*Radiol Oncol 2003; 37(2): 101-7.*

# Effect of electroporation on radiosensitization with cisplatin in two cell lines with different chemo- and radiosensitivity

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***Aim.*** *Radiosensitization with cisplatin can be enhanced by electroporation of cells and tumours. The aim of this study was to extend our previous studies on two carcinoma tumour models with different chemo- and radiosensitivity in order to evaluate whether this treatment is effective also on less chemo- and radiosensitive tumour cells.*

***Materials and methods.*** *This in vitro study was performed on carcinoma SCK and EAT-E cells. The cytotoxicity of three-modality treatment consisting of cisplatin, electroporation and irradiation was determined by the clonogenic assay.*

***Results.*** *The radiosensitizing effect of cisplatin on the two cell lines was greatly enhanced by electroporation. By this combined treatment, less chemo and radiosensitive EAT-E cells were rendered as sensitive as more chemo and radiosensitive SCK cells.*

***Conclusion.*** *The enhancement of cisplatin-induced radiosensitization of cells by electroporation could be beneficially used in the treatment of intrinsically less chemo- and radiosensitive tumours.*

*Key words: tumour cells cultured - drug effects; electroporation; drug delivery systems; cisplatin; radiation tolerance*

## Introduction

Electrochemotherapy combines administration of non-permeant or poorly permeant chemotherapeutic drug with the application of electric pulses to the tumours in order to facilitate the drug delivery into the cells.[1]

Received 9 May 2003
Accepted 29 May 2003

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Thus, the enhanced drug delivery locally potentiates chemotherapeutic drug effectiveness at the site of electric pulses application. So far, two chemotherapeutic drugs have proved to be effective in electrochemotherapy, bleomycin and cisplatin. Several fold increase in bleomycin and cisplatin cytotoxicity and several fold increase in antitumour effectiveness have been shown in many preclinical electrochemotherapy studies.[2-9] The increased drug delivery, both into the cells *in vitro* and tumours *in vivo*, was shown to be a predominant underlying mechanism.[4,10] Clinical trials were conducted also on the pa-

tients with different malignancies in whom electrochemotherapy proved to be effective in local tumour control.[11-19]

Based on the data that cisplatin is a radiation sensitizer when bound to DNA, several studies have been conducted using different drug delivery systems in order to increase the amount of cisplatin in tumour cells.[20-27] Our group was the first to demonstrate that the electroporation of tumours increases the radiosensitizing effect of cisplatin in tumours. Radiosensitizing effect of cisplatin increased tumour cures from 72% to 92% when cisplatin was combined with electroporation of EAT murine tumours.[26] The results of this first study were additionally confirmed on another tumour model, LPB fibrosarcoma *in vitro* and *in vivo*.[27] When electrochemotherapy with cisplatin preceded tumour irradiation, tumour curability was enhanced by a factor of 1.6 compared to tumour irradiation alone, and by a factor of 1.4 when compared to cisplatin-induced radiosensitization of tumours without tumour electroporation.[27] Furthermore, in that study, we demonstrated that the increased radiosensitizing effect of cisplatin was due to the increased electroporation-mediated cisplatin delivery into the tumours.[27]

The aim of this study was to extend our previous study in two carcinoma tumour models with different chemo- and radiosensitivity, in order to evaluate whether this treatment is effective also in less chemo- and radiosensitive tumour cells. The study was performed in EAT-E and SCK carcinoma cells with different chemo- and radiosensitivity.

## Materials and methods

*Tumour cell lines*

In the study, two mouse-tumour cell-lines were used, SCK mammary carcinoma cells and EAT-E (Ehrlich ascites carcinoma cells) cells. SCK cells were grown in RPMI medium (RPMI, Sigma, St. Louis, USA) supplemented with 10% heat-inactivated foetal calf serum (FCS, Sigma). EAT-E cells were grown in Eagle minimum essential medium (EMEM) supplemented with 10% FCS. Both cell lines were routinely subcultured twice per week and were maintained in a humidified atmosphere with 5% $CO_2$ at 37°C.

*Drug*

cis-Diamminedichloroplatinum (II) (cisplatin) was obtained from Pharmacia&Upjohn S.p.A. (Milan, Italy) as a crystalline powder. It was dissolved in sterile $H_2O$ at a concentration of 1 mg/ml. Final concentration was prepared in EMEM. For each experiment, a fresh solution of cisplatin was prepared.

