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Original article

The feasibility of low level direct current electrotherapy for regional cancer treatment

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Abstract. The feasibility of electrotherapy with low level direct current for cancer treatment was tested on fibrosarcoma SA-1 and malignant melanoma B-16 experimental tumor models. Treatment was performed with Pt/Ir electrodes inserted into the solid tumors and subcutaneously in the vicinity of the tumors, with currents ranging from 0.6 to 1.8 mA. Regardless of the electrode polarity or electrode configuration, significant antitumor effect was observed on both tumor models. Treatment with cathodic electrodes inserted into the tumor induced higher extent of tumor necrotization than with anodic electrodes. Treatment effectiveness was enhanced by delivery of the current through multiple electrodes and was direct current intensity dependent. The highest current employed (1.8 mA) induced the highest tumor growth delay; 11.8 ± 0.9 days (AM ± STD) on SA-1 and 16.8 ± 0.8 days on B-16 tumors. The difference in susceptibility of the two tumor models to electrotherapy remains to be explained. Nevertheless electrotherapy with direct current is suggested as effective, relatively easy and inexpensive for local/regional cancer treatment.

Key words: Electric stimulation therapy – Sarcoma experimental – Melanoma experimental

Introduction

Many different forms of electrical current with respect to frequencies, pulse-shapes and amplitudes have been employed in biomedicine with the aim of tissue remodelling by enhancing or suppressing cell proliferation [2, 3, 5, 10, 28]. One of the scopes was also employment of electrical direct current as an antitumor agent [29]. First reports date back in 18th century, but solid experimental data were published recently [4, 8]. Many studies have reported antitumor effect of direct current on experimental tumor models [4, 6, 8, 11, 14, 24] as well as in clinical studies [1, 18, 19, 20].

The mutual observation was that with direct current tumor growth could be delayed and that antitumor effect of direct current was current intensity and treatment time dependent [7, 11, 15, 16]. The underlying mechanisms remain unrecognised, although electrochemical reactions in the vicinity of electrodes were proposed as one of the main mechanisms [4, 22, 23, 29].

Because electrotherapy was recognised as local/regional treatment, several studies have dealt with its potentiation by either chemotherapy [12, 13, 20], radiotherapy [9, 23] or immunotherapy with biological response modifiers [25, 26]. Most of these reports have found at least additive effects in the combined treatments.

Because these studies differ in treatment approaches with respect to material used for electrodes, their shape and placement, schedule of treatment, current intensity and tumor models, it is difficult to compare them. None of the studies reported effects of different electrotherapy treatment schedules on the same tumor model, or compared its effect on different tumor models. Therefore the aim of the study was to test the antitumor effects of electrotherapy with low level direct current, with respect to current polarity, electrode configuration and current intensity on two different tumor models.

Materials and methods

Animals. Female and male mice of A/J and C57Bl/6 strains were purchased from Rudjer Boskovic Institute, Zagreb, Croatia. Animals were maintained at constant room temperature 24° Centigrade at natural day/night light cycle in conventional animal colony. Mice in good condition, without signs of fungal or other infection, eight to ten weeks old, were included in experiments.

Tumors. Melanoma B-16 was maintained in C57Bl/6 mice by serial intramuscular transplantation. Tumor cells from fourth isotransplantat

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generation were prepared by gentle mechanical disagregation of vital parts of the tumors. Fibrosarcoma SA-1 cells syngeneic to A/J mice were obtained from ascitic form of the tumor. Solid subcutaneous tumors, dorsolaterally in animals, were initiated by injection $5x10^5$ viable SA-1 cells and $7x10^5$ viable B-16 melanoma cells. When the tumors have reached 40 to 50 mm^3 in volume, animals were marked individually and randomly divided into smaller groups subjected to specific experimental protocol on day 0. On each consecutive day the tumor volume was calculated as $V = \pi \cdot e_1 \cdot e_2 \cdot e_3/6$ where e_j were three mutually orthogonal tumor diameters measured by vernier calliper gage. Experimental tumors were measured until the first tumor in the group reached 400 mm^3. Each day arithmetic mean and standard error of the mean were calculated for each experimental group. Tumor doubling time (DT) was determined for individual tumors and tumor growth delay (GD) from mean DT of experimental groups [26]. Animals were considered cured if being tumor free fifty days after the treatment.

