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Tumor treatment by direct electric current—tumor temperature and pH, electrode material and configuration

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Abstract

Electrotherapy with low-level direct current has been suggested as an effective regional cancer treatment. A great variety of electrode materials, their placement with respect to tumor (i.e. electrode configurations) current levels and therapeutic schedules have been employed to date. In our paper the impact of electrode materials and configurations was studied, employing the same treatment parameters and schedule, on the subcutaneously grown solid tumor fibrosarcoma SA-1 in A/J mice. The effect of electrotherapy was assessed by tumor volume determination on each consecutive day and by determination of the extent of necrosis along the tumor's greatest diameter immediately after electrotherapy, 24, 48 and 72 hours later. The development of tumor necrosis after electrotherapy application differed for different electrode configurations. Intratumoral temperature and pH were measured before and after the application of electrotherapy in all electrode configurations used. Irrespective of the electrode configuration the intratumoral temperature was unchanged by electrotherapy. Considerable variations in pH were revealed for configurations where one electrode was inserted into the tumor, whereas in configurations with both electrodes outside, the tumor pH remained unchanged by electrotherapy. Electrodes made of platinum, platinum (90%):iridium (10%) alloy, gold, silver and titanium were used for current delivery, and significant tumor growth retardation was achieved irrespective of electrode configuration and material ($p < 0.001$, Mann–Whitney Rank–Sum test). In addition to possible toxicity arising from dissolved metal ions and products of electrochemical reactions, which may result from electrolysis, direct effects of current on tumor and immune cells are suggested.

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INTRODUCTION

Although reports on the use of electrotherapy (ET) with low-level direct current in tumor treatment are scarce, several different approaches for current delivery have been employed. Different electrode shapes, materials and placement (i.e. configuration with respect to tumors treated), in addition to different current intensity, treatment schedules and tumor models make comparison of the results reported very difficult, if not impossible.

In the experiments reported to date, metal needle electrodes have been employed with one electrode introduced into the tumor and another needle or larger plate electrode placed away from the tumor. Alternatively, both were introduced into the tumor [1–3]. In one report only [4] the tumor was placed between a pair of surface electrodes. A great variety of metals have been used for electrode material such as stainless steel, Pt, Pt:Ir alloy, Rh, Cu and others, and in all cases tumor growth retardation or even tumor remission has been achieved. The currents used have been in the range from microamps [5,6] to tens of milliamps [2,3], delivered continuously for 24 h per day, or with interruptions, or as a single shot treatment. Treatment time has ranged from five minutes to several hours or even days. In some reports the “dosage” is defined as current charge (current multiplied by treatment time) [2]. In most reports both current and voltage were measured during the treatment. However, the current was sometimes held constant by voltage adjustment, and sometimes the voltage was fixed by varying the current. In addition to the enormous variation in technical details, different tumor models have been employed and tumors of different size have been treated [7].

The proposed electrotherapy, although proven to be effective, lacks sufficient information on the mechanisms involved. The most frequently mentioned mechanisms which could play a significant role in observed phenomena are temperature rise and local pH change due to current flow, changes in tumor bioelectric potential, and deposition of metal ions. In this work we have attempted to clarify and determine the impact of electrode configuration and electrode material on tumor growth retardation, as achieved by electrotherapy, by using a consistent tumor model, current level, treatment time, and therapeutic schedule. In addition we have addressed the hitherto proposed mechanisms, in the light of experimental results, on tumor temperature and pH. These have been measured *in vivo* prior to, during and after the application of ET in different electrode configurations.

MATERIALS AND METHODS

Animals and cells

Subcutaneous solid tumors were initiated by injecting 5×10^5 viable fibrosarcoma SA-1 cells syngenic to A/J mice. The animals were purchased from Rudjer Bošković Institute (Zagreb, Croatia) and kept in a standard mice colony at a constant temperature of 24°C with a natural day/night cycle and fed *ad libitum*.

Tumor cells were prepared from the ascitic fluid of tumor-bearing animals; the viability, determined by the trypan blue exclusion test, was greater than 95%. Animals were randomly divided into designated experimental groups and marked individually after the tumors had reached approximately 40 mm³ in volume.