*Irradiation of cells*

The cells were irradiated using Darpac 2000 X-ray unit (Gulmay Medical Ltd, Shepperton, UK), operated at 220 kV, 10 mA, using 0.55 mm Cu filtration and 1.8 mm Al filtration. Cells ($1x10^6$ cells/ml of EMEM) were irradiated in low attachment 24-well plates (Corning, Badhoevedorp, The Netherlands) at a dose rate 2 Gy/min with graded doses (2-8 Gy) and thereafter plated in Petri dishes (Corning) for clonogenic assay. $D_0$ values were used as the measure of cell radiosensitivity. The data were pooled from three independent experiments and normalised to the control non-irradiated cells.

*Electrochemotherapy protocol*

To determine the survival of SCK or EAT-E cells after the combined treatment with cisplatin and electroporation, 90 μl of cell suspension ($2.2x10^7$ cells/ml) was mixed with 10 (μl of cisplatin of different stock concentrations, ranging from 4.0 to 800.0 μg/ml. One half of the mixture was exposed to 8 electric pulses with electric field intensity 1000 V/cm, pulse duration 100 μs, and frequency 1 Hz. These parameters were optimal for electropermeabilisation of the two cell lines and were determined by measuring the uptake of pro-

pidium iodide used as a measure of electropermeabilisation and by determining the cell survival after exposuring the cells to different electric field intensities used as a measure of electrosensitivity (unpublished data).[28] Electric pulses were generated by Jouan GHT 1287 electroporator (St. Herblain, France). Other half of cell suspension served as a control for cisplatin treatment alone. The cells were then incubated for 5 min at room temperature in low attachment 24-well plates, diluted and plated on Petri dishes for clonogenic assay. The survival of EAT-E cells treated with electric pulses alone was 84.7±3.0% and the survival of SCK cells after electroporation was 79.7±3.0%.

*Electrochemotherapy combined with irradiation of EAT-E and SCK tumour cells*

To evaluate whether electroporation increases the radiosensitizing effect of cisplatin *in vitro*, EAT-E and SCK cells were electroporated in the presence of cisplatin and then irradiated. After electrochemotherapy, the cells were diluted in a fresh serum-free medium and 5 minutes later exposed to irradiation (4 Gy) (Figure 1). For clonogenic assays, the cells were plated in Petri dishes. The survival of SCK cells and of EAT-E cells after electroporation combined with irradiation was 12.1±2.0% and 26.8±3.0%, respectively. All data were pooled from three independent experiments performed in triplicates. From normalised survival curves, $IC_{50}$ value (drug concentration required to reduce cell survival for 50%) was determined for each treatment group. The statistical differences, using t-test, in sensitivity of cells to different treatments were calculated at the $IC_{50}$ level.

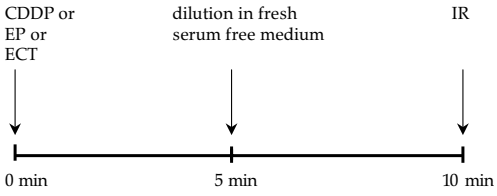


**Figure 1.** Treatment schedule *in vitro*. Cells were treated either with cisplatin (CDDP), electroporation (EP), electrochemotherapy (ECT), and/or irradiation (IR).

## Results

*Radiosensitivity of SCK and EAT-E cells*

SCK and EAT-E carcinoma cells were irradiated with graded single doses (2-8 Gy) and the surviving fraction was determined by clonogenic assay. From the survival curve $D_0$ was determined (Figure 2). SCK cells were more radiosensitive with $D_0$ = 1.2 Gy than EAT-E cells where $D_0$ was 2.0 Gy. According to the shape of the survival curves, SCK cells were less prone to repair radiation damage than EAT-E cells. Based on these results, a dose of 4 Gy was chosen for subsequent studies to determine the effect of electroporation on radiosensitization induced by cisplatin. The treatment of SCK and EAT-E cells with 4 Gy reduced their survival to 31.8±6.0% and 53.9±6.0%, respectively.

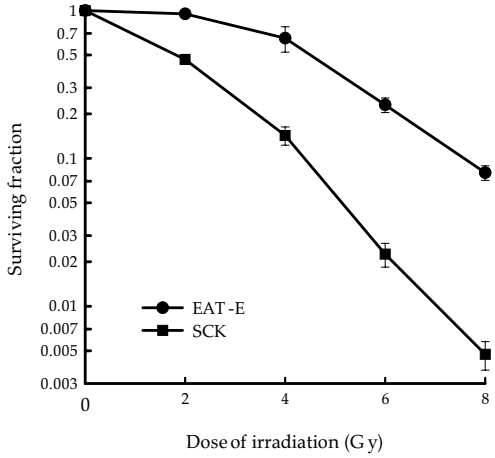


**Figure 2.** Surviving fraction of SCK and EAT-E carcinoma cells was determined after graded doses of irradiation by clonogenic assay. Values are mean ± SEM (n=9).

