*Radiol Oncol* 1995; 29: 121–7.

# Improved therapeutic effect of electrochemotherapy with cisplatin by intratumoral drug administration and changing of electrode orientation for electropermeabilization on EAT tumor model in mice

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*Antitumor effectiveness of cisplatin can be improved either by intratumoral administration of the agent or by increased drug delivery into the cells by exposing the tumor to short intense electric pulses. Electric pulses increase plasma membrane permeability (electropermeabilization) of tumor cells and thus allow the chemotherapeutic agent intracellular access. This combined use of electric pulses and chemotherapy is termed electrochemotherapy. Also, we recently demonstrated that efficacy of electrochemotherapy can be improved by changing of electrode orientation for electropermeabilization. Therefore, the aim of this preliminary study was to determine whether intratumoral cisplatin administration and changing of electrode orientation for electropermeabilization can improve therapeutic effect of electrochemotherapy. For this purpose electrochemotherapy with intratumoral versus intravenous cisplatin administration and electrochemotherapy with train of 8 electric pulses versus two trains of 4 pulses, given perpendicularly to each other (4 + 4 pulses), were tested on EAT subcutaneous tumors in mice. Electrochemotherapy with intratumoral cisplatin administration was more effective than electrochemotherapy with intravenous cisplatin administration. In addition, antitumor effectiveness of electrochemotherapy with intratumoral cisplatin administration was improved with changing of electrode orientation (4 + 4 pulses), since with this treatment 18 % of mice were cured in contrast to other treatment combinations tested, where only some partial responses were observed.*

*Key words:* neoplasms, experimental-therapy; cisplatin; electric stimulation therapy; cell membrane permeability

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UDC: 616-006.6-085.84-092.9

## Introduction

Cisplatin is chemotherapeutic agent widely used against variety of malignancies. Like all chemotherapeutic agents, its effectiveness is limited by normal tissue toxicity.[1] One of the ways to reduce toxicity to normal tissues is local, intratumoral (i.t.) cisplatin administration. By this

route of administration higher cisplatin concentration in the tumor and lower concentration in the organism is achieved, and thus therapeutic gain is increased.[2]

Also, one of the ways of potentiating cytotoxic action of chemotherapeutic drugs is increased drug delivery into the tumor cells. This can be done by use of short intense electric pulses, which nonselectively increase plasma membrane permeability (electropermeabilization), without impairing cell viability and thus allowing chemotherapeutic drugs to diffuse into the cells and act on their intracellular targets.[3–10] This principle of increased drug delivery was termed electrochemotherapy. Electrochemotherapy with bleomycin and cisplatin was elaborated *in vitro, in vivo* and in clinical trials.[8, 11–22]

In all of the studies on electrochemotherapy reported electric pulses were delivered in one direction.[11, 12, 14–17, 19, 21, 22] However, according to our observations on electrochemotherapy with bleomycin, therapeutic response to treatment can be improved if train of 8 electric pulses used for electropermeabilization is split into two and the second train given perpendicularly to the first one (4 + 4 pulses).[23] By this changing of electrode orientation whole tumor mass is encompassed by electrodes and more tumor cells are exposed to electric field over critical threshold value for effective electropermeabilization.

The aim of this preliminary study was to determine whether intratumoral administration of cisplatin and changing of electrode orientation for electropermeabilization can improve therapeutic effect of electrochemotherapy with cisplatin on EAT tumor model in mice. For this purpose we compared electrochemotherapy with i.t. versus intravenous (i.v.) cisplatin administration and electrochemotherapy with train of 8 electric pulses given in one direction versus two trains of 4 pulses given perpendicular to each other for electropermeabilization. Tumor response to electrochemotherapy was assessed by tumor growth delay and therapeutic responses to treatment.