Statistically the differences between the experimental groups were evaluated by nonparametric Mann-Whitney Rank-Sum test. Differences in response of both tumor models to treatment performed was evaluated by Student's t-test, after F test was performed and fulfilled.

Electrotherapy. During 60 minutes electrotherapy, current and voltage were continuously monitored. Direct current (DC) source was designed and manufactured at Faculty of Electrical and Computer Engineering, Ljubljana, Slovenia. Current (0.6 to 1.8 mA) was delivered through platinum-iridium (Pt-Ir; 90-10 %) alloy needle electrodes of 0.7 mm diameter, with rounded tips. Different electrode configurations were used. When single electrode was inserted centrally into the tumor the other electrode of opposite polarity was placed subcutaneously in the vicinity of the tumor (SEET). Multiple array electrode configuration was performed with three electrodes inserted into the tumor and two electrodes in subcutaneous tissue (MEET). Depending on the polarity of the electrode(s) inserted into the tumor, electrotherapy was considered anodic or cathodic. The animals in the control groups were treated in the same way as animals in the experimental groups with electrodes introduced into the tumors and subcutaneously, except that no current was delivered.

Determination of tumor necrosis. Tumors were excised, fixed in 10 % phosphate-buffered formalin, embedded in paraffin for histological sectioning and then stained with hematoxylin and eosin. The extent of necrosis along the tumor greatest diameter was determined as the percentage of necrotic regions with respect to whole area of the tumor. Tumors were excised 24 hours after electrotherapy. For comparison between different experimental protocols nonparametric Mann-Whitney Rank-Sum test was employed.

Results

Antitumor effect of direct current with SEET was determined on subcutaneous SA-1 and B-16 melanoma tumors with both current polarities. Both tumor models were rapidly growing in subcutaneous tissue, SA-1 tumors being faster (DT = 1.8 ± 0.6 days; AM STD) than B-16 melanoma tumors (DT = 2.5 ± 0.7 days, $p < 0.005$). Antitumor effect of electrotherapy, on both tumor models, with 0.6 mA delivered for 60 minutes, was significant compared to controls regardless of the polarity used ($p < 0.001$). The cathodic electrotherapy was more effective than anodic though there was no statistical significance between the effectiveness of the two polarities in tumor growth delay ($p > 0.05$), except that tumors treated with cathodic electrotherapy regressed quicker than tumors treated with anodic electrotherapy (Figure 1).

This single shot electrotherapy induced different amount of necrotisation in fibrosarcoma SA-1 tumors. Twenty four hours after electrotherapy with 0.6 mA for 60 minutes produced the highest degree of necrotization in tumors subjected to cathodic electrotherapy (68.3 ± 11.8 %), but less in tumors after anodic electrotherapy (40.6 ± 15.0 %, $p < 0.001$). The necrotic areas were sharply delineated and centrally located at the site of electrode insertion. Both polarities of electrotherapy produced significantly higher extent of tumor necrotization centrally located, compared to untreated controls where the necrotic areas were evenly distributed over the tumors (17.8 ± 5.9 %, $p < 0.008$).