*Radiosensitization with cisplatin*

To determine the effect of electroporation on radiosensitizing effect of cisplatin, the cells were irradiated with 4 Gy following the pretreatment with different concentrations of cisplatin alone or combined with electroporation. SCK cells were more sensitive to the cytotoxic effects of cisplatin than EAT-E cells (Figure 3,4, Table 1). More then 3-times lower cisplatin dose was needed for the same cell kill. When the cells were irradiated 5 minutes after a 5-minute incubation with cisplatin, the cisplatin cytotoxicity was equally enhanced in both cell lines and was approximately 2-fold.

Electroporation increased cisplatin cytotoxicity (electrochemotherapy) in both cell lines. The survival curve was biphasic, which is due to the electroporation of cells. SCK cells were electroporated in 70.0±8.0%, EAT-E cells in 80.0±9.0%. The reduction in cell survival was therefore steep, declining to the level of 0.3 in SCK cells and to 0.2 in EAT-E cells, which is in accordance with the number of electropermeabilised cells. The remaining part of the cell survival curve had the same slope as the survival curve of cells treated with cisplatin alone (Figure 3,4). At the cell survival level of 0.5, the potentiation of cisplatin cytotoxicity for SCK cells by electroporation was approx 4-fold. In contrast, in EAT-E cells this potentiation was more than 20-fold, suggesting that the cell membrane is the major barrier for cisplatin cytotoxic action. Consequently, when the electroporation was used as a drug delivery system for cisplatin, $IC_{50}$ was almost equal for both cells lines (Table 1).

The cell survival curve of the cells treated with electrochemotherapy and irradiation declined to a lower level of survival in both cell lines, thus proving the radiosensitizing effect of cisplatin (Figure 3,4).

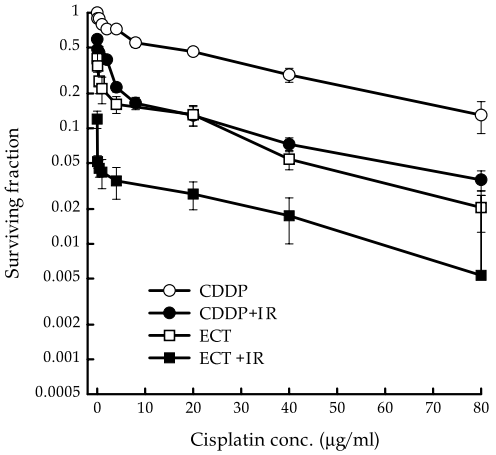


**Figure 3.** Survival curves of SCK cells after treatment with different cisplatin concentrations only (CDDP), cisplatin combined with electroporation (ECT) or single dose of irradiation (CDDP + IR) and combination of all the three treatment modalities (ECT + IR). Immediately after addition of cisplatin to cell suspension, cells were electroporated and 10 minutes later irradiated. Values are mean ± SEM (n=9).

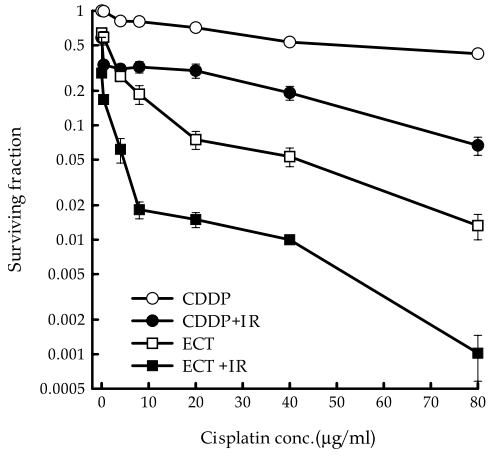


**Figure 4.** Survival curves of EAT-E cells after treatment with different cisplatin concentrations only (CDDP), cisplatin combined with electroporation (ECT) or single dose of irradiation (CDDP + IR) and combination of all the three treatment modalities (ECT + IR). Immediately after addition of cisplatin to cell suspension, cells were electroporated and 10 minutes later irradiated. Values are mean ± SEM (n=9).

