

Original Article



Repeated electroporation enhances the responsiveness to cisplatin-based intra-tumoral chemotherapy in murine breast cancer: revival of the electro-chemotherapy concept

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Abstract

Background and aims: Electroporation demonstrated certain modulable actions on tumoral cell membrane permeability to increase the intracellular bioavailability of chemotherapeutic drugs. The current *in vivo* study aimed to investigate the synergic effect of concomitant electroporation application to the intratumoral administration of cisplatin on murine invasive ductal adenocarcinoma breast cancer.

Methods: The fragments of the extracted tumor were implanted subcutaneously in healthy female Balb/C mice. Having reached the determined tumoral volume, the mice were randomly divided into five groups, including control, intratumoral cisplatin injection, tumoral electroporation application, electrochemotherapy (ECT), and double course ECT. The normalized tumoral volume and the inhibition ratios were calculated during a 30-day follow-up period. The data were tested by ANOVA, and a statistically significant level was set at $P < 0.05$.

Results: The inhibition ratio was significantly different between the intra-tumoral cisplatin administration and tumoral electroporation application groups compared to the control group ($P < 0.05$). ECT displayed superior results in comparison to the two later groups ($P < 0.05$). The double-course ECT group even represented a significant difference compared to the ECT group ($P < 0.05$).

Conclusion: Concomitant ECT to the cisplatin intratumoral administration indicated contributive anti-tumoral impacts in an *in vivo* murine model of invasive ductal adenocarcinoma breast cancer. ECT promises further applications to overcome the occurrence of therapeutic resistance to chemotherapeutic drugs.

Keywords: Electroporation, Electrochemotherapy, Cisplatin, Ductal carcinoma

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Introduction

Increasing the cell membrane permeability to enhance the intracellular entrance of any cytotoxic or cytostatic drugs was considered a potential adjunctive therapeutic approach promising more pronounced chemotherapeutic anti-tumor responsiveness (1). Considering that the cell membrane permeability is a bi-directional substance-transferring process, making a given drug enter into cells should act as an intra-cellular drug trap that aborts its consequent extra-cellular expulsion (2). The latter perspective does augment the intra-cellular drug concentration (intracellular bioavailability) while lessening the required systemic chemotherapeutic drug doses, affording the ever-sought clinical view as to how to

minder the chemotherapeutic side effects (3,4).

Electroporation was advanced as a physical adjunctive method to increase the cell membrane permeability in the face of chemotherapeutic drugs; thereafter, it was deemed "electrochemotherapy" (ECT) (5,6). It consists of exogenous induction of an alternated cell membrane potential on tumoral cells through the application of short frequencies-high amplitudes repetitive electrical pulse bouquets in adjunct to chemotherapy and is applied in a precise frame-time with regard to the latter administration (6). The presumed ECT effectiveness was previously reported by the means of *in vitro* and *in vivo* experiments and it was extended to the preclinical and clinical trials intended to treat tumors such as sarcoma,

carcinoma, or melanoma (7, 8). Nevertheless, ECT displayed limited efficiency in the case of voluminous solid tumors (9). The present study was designed to investigate the *in vivo* additive promoting the anti-tumoral effect of the electroporation to the intratumoral cisplatin-based chemotherapy application on a subcutaneously implanted murine breast cancer cells' tumor and in mice.

Materials and Methods

Mice population

Healthy female Balb/C mice (6-8 weeks old) were purchased from Pasteur Institute (Tehran, Iran); they were adapted for 10 days to an ambient temperature of 25°C and a natural light/dark cycle.

Tumor implantation

Affected mice by the spontaneous mouse mammary tumor (invasive ductal carcinoma) were purchased from the Immunology Department of Tarbiat Modares University (Tehran-Iran). Its tumor was removed surgically and chopped into fragments. Subsequently, the tumor fragments were subcutaneously implanted at the flank of the healthy mice. Once the implanted tumor reached a diameter of 12-15 mm in approximately 2 weeks (630 mm³ in volume), the mice were randomly divided into three groups (8-10 animals for each group).

Cisplatin: administration dose and inject a preparation

The application dose of the intratumoral injection of cisplatin was 8 mg/kg for each mouse. The cisplatin vial (50 mg/mL, Ebewe Pharma, Austria) was performed by sodium chloride 0.9% on the day of injection. A volume of 0.02 mL/g of mouse body weight of the prepared cisplatin was injected intratumorally.

Electroporation process

Eight square-wave electric pulses of 1000 V/cm amplitude, with a pulse duration of 100 μs and repetition frequency of 1 Hz, were delivered by two flat, parallel stainless-steel electrodes which were placed on the skin at the opposite sides of the tumor. The adequacy of contact between the skin contacts of the electrodes was assured using a conductive gel. Electric pulses were delivered by an ECT-SBDC, a pulse generator. Electroporation was delivered one minute after the intratumoral cisplatin injection.