## Materials and methods

*Drug formulations*

Cisplatin (Platimit) was obtained from Pliva (Zagreb, Croatia) as crystalline powder and dissolved in sterile $H_2O$ at a concentration 1 mg/ml. The final cisplatin dose (1 mg/kg) was prepared in 0.9 % NaCl solution. The cisplatin solutions were injected systemically, i.v. into the lateral tail vein of the mice or locally, i.t.. Injection volume was 0.02 ml/g body weight for i.v. administration and 0.1 ml/tumor for i.t. administration. For i.t. cisplatin administration "fan" pattern was used which facilitates drug distribution throughout the tumor.[2] Cisplatin solution was injected while the needle was slowly withdrawn. For each experiment fresh cisplatin solution was prepared.

*Animals*

In the experiments CBA mice of both sexes, 8–12 weeks old, weighing 20–30 g, in good condition, without fungal or other infections were used. Mice were purchased from the Institute of Pathology, University of Ljubljana and were kept at constant room temperature (24 °C) under natural day/night light cycle, fed with standard mouse chow and tap water *ad libitum*.

*Tumor model*

Ehrlich ascites tumor (EAT) cell suspension, syngeneic to CBA mice was prepared from ascitic form of the tumor. Solid subcutaneous tumors were initiated in the right flank of the mice by injection of $5 \times 10^6$ EAT cells. The viability of the cells injected was over 95 % as determined by trypan dye exclusion assay. After 6–8 days when the tumors reached approximately 40 $mm^3$, mice were randomly divided into experimental groups comprising 5–11 mice and subjected to specific treatment protocol.

*Electrochemotherapy protocol*

Electric pulses were delivered through two parallel plate electrodes 8 mm apart (two stainless

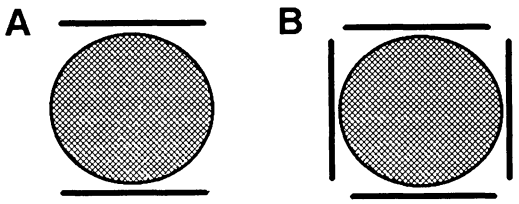


**Figure 1.** Schematic presentation of the electrode orientation at the two electric pulses treatment protocols. A. Electric pulses were given by two electrodes positioned on the two opposite margins of the tumor in the train of 8 consecutive pulses; B. In the protocol B the 8 pulses were split into two trains of 4 pulses with one second interval (4 + 4 pulses). Each train of 4 pulses was given to the tumor by the placement of the electrodes at the opposite margins of the tumor in the two perpendicular directions.

steel strips with rounded corners, 7 mm in width) placed at the opposite margins of the tumor. Conductive gel was used to assure good contact between electrodes and the skin. Eight square wave pulses; 100 $\mu$s pulse width, repetition frequency 1 Hz and 1040 V amplitude were generated by electropulsator Jouan GHT 1287 (St. Herblaine, France). Tumor bearing mice were treated either with 8 square wave pulses given in one direction or with 8 pulses split into two train of 4 pulses. In this experimental group second train of 4 pulses was given perpendicularly to the first one (Figure 1). Cisplatin was injected either i.v. or i.t. In electrochemotherapy with i.v. cisplatin administration mice were treated with electric pulses 3 min after cisplatin injection and in electrochemotherapy with i.t. injection mice were treated with electric pulses 10 min after i.t. cisplatin injection. Mice in control group and in electric pulses groups were injected with 0.9 % NaCl solution instead of cisplatin solution.

*Tumor response and statistical analysis*

Tumor growth was followed every 2 days by measuring three mutually orthogonal diameters ($e_1$, $e_2$, $e_3$) with vernier calliper and tumor volume calculated by the formula $e_1 \times e_2 \times e_3 \times \pi/6$. From the measurements, the arithmetic mean (AM) and standard error of the mean (SE) were calculated for each experimental group, pooled from two separate experiments. Tumor doubling time (DT) was determined as time in days for tumors to double their volume from the beginning of the treatment. For each individual tumor in all experimental groups DT and tumor growth delay from the DT of each individual tumor in experimental groups minus mean DT of control group were calculated.