**Table 1.** Cytotoxic effect of cisplatin, electric pulses, irradiation and the combination of these three treatments on SCK and EAT-E cells *in vitro*

| Group | $IC_{50}$ (μg/ml)[a] | |
|---|---|---|
| | EAT-E | SCK |
| CDDP[b] | 48.5±1.5 | 14.8±1.0 |
| ECT[c] | 2.2±0.9 | 3.4±0.7 |
| CDDP+IR[d] | 22±2.0 | 8.0±0.9 |
| ECT+IR[e] | 0.9±0.3 | 0.9±0.2 |

[a]$IC_{50}$ - Drug concentration to reduce cell survival for 50%.
[b]CDDP - Cisplatin. Survival of cells treated with cisplatin was normalised to the untreated control cells.
[c]ECT - Cells were treated with electroporation (EP) and CDDP. Survival of cells in this group was normalised to the effect of electroporation alone.
[d]CDDP+IR - Cells were irradiated with 4 Gy 10 minutes after incubation with CDDP. Survival of cells in this group was normalised to the effect of irradiation alone.
[e]ECT+IR - Cells were irradiated with 4 Gy 10 minutes after EP. Survival of cells in this group was normalised to the effect of electroporation in combination with irradiation.

## Discussion

The results of this study show that the radiosensitization of cisplatin in the two carcinoma cell lines used was greatly enhanced by electroporation. By this combined treatment, the less chemo- and radiosensitive EAT-E cells were rendered as sensitive as more chemo- and radiosensitive SCK cells.

First reports on improvement of combined modality therapy with cisplatin and radiation using electroporation of cells and tumours have already been published.[26,27] In our first study we showed that the delivery of cisplatin into the cells by electroporation of tumours increased radiosensitizing effect of cisplatin.[26] When electrochemotherapy preceded irradiation of EAT tumours, the curability rate of the tumours increased from 27% to 92%. This combined treatment was also better than the cisplatin therapy combined with local tumour irradiation, and irradiation combined with application of electric pulses. This study confirmed that cisplatin is a well known chemotherapeutic drug with radiosensitizing effect and that with increasing cisplatin delivery into the tumour cells, radiosensitizing effect of cisplatin also increases.[26] The improved therapeutic effect was demonstrated in several studies, where intratumoural drug solution in slow release device or polymer implant were combined with irradiation of murine tumours.[20-25]

In our second study, we showed that the electroporation of tumours increased radiosensitizing effect of cisplatin also in LPB sarcoma and that the increased platinum delivery into the tumours with electroporation was a predominant underlying mechanis.[27] This was a confirmation of previous observations that radiosensitization occurs only when cisplatin is present in tumour cells in sufficient amount.[26] The study showed that, when electrochemotherapy preceded irradiation, tumour curability rate was increased compared to irradiation only by EF=1.6, as well as compared to radiosensitization of tumours treated with cisplatin alone (EF=1.4). Radiosensitization was demonstrated also in LPB cells *in vitro*. Irradiation of cells pretreated with electrochemotherapy shifted the survival curve 2-fold further to the left compared to electrochemotherapy treated cells.[27]

In the present study, we extended our previous studies on two carcinoma tumour models EAT-E and SCK with different chemo- and radiosensitivity, in order to evaluate whether this treatment is effective also on less chemo- and radiosensitive tumour cells. The results of this study are in accordance with the results of previous study on LPB sarcoma cells.[27] We found that, when electroporation is used as a drug delivery system, the same cisplatin concentration yields the same cell kill on different cell lines. Specifically cisplatin concentration, when combined with electroporation that caused 50% reduction in cell survival for EAT-E cells, was 2.2±0.9 μg/ml, 3.4±0.7 μg/ml for SCK cells, and 4.0±0.5 μg/ml for LPB cells. Furthermore, the

same effect was observed when irradiation was combined with electrochemotherapy. Electroporation-enhanced cisplatin-induced radiosensitization was approximately the same for all cell lines. If we take into account that EAT-E ($IC_{50}$ = 48.5±1.5 μg/ml) and LPB ($IC_{50}$ = 120.0±3.0 μg/ml) cells are less sensitive to cisplatin than SCK ($IC_{50}$ = 14.8± 1.0 μg/ml) cells and that EAT-E cells are more radioresistant ($D_0$ = 2.0 Gy) than LPB ($D_0$ = 1.6 Gy) and SCK ($D_0$ = 1.2 Gy) cells, then we can conclude that the electrochemotherapy combined with irradiation radiosensitizes the cells to the approximately the same level, regardless of the intrinsic sensitivity of cells to cisplatin or irradiation.

In conclusion, by this combined treatment, the less chemo- and radiosensitive EAT-E cells were rendered equally sensitive as more chemo and radiosensitive SCK cells. Therefore, this enhancement of cisplatin-induced radiosensitization by electroporation of cells could be beneficially used for the treatment of less chemo and radiosensitive tumours.

## Acknowledgement

This work was supported by the Ministry of Education, Science and Sport of the Republic of Slovenia.

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