Experimental group assignment

The randomly designed mice experimental groups were labeled as follows:

- CG: The control group,
- CTG: The chemotherapy group receiving cisplatin intratumoral injection
- EPG: The electroporation group receiving electric pulses
- ECT: The electrochemotherapy receiving cisplatin intratumoral injection and receiving electrical pulses
- DECT: Receiving a second ECT in a 15-day interval

from the first ECT

Experimental assessment

Tumor growth was daily assessed through mutual measurements of the two orthogonal tumor diameters (e1 is the larger tumor diameter, and e2 is the largest diameter orthogonal to e1). Tumor volumes were measured using the formula $V = \pi/6 \times e_1 \times e_2^2$, leading to tumor-doubling time (DT) calculations for each individual treated tumor. Tumor growth delay (GD) was measured by subtracting the mean tumor volume DT from that of the tumors in the control group and dividing the mean tumor volume DT of each experimental group. The inhibition ratio was calculated on day 30 after the treatment by formula $(1 - \text{the treated tumor average volume}/\text{untreated tumor average volume}) \times 100\%$. Partial response was considered a decrease of more than 50% of the tumor volume. Complete response was considered the absence of any detectable tumor for more than 100 days. Normalized tumoral volume (V_n/V_0) for each mouse was calculated by dividing the tumor volume at day N after the treatment (V_n) by the tumor volume on the treatment day (V_0).

Statistical analysis

All data were tested for the normality of distribution. The ANOVA with repeated measures was used to evaluate the statistical significance of differences between experimental and control groups at different times, and a $P < 0.05$ was considered significant in the statistical tests ($P < 0.05$).

Results

Tumor growth

Cisplatin or cis- diamminedichloroplatinum: The intratumoral injection of cisplatin at a dose of 8 mg/kg delayed the tumor growth up to 5.5 days (Figure 1), resulting in an inhibition ratio of 33%, which is significantly different in comparison to CG ($P < 0.05$, Figure 2). The tumor growth restarted on day 3 after intratumoral injection.

EPG: The tumoral application of electroporation protocol displayed a tumor GD of only 2 days (Figure 1) with an inhibition ratio was 32% (Figure 2), which is noticeably different in comparison to CG ($P < 0.05$). The tumor

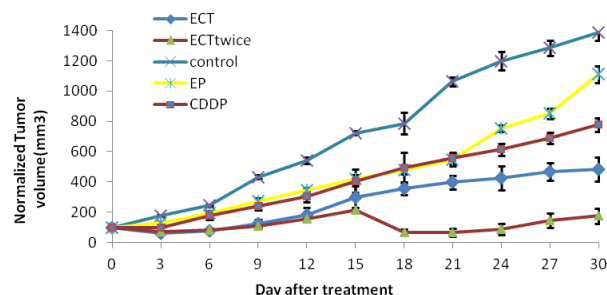


Figure 1. Time course of tumor growth in experimental groups. Note: Control: Control group; Cisplatin: Chemotherapeutic; EP: Electroporation; ECT: Electrochemotherapy; ECT twice: Double electrochemotherapy. Data are expressed as means \pm standard errors; CDDP, cis-diamminedichloroplatinum(II)

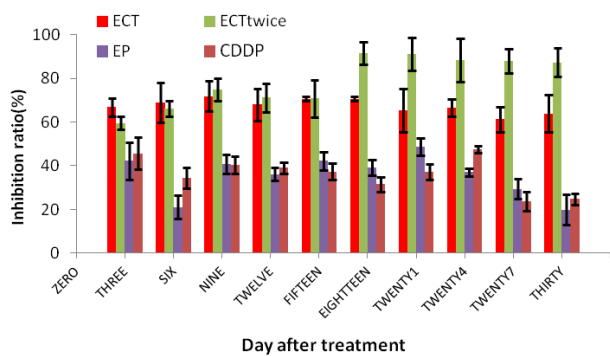


Figure 2. Inhibition ratio in comparison to the control group for treatment groups. Note. EP: Electroporation; Cisplatin: Chemotherapy; ECT: Electrochemotherapy; ECTtwice: Double electrochemotherapy; CDDP, cis-diamminedichloroplatinum(II)

growth restarted on day 3 after the electroporation application. No tumor was cured after either of these treatments alone.

ECT: A prolonged tumor GD up to 15.5 days was observed (Figure 1), and the inhibition ratio reached 61.2%, which is significantly different compared to CG ($P < 0.05$). With a 30% increase in the inhibition ratio (Figure 2), ECT was significantly more effective in comparison to CTG and EPG for the first 9 post-procedural days ($P < 0.05$)

ECTtwice: A more prolonged delay to tumor growth up to 18 post-procedural days (or 3 days after the second ECT) was noticed (Figure 1), resulting in an inhibition ratio of 79% (Figure 2), which is significant compared to ECT ($P < 0.05$). Tumor GD was significantly postponed until day 30 of the initial ECT application (Figure 1). Table 1 highlights tumor growth in the experimental groups.