Therapeutic response was scored according to WHO guidelines as progressive disease (PD) if tumor volume increased, no change (NC) if tumor volume reduced less than 50 %, partial response (PR) if tumor volume reduced more than 50 % and complete response (CR) if tumor became unpalpable. Mice, tumor free 100 days after the treatment, were termed as cured and were not included in tumor growth curves and tumor growth delay calculations.

The significance of the differences between the mean DT and tumor growth delay of the experimental groups was evaluated with Newman-Keuls method for multiple comparison after one way analysis of variance was performed and fulfilled. Levels of P less than 0.005 were taken as statistically significant.

## Results

In this study electrochemotherapy with i.t. cisplatin administration was compared to electrochemotherapy with i.v. administration. In addition, electrochemotherapy with train of 8 electric pulses given in one direction was compared to electrochemotherapy with two trains of 4 pulses given perpendicularly to each other (4 + 4 pulses).

Cisplatin treatment alone as single treatment was more effective after i.t. administration than after i.v. administration. Intratumoral cisplatin administration had marked antitumor effect, tumor growth delay was significantly prolonged compared to i.v. cisplatin treated group (Table 1, Figure 2).

Electric pulses treatment alone as single treatment, in both orientations (8 pulses and 4 + 4

**Table 1.** Antitumor effectiveness of electrochemotherapy with 1 mg/kg cisplatin. Comparison of i.t. versus i.v. cisplatin administration and train of 8 pulses given in one direction versus two times of 4 pulses (4 + 4 pulses) given perpendicularly to each other for electropermeabilization.

| Experimental groups | n | DT[2] (days) | Tumor growth delay[3] (days) | Therapeutic response[4] (n) | Cures (n, %) |
|---|---|---|---|---|---|
| Control | 21 | 4.1 ± 0.3 | | | 0 |
| Cisplatin i.v. | 14 | 5.1 ± 0.3 | 1.0 ± 0.3 | PD(14) | 0 |
| Cisplatin i.t. | 10 | 13.5 ± 1.6 | 9.4 ± 1.6 | PD(8)/NC(2) | 0 |
| Electric pulses 8 p. | 15 | 6.5 ± 0.5 | 2.4±0.5 | PD(15) | 0 |
| Electric pulses 4+4 p. | 10 | 6.4 ± 0.8 | 2.3 ± 0.8 | PD(10) | 0 |
| ECT[1] 8p.-i.v. cisplatin | 16 | 8.1 ± 0.6 | 4.0 ± 0.6 | PD(14)/NC(2) | 0 |
| ECT 4+4p.-i.v. cisplatin | 10 | 10.4 ± 1.0 | 6.3 ± 0.8 | PD(4)/NC(6) | 0 |
| ECT 8p.-i.t. cisplatin | 11 | 17.8 ± 1.4 | 13.7 ± 1.4 | NC(7)/PR(4) | 0 |
| ECT 4+4p.-i.t. cisplatin | 11 | 20.5 ± 1.1 | 16.4 ± 1.1 | NC(4)/PR(5)/CR(2) | 2 (18 %) |

[1] ECT – electrochemotherapy
[2] DT – tumor doubling time (AM ± SE)
[3] Tumor growth delay compared to control group (AM ± SE).
[4] Therapeutic response to treatment was scored according to the WHO guidelines as PD-progressive disease; NC – no change; PR – partial response; CR – complete response.

pulses), was equally effective and had moderate effect on tumor growth (Table 1, Figure 2).

Electrochemotherapy treatment with i.v. cisplatin administration was more effective than treatment with i.v. cisplatin alone as single treatment, demonstrated by significantly prolonged tumor growth delay (Table 1, Figure 2). Changing of electrode orientation (4 + 4 pulses) for electropermeabilization did not potentiate antitumor effectiveness of i.v. cisplatin administration compared to treatment with 8 electric pulses given in one direction. The small difference between the two electrochemotherapy treatment protocols with i.v. cisplatin administration was observed only in the first 4 days after the treatment. Electrochemotherapy with i.v. cisplatin administration and 4 + 4 pulses arrested tumor growth in the first 4 days after the treatment, while after electrochemotherapy with i.v. cisplatin administration and 8 pulses no arrest of the tumor growth was observed.