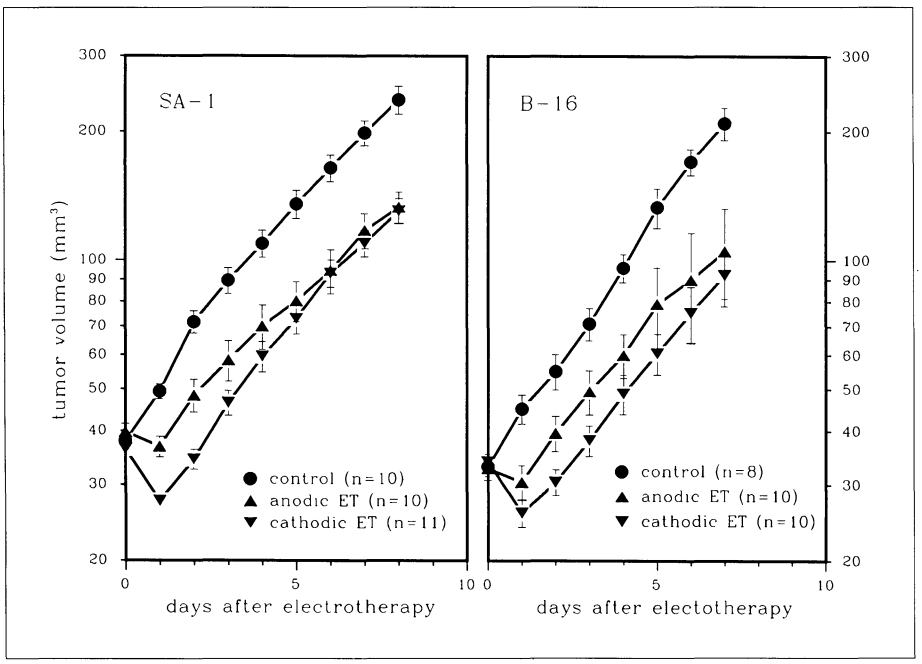

Fig. 1: Growth curves of fibrosarcoma SA-1 and melanoma B-16 tumors following anodic or cathodic single shot electrotherapy on day 0 with 0.6 mA of one hour duration. One electrode was inserted centrally into the tumor and the other subcutaneously in the tumor vicinity (SEET). Vertical bars: standard error of the mean.

Because antitumor effect of electrotherapy was concentrated at the site of electrode insertion, distribution of electrical current through several electrodes could be beneficial. Therefore electrotherapy on SA-1 tumors with 0.6 mA cathodic direct current for 60 minutes performed with single electrode located centrally in the tumor (SEET) was compared with electrotherapy performed through three electrodes distributed over the tumor mass with equal distances between them (MEET). The delivery of direct current by three electrodes produced significantly better antitumor effect ($p < 0.05$) from day 3 to day 9 in comparison to single electrode (Figure 2).

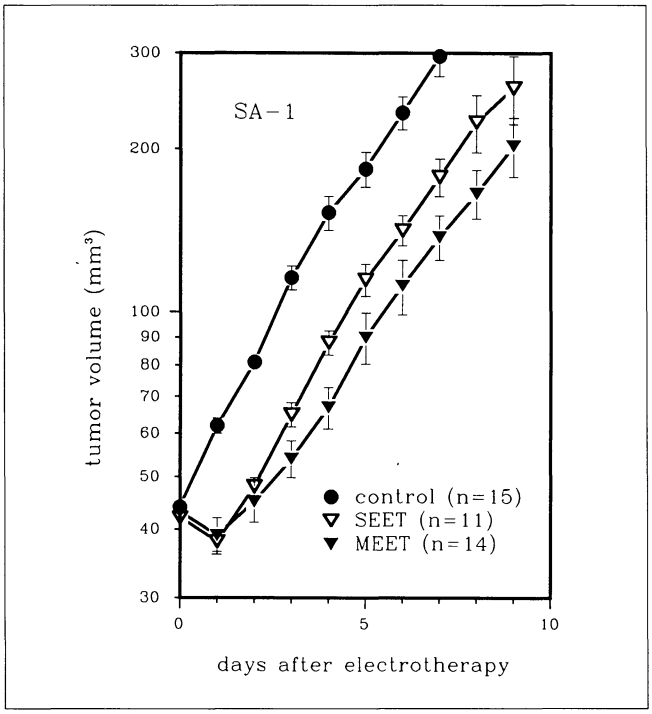

Fig. 2: Tumor growth curves following the cathodic electrotherapy on SA-1 tumor model of one hour duration on day 0 with 0.6 mA. The current was delivered through one electrode inserted directly into the tumor and the other subcutaneously in tumor vicinity (SEET) or through three electrodes inserted into the tumor site and two subcutaneously in its vicinity (MEET). Vertical bars: standard error of the mean.