Discussion

Modulating the tumoral cell membrane permeability given increasing the intracellular uptake of chemotherapeutic drugs has been an ever-tremendous perspective in the field of anti-cancer therapeutic research (3). The cellular trans-membrane passage is an actively and intelligently regulatory process that can be upregulated or downregulated toward any given substance. Such an innate mouldability confers to any tumoral cell the ability to develop trans-membranous resistance to the intra-cellular transfer of chemotherapeutic drugs (10). The iatrogenic capability to take control of tumoral cells' trans-membrane transferring to the chemotherapeutic drugs does sustain an increased intracellular drug concentration. The latter should act as an intra-cellular drug trap that augments the intra-cellular drug bioavailability and diminish drug extracellular washout accordingly, resulting in the enhancement of the cytotoxic or cytostatic sought effect (11). Increasing the intracellular drug concentration does come to reduce the required drug therapeutic doses with its attendant corollaries to lessen the clinical chemotherapy drug side effects that may lead to therapeutic penalties. A myriad of

Table 1. Tumor growth in the experimental groups

Experimental Groups	N	DT (Days) Mean \pm SE	GD (Days)	Surviving Days
Control	8	4.46 \pm 0.46	-	25
Cisplatin	8	10.16 \pm 1.21	5.54	30
EP	8	6.62 \pm 0.48	2	25
ECT	10	20.15 \pm 1.18	15.53	45
ECTtwice	8	38.75 \pm 2.57	34.13	80

Note. GD: Growth delay; DT: Doubling time; EP: Electroporation; ECT: Electrochemotherapy; ECTtwice/DECT: Double electrochemotherapy. Data are expressed as the mean \pm standard error.

herbal, chemical, nano-chemical, and physical concepts has been advanced, experienced, and undertaken to take therapeutic dominance on the tumoral cell membrane function (12,13).

The intricated cell membrane function is actively sustained by the electro-chemical interactions, resulting in the formation of the membrane's electrical action potential. Therefore, modulating or perturbing the membrane potential action, and that concomitantly to the chemotherapy, does sound attractive to be dogged as a strategy in increasing the intracellular drug uptake.

Electroporation, which is known for its ability to transiently reduce the regional blood flow, was advanced as an adjunct physical strategy to modulate the tumoral cell permeability, concomitant to chemotherapy (5,14). The latter consists of locally delivering high-amplitude and short-frequency bouquets of electrical pulses in a predetermined chronological frame-time with regard to chemotherapy administration (15). It was previously reported that electroporation can facilitate the uptake of any potentially permeate molecule through the cell membrane (16-18).

Cisplatin is a pillar chemotherapeutic drug employed to treat a myriad of human malignancies (19). However, the resistance to cisplatin administration was fraught with the occurrence of tumoral resistance, early after the first observed tumoral responsiveness, raising the issue of the gradual decrease in further tumoral responsiveness (20,21). Hence, diverse methods acting as trans-membranous drug transporting systems have been explored given overcoming the tumoral cell membrane resistance to cisplatin (22,23).

Electroporation has demonstrated a certain modular role in tumoral cell membrane permeability to enhance intracellular cisplatin bioavailability. The *in vitro* cytotoxicity of cisplatin was boosted using concomitant electroporation, and the latter was boosted by several folds (24,25). Electroporation concomitantly to cisplatin administration displayed enhanced *in-vitro*, *in vivo*, and even clinical anti-tumoral effectiveness in the setting of cutaneous tumor nodules (25).

In the current experimental study, it was sought to investigate the *in vivo* additive anti-tumoral effects of electroporation application to intratumoral cisplatin administration on the murine invasive ductal carcinoma

tumor model in mice. The present findings represented the significant additive anti-tumoral contribution of ECT to tumoral electroporation and intratumoral cisplatin administration alone, as well as the superiority of applying two courses of ECT (ECTtwice) over a single course ($P < 0.05$) expressed using the respective tumoral inhibition ratios. The normalized tumor volumes indicated a significant tumoral growth inhibition over a 30-day follow-period, with the ECTtwice group displaying the most pronounced temporal effect (Figure 1). In parallel to previous reports, it can be argued that the noticed additive anti-tumoral actions of ECT result from the ability of electroporation to modulate tumoral cell membrane permeability with regard to cisplatin, increasing the latter intracellular bioavailability by trapping it inside the tumoral cell (25-27).

Conclusion

Through its ability to modulate tumoral cell membrane permeability, electroporation represented an *in vitro* contributive anti-tumoral impact on the murine invasive ductal adenocarcinoma breast cancer presumably by increasing intracellular cisplatin bioavailability. ECT remains a promising anti-cancer therapeutic pathway to effectively overcome the clinically vexing issue of therapeutic resistance occurrence and subsequently envisage reductions in required doses of chemotherapeutic drugs.

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Competing Interests

The second author of this article is the Deputy Editor of the journal, the entire process of evaluating and reviewing the article was the same as that of the other authors and there is no conflict of interest.

Ethical Approval

The current study was approved by the Research and Medical Ethics Committee of Shahid Beheshti University with the code 143057 in accordance with the locally elaborated and approved Guidelines for Care and Use of Laboratory Animals.

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