Electrochemotherapy with i.t cisplatin administration was more effective than treatment with i.t. cisplatin treatment alone as single treatment, demonstrated by significantly prolonged tumor growth delay (Table 1, Figure 2). Changing of electrode orientation for electropermeabilization (4 + 4 pulses) was more effective than treatment with 8 pulses in one direction. Electrochemotherapy with 8 pulses resulted in 36 % of PR, whereas changing of electrode orientation (4 + 4 pulses) resulted in higher percentage of PR (45 %) and also in 18 % of cured mice.

Electrochemotherapy with i.t. cisplatin administration was more effective than electrochemotherapy with i.v. cisplatin administration (Table 1, Figure 2). Specifically, tumor growth delay after electrochemotherapy with i.t. cisplatin administration was significantly prolonged compared to tumor growth delay after electrochemotherapy with i.v. cisplatin administration. Also, electrochemotherapy with i.t. cisplatin administration resulted in high percentage of PR and CR, in contrast to electrochemotherapy with i.v. cisplatin administration where only NC therapeutic response was recorded.

## Discussion

This study shows that i.t. cisplatin administration and 4 + 4 pulses given perpendicularly to each other for electropermeabilization improve therapeutic effect of electrochemotherapy with 1 mg/kg cisplatin on EAT tumor model in mice.

In our previous study on three different tumor models in mice (EAT, SA-1 fibrosarcoma and B 16 melanoma) antitumor effectiveness of electrochemotherapy with i.v. cisplatin administration was tested with respect to electric pulses amplitude, cisplatin dose and sequencing and timing of cisplatin administration relative to electric pulses application.[22] In that study