Effectiveness of multiple array electrode configuration with cathodic electrotherapy was compared on SA-1 and melanoma B-16 tumor models. Electrotherapy with escalating levels of direct currents resulted in better antitumor effect on B-16 melanoma than on SA-1 fibrosarcoma tumors (Figure 3, Table 1). The highest current level (1.8 mA) induced on B-16 melanoma 16.8 ± 0.8 days tumor growth delay and even 40 % animals were cured.

Discussion

In the experiments antitumor effectiveness of electrotherapy with direct current, depending on the polarity and current intensity is described. Therapy with 0.6 mA resulted in significant antitumor effect on both tumor types, fibrosarcoma and malignant melanoma. Depending on the polarity of single electrode inserted into the tumors, anodic and cathodic treatment did not differ significantly in their effectiveness. This is in accordance with some experimental data published previously, although very few authors compared effectiveness of both polarities. David et al. [4] reported no obvious difference

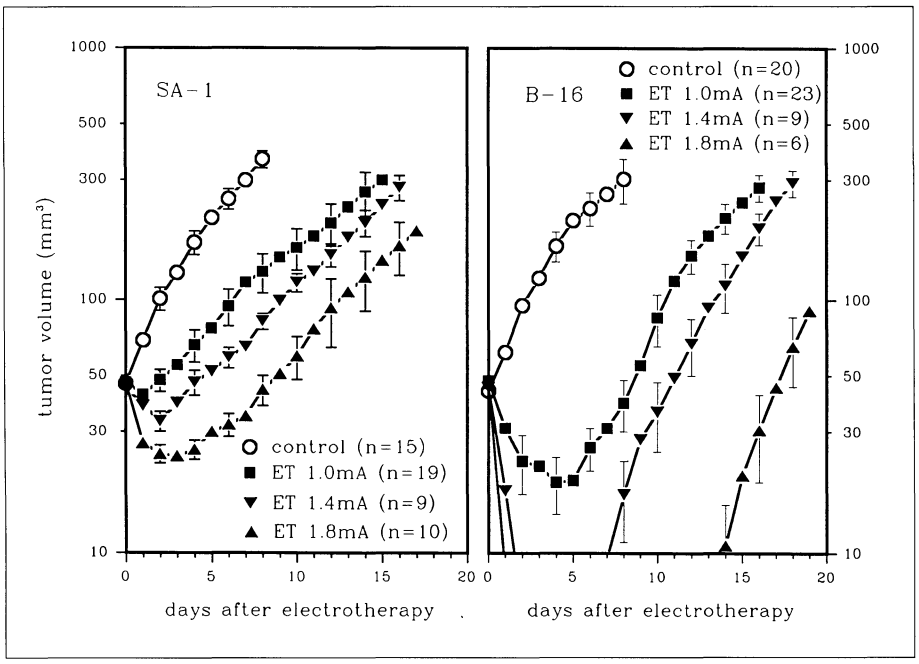

Fig. 3: Tumor growth curves after multiple array electrotherapy (MEET) with 1.0, 1.4 and 1.8 mA of one hour duration on fibrosarcoma SA-1 and malignant melanoma B-16 tumors. Vertical bars: standard error of the mean.

Table 1: Antitumor effect of electrotherapy on fibrosarcoma and melanoma tumors.