Electrodes and current delivery

Electrodes in the shape of a needle 0.6 mm in diameter and 18–20 mm long, with a spherical hub of different metals were used (i.e. Pt 90%: Ir 10%), Pt, Au, Ag and Ti) all of 99.999% purity. Electrodes were attached to the direct current source with copper wire. The current supply device was designed to deliver a constant 0.6 mA, by adjusting the voltage. This current level was chosen as a result of our previous experience [8]. Current and voltage were monitored throughout the treatment. At the start of ET the current was linearly raised from zero to the preset value in one minute and was similarly decreased to zero at the end of ET. The duration of ET was one hour,

Two different electrode configurations were used for current delivery. In the first, one electrode was inserted directly into the tumor (approximately 2 mm deep) with the second electrode placed subcutaneously (the whole length) in healthy tissue approximately 5–10 mm from tumor edge. When the electrode in the tumor was connected to the positive terminal ET was considered as being anodic (and as cathodic vice versa). The second electrode configuration effected “field” electrotherapy in which both electrodes were introduced subcutaneously for their whole length, to either side of the tumor, and 5–10 mm away from it.

Assessment of therapy efficacy

The day when tumors reached approximately 40 mm³ was considered as day zero (D0), when a single shot ET was performed. On D0 and each following day until D9, the volume of each tumor was determined by measuring its three mutually orthogonal diameters with a vernier calliper gage and calculating $V = \pi/6 abc$ [9] (where V is the tumor volume, and a , b and c the measured tumor diameters). Each day the mean and standard error of the mean were calculated for each experimental group. The values were plotted as growth curves on a logarithmic scale. Every day the nonparametric Mann–Whitney Rank–Sum test was performed (two-tailed on D0, single-tailed on D1 to D9) in order to evaluate statistically the differences between experimental groups.

Tumor growth delays, with standard deviations, were calculated from mean tumor doubling times and standard deviations obtained by determining doubling times of individual tumors in each experimental group [10,11]. To compare growth delays a two-tailed Student's t -test was employed.

The extent of necrosis along the tumor's greatest diameter was determined by two independent histologists. The preparations they were asked to estimate were coded and they had no knowledge of the prior treatment. Their estimates were

pooled afterwards. The extent of necrosis was determined as the percentage of necrotic region with respect to the whole tumor area. Tumors for histological examination were excised either before or immediately after ET, or 24, 48, and 72 h after the start of ET. To enable comparison between different experimental protocols a nonparametric Mann–Whitney Rank–Sum test was conducted at each given time point.

Tumor temperature measurement

Tumor temperature was measured by introducing a Ni:Cr–Ni thermosensor TK127, AMR, Holzkirchen, Germany) into the tumor. The exposed tip of the sensor was 0.5 mm in diameter and 1.5 mm in length; the remainder of the tip was insulated by medical grade silicon tube. The thermosensor was connected to a thermometer 2280-8 (AMR) which gave readings averaged over 10 s. Ambient temperature was monitored during experiments since fluctuations in ambient temperature greater than 2°C were observed to affect results of tumor temperature measurements.

Tumor temperature was recorded for 15 min prior to commencement of ET and at the end of ET. Tumor temperature was subsequently measured every morning on four consecutive days for the control group and the three experimental groups (subjected to cathodic, anodic and “field” ET).

Tumor pH measurement

Tumor pH was measured by the introduction of a miniature glass electrode (MI-408; Microelectrodes Inc., Londonderry, New Hampshire, USA) encased by a 21-gage stainless steel needle designed for pH measurement over the range 1 to 13 pH units. A reference electrode (HEK 0301, ISKRA Horjul, Slovenia) was placed caudally, approximately 25 mm from the tumor edge in subcutis. A pH meter (MA5740, Iskra, Horjul, Slovenia) with automatic calibration at two points was used.

The pH was measured prior to ET, at 5, 15, 30, 45 and 60 min during ET, and at 15, 30 and 60 min after one hour’s ET. Tumor pH was subsequently measured on four consecutive days for the control group and the three experimental groups.

RESULTS

Regardless of electrode configuration statistically highly significant ($p < 0.001$) tumor growth retardation was achieved by single shot ET. With one electrode in the tumor and the other placed subcutaneously, variation in growth retardation was obtained with respect to current polarity. However, this difference was manifested for only three or four days after ET, becoming indistinguishable later (Fig. 1(a)). With both electrodes placed subcutaneously virtually the same results were obtained. Only on the first day after single shot ET was the response for “field” ET between cathodic and anodic ET (Fig. 1(a)).