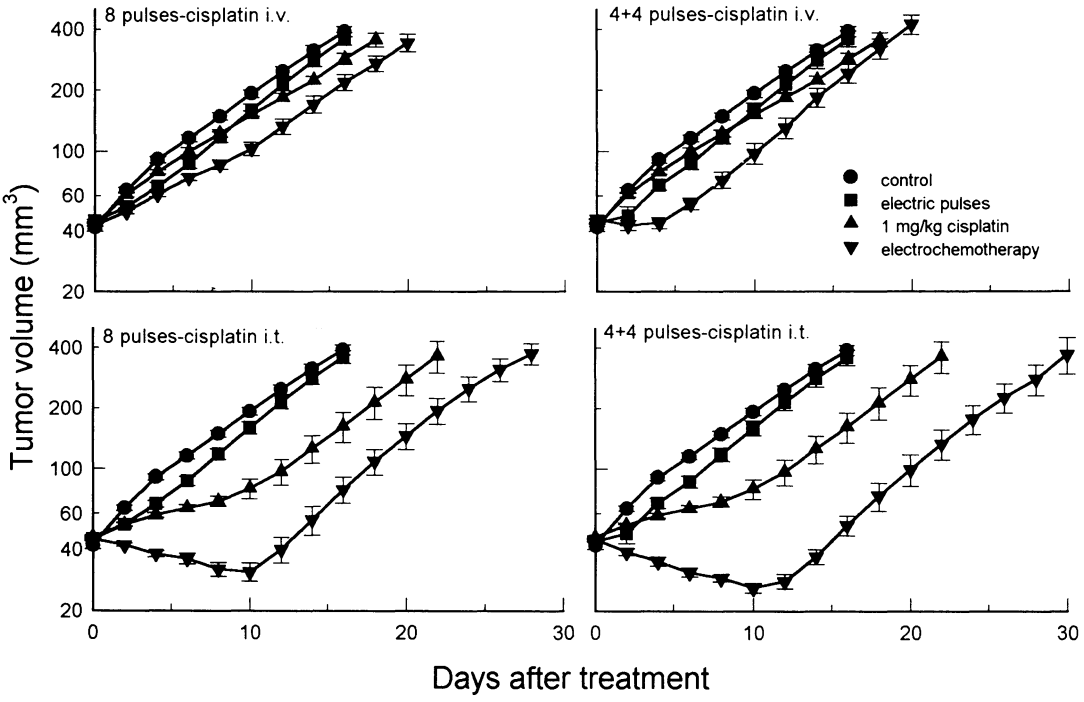


**Figure 2.** Growth curves of EAT tumors after treatment with 1 mg/kg cisplatin administered either i.v. or i.t. and/or electric pulses (8 pulses or 4 + 4 pulses; pulse length 100 $\mu$s, repetition frequency 1 Hz, pulse amplitude 1040 V, electrode distance 8 mm). In electrochemotherapy group with i.v. cisplatin administration mice were treated with cisplatin 3 minutes before electric pulses application and in electrochemotherapy with i.t. cisplatin administration 10 minutes before electric pulses. Values are AM ± SE for 9–21 mice.

antitumor effectiveness of electrochemotherapy with i.v. cisplatin administration was demonstrated by prolonged tumor growth delay compared to cisplatin treatment alone. Also, electrochemotherapy with 8 mg/kg (the highest dose tested) resulted in 14% of cured mice bearing B 16 melanoma tumors. However, no mice bearing EAT or SA-1 tumors were cured. In addition, in our previous study on EAT tumor model we have demonstrated that changing of electrode orientation for electropermeabilization improves therapeutic effect of electrochemotherapy with bleomycin.[23] Therefore, in this preliminary study both i.t. cisplatin administration and changing of electrode orientation for electropermeabilization were tested for ability to improve therapeutic effect of electrochemotherapy with cisplatin.

For electrochemotherapy with i.v. cisplatin administration 3 minute time interval between the cisplatin administration and electric pulses application was chosen. In our previous study we demonstrated that at this time interval the most pronounced antitumor effect of electrochemotherapy with cisplatin is achieved.[22] Also, for electrochemotherapy with i.v. bleomycin it was demonstrated that the best antitumor effect is achieved when bleomycin is injected 3 minutes before electric pulses application.[21] Therefore, it seems that both chemotherapeutic drugs have similar accumulation properties in tumors of mice. According to the experiments performed on electrochemotherapy with i.t. bleomycin administration, where the best antitumor effect was achieved with 10 minute interval (Heller, personal communication), the same time interval (10 minutes) was used for electrochemotherapy with i.t. cisplatin administration. However, a time response relationship studies for electrochemotherapy with i.t. cisplatin administration need to be performed to confirm the choice of timing.

The dose of cisplatin used in our preliminary study was a subtoxic dose (toxic dose 10-15 mg/kg), well tolerated by the animals, which injected systemically did not induce significant antitumor effect. The results demonstrate that electrochemotherapy with i.v. cisplatin administration was moderately effective and suggest that cisplatin concentration achieved in the tumor is not sufficient for pronounced antitumor effect. However, electrochemotherapy with i.t. cisplatin administration was more effective than electrochemotherapy with i.v. cisplatin administration at the same dose of the drug. Antitumor effectiveness of electrochemotherapy with i.t. administration is comparable to electrochemotherapy with i.v. administration, but in 8-fold higher dose.[22] In addition, electrochemotherapy with i.t. cisplatin administration and 4 + 4 pulses for electropermeabilization resulted in some cured mice with long lasting CR (18%), indicating that with increased cisplatin concentration in the tumor and changing of electrode orientation more clonogenic tumor cells are electropermeabilized and thus also sterilized by the chemotherapeutic drug.

In conclusion, electrochemotherapy with i.t. cisplatin administration and 4 + 4 pulses given perpendicularly to each other for electropermeabilization improves therapeutic effect of electrochemotherapy on EAT tumors in mice. Therefore, electrochemotherapy with i.v. cisplatin administration can be used as adjunct to ongoing cisplatin-based chemotherapy in patients who have tumor lesions accessible to application of short intense electric pulses, so that antitumor effectiveness of chemotherapy is potentiated locally. On the other hand, since electrochemotherapy with i.t. cisplatin administration is more effective than electrochemotherapy with i.v. cisplatin administration, it could be used as a single treatment modality.

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