	Fibrosarcoma SA-1			Melanoma B-16		
	GD[a] (days)	n	Cures	GD (days)	n	Cures
ET[b] 1.0mA	4.1 ⌒ 0.8	(19)	0%	8.6 ± 0.6	(23)	4%
ET 1.4mA	7.2 ⌒ 0.7	(9)	0%	11.5 ± 1.1	(9)	10%
ET 1.8mA	11.8 ⌒ 0.9	(10)	0%	16.8 ± 0.8	(6)	40%

[a]growth delay, [b]electrotherapy

in effectiveness of anodic or cathodic treatment, whereas Schauble et al. [24] reported better effect with anodic electrotherapy. These differences in susceptibility to different electrical current polarities could be ascribed to different tumor models employed in the studies. Also Nordenström et al. [19], reported variable responses of tumors in the clinical study to anodic or cathodic fields.

Although no significant differences in tumor growth delay has been observed, cathodic electrotherapy was moderately more effective than anodic on fibrosarcoma and on melanoma tumors. Also tumor tissue destruction around the cathode was more extensive than around anode with better delineated margins. Therefore it was assumed that with cathodic treatment is easier to control the extent of tissue destruction.

As a consequence of previous observations multiple array electrode configuration was suggested and confirmed to be more effective than single electrode treatment. If the electric current was distributed over broader area, the same current intensity produced better antitumor effect. As the result of optimal current distribution with sufficiently high current intensities we were able to eradicate the tumors and cure the animals. Single shot electrotherapy for one hour with 1.8 mA cured 40 % of malignant melanoma bearing animals. Therefore optimal current distribution over the entire tumor mass seems essential, in order to eradicate also the clonogenic cells in the periphery of the tumor mass. This problem has been reported also by other authors [4], because central portion is easy to destruct, while in the periphery always remain some clonogenic cells. This curability rate was reported also by Marino et al. [11] on Lewis lung carcinoma, but with much higher current (3.0 mA), which further supports the hypothesis of even distribution of currents over the whole tumor mass.

The mechanisms governing the antitumor effect of electrotherapy with direct current could be multiple: electrochemical reactions at the tissue-electrode interface, temperature rise in tumors, or modified cell proliferation due to electrical currents. Some of them could be ruled out, such as overheating of tumors, since our temperature measurements in the tumors before, during and after electrotherapy show that there is no difference [17]. Other authors also reported that energy delivered into the tumor with electrotherapy is too small, theoretically and on the basis of measurements to heat the tumors up to the level where the cytotoxic effects are to be expected [4, 7, 15]. Due to electrochemical processes dramatic changes in pH occur in the immediate vicinity of electrodes, acidification around anode and basiphication around cathode [7, 17, 22, 23]. This was considered as one of major antitumor mechanisms of electrotherapy. Besides cytotoxicity of metal ions as a consequence of metal corrosion is possible [23]. This is of special importance since Pt which was used as a material for electrodes is known as cytotoxic agent [21]. On the basis of our experiments different metals (Au and Ag) as electrode material in electrotherapy have virtually the same antitumor effect [16]. Exact measurements of the metals dissolved in the tissue have not been done yet, but on the basis of our preliminary data and on the basis of other publications metal ions produced during electrotherapy can not be one of the major antitumor mechanisms [29]. Beside these effects of electrotherapy, electrophoretic influences on cell morphology and ionic composition, as well as electroosmotic influences on water content and tissue may appear [18, 22, 23, 27]. The electric field strength may result in hyperpolarisation and depolarisation of the cell membrane on cathodic and anodic facing side of the cell. The changes in transport across the cell membrane and consequent changes in transmembrane potential may lead to the conformational changes, and together with ionic redistribution of simple ions in extracellular space the ion flux across the cell membrane may be changed. The consideration of the latter was elaborated in our hypothesis of static electric field on cell cycle regulation [28].

The described antitumor effects of electrotherapy on experimental tumor models and the first clinical reports may further promote investigations in this interesting field. The application of electrotherapy can be envisioned in local/regional treatment of solid tumors especially in combined modality treatment with other cytotoxic treatments or biological response modifiers.

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