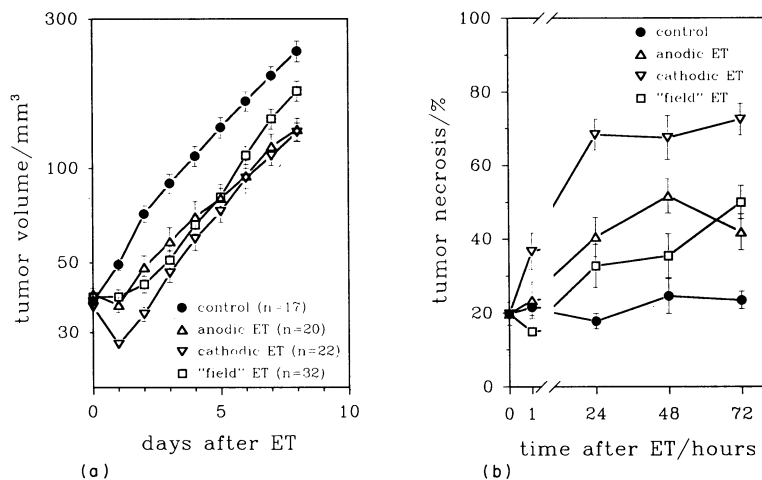


Fig. 1. (a) Tumor growth curves as means, with vertical standard error bars, for the control and experimental groups subjected to ET of 0.6 mA for one hour. The number of animals in each group is given in parentheses. ET was performed as single shot treatment on day 0. Tumor growth retardation for all three experimental groups was statistically highly significant ($p < 0.001$) in comparison to the control group. (b) The extent of necrosis along the tumor's greatest diameter, assessed before and immediately after one hour's ET with 0.6 mA, for all configurations. For the control electrodes were inserted, as in the ET groups, but no current flowed. Each data point on the graph presents the mean of ten or more tumors with vertical bars showing the standard error.

The effect of ET in both electrode configurations was further assessed by evaluating the extent of necrosis. The percentage of necrosis developed after cathodic ET increased dramatically immediately after ET and remained very high throughout the observed period, whereas for anodic ET this effect was not statistically significant. However, one day after anodic ET and subsequently, the percentage of necrosis was higher than for the controls, but lower in comparison to that for cathodic ET. The dynamics of tumor necrosis development following "field" ET differed, with a rise in percentage of necrosis occurring only the second and third day after ET (Fig. 1(b)). After anodic and cathodic ET tumor necrosis development was located centrally around the electrode within the tumor, whereas for "field" ET necrosis development was evenly distributed over the entire cross-section of the tumor.

The effect of ET for the "field" configuration with different electrode materials was assessed by growth curves (Fig. 2) and by tumor growth delay. For all materials used growth retardation was statistically highly significant ($p < 0.001$) when compared to the corresponding controls. In an attempt to assess the responses achieved by different metals we found Pt:Ir the least and Ag electrodes the most effective (Table 1). Differing degrees of tissue injury in the immediate vicinity of the electrodes was noted, the most severe being when titanium electrodes were employed. Since the injury did not heal in 2 days the experiment with titanium

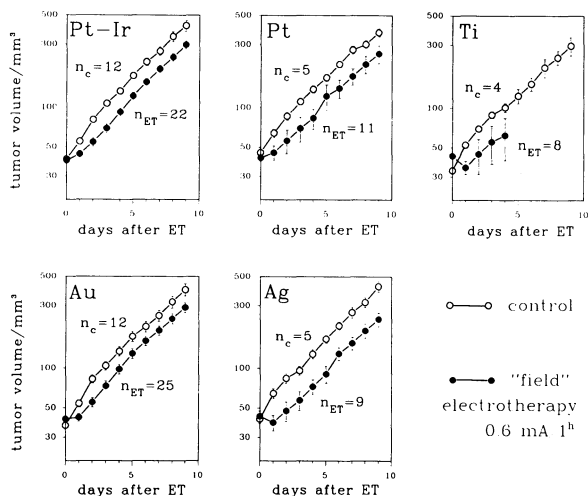


Fig. 2. Tumor growth curves with mean data points showing tumor volume for group subjected to “field” electrotherapy of 0.6 mA for 1 h. Different metal electrodes (Pt (90%):Ir (10%); Pt; Ti; Au; and Ag were used. In all groups subjected to single shot ET tumor growth retardation was statistically highly significant ($p < 0.001$) in comparison to the controls.

