treatment efficiency. Therefore, as well membrane electropermeabilization which facilitates drug transport and its accumulation in the tumor cells, change in tumor blood flow is involved in antitumor effective of electrochemotherapy and combination these two mechanisms can inhibit tumor growth.

Many researchers have tried to improve effectiveness of electrochemotherapy. For this reason three different approaches were performed: 1- changing electrode or electric field orientation could improve electric field distribution, 2- intratumoral injection of chemotherapy drug causes to increase aggregation of drug, and 3- by repetitive treatment of tumors that regrow after one therapy session could find better control over the tumors (ECT: animal models)[20]. In present study we injected bleomycin intratumoral but all tumors start growing. In ECT group with 1 Hz pulse repetition frequency in 15th day after the first treatment and in group treated with 5 kHz repetition frequency in 12th day after treatment. Therefore we let tumors grows for 3 days after regrowing day and then repeated our electrochemotherapy such as the first session. Our data demonstrated with repetitive treatment could improve effectiveness of electrochemotherapy in treatment of tumors that resist to it (table 1).

5 Conclusion
In summary we showed that the effect of 1 Hz and 5 kHz pulse repetition frequency is comparable in treatment of invasive ductal carcinoma, but different mechanisms in two conditions have main roles and in the days after treatment the rate of tumor growth is different. Therefore maybe studying of changes in tumor blood flow may be in different and long times after electrochemotherapy is desirable.

References:
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invasive or non-invasive electrodes.