electrodes was discontinued. With silver electrodes the formation of AgCl was detected, whereas electrodes of gold, platinum and platinum:iridium alloy were shown to be suitable for current delivery. With the latter electrodes very small injuries were observed, which then healed without delay.

Tumor temperature during or immediately after ET was not changed in either configuration (Table 2). Tumor temperature increased by about 1°C one day after ET and remained elevated for all groups including the control group (Fig. 3). This rise in tumor temperature therefore was due to inflammation processes caused presumably by tumor tissue irritation resulting from temperature probe introduction.

TABLE 1

Tumor growth delay (GD) with standard deviations (std.) and degrees of freedom (ν) achieved by single shot “field” electrotherapy of 0.6 mA direct current for 1 h using different electrode materials. Metal needle electrodes were introduced subcutaneously 5–8 mm from the tumor edge, diametrically with respect to the tumor.

Electrode material	GD \pm std.	ν	Student's two-tailed <i>t</i> -test			
			Pt:Ir	Pt	Au	Ag
Pt:Ir	1.3 \pm 0.3	32	–	< 0.1	< 0.001	< 0.001
Pt	1.9 \pm 1.2	14	< 0.1	–	1	< 0.1
Au	1.9 \pm 0.6	35	< 0.001	1	–	< 0.02
Ag	2.8 \pm 1.1	12	< 0.001	< 0.1	< 0.02	–

TABLE 2

Intratumoral temperature measured prior to and immediately after single shot electrotherapy of 0.6 mA for 1 h. Given as mean \pm standard deviation of measurements in 20 animals.

Treatment	Before ET/ $^{\circ}$ C	After ET/ $^{\circ}$ C
Control	34.6 \pm 0.3	34.5 \pm 0.2
Cathodic ET	34.4 \pm 0.3	34.4 \pm 0.3
Anodic ET	34.4 \pm 0.2	34.5 \pm 0.5
"Field" ET	34.3 \pm 0.3	34.4 \pm 0.2

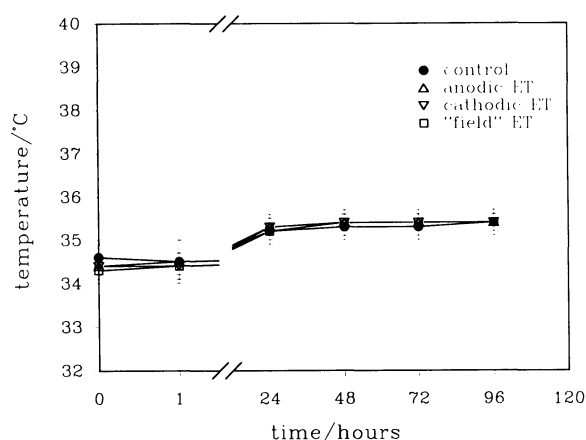


Fig. 3. Intratumoral temperature measured before, and immediately after 1 h electrotherapy with 0.6 mA, and on four subsequent days. Each data point represents the mean of 15 or more tumors measured, with vertical bars to show the standard error.

The pH measurements resulted in extreme changes toward acidic and basic values immediately after the start of anodic and cathodic ET respectively (Table 3). Initial tumor pH was restored in one day after anodic ET, whereas for cathodic ET restoration took three to four days (Fig. 4). In the experimental group where

TABLE 3

Intratumoral pH measured prior to and immediately after single shot electrotherapy of 0.6 mA for 1 h. Given as mean \pm standard deviation of measurements in 20 animals.

Treatment	Before ET	After ET
Control	6.7 \pm 0.2	6.8 \pm 0.2
Cathodic ET	6.7 \pm 0.2	10.8 \pm 0.7
Anodic ET	6.6 \pm 0.2	0.9 \pm 0.2
"Field" ET	6.8 \pm 0.3	6.6 \pm 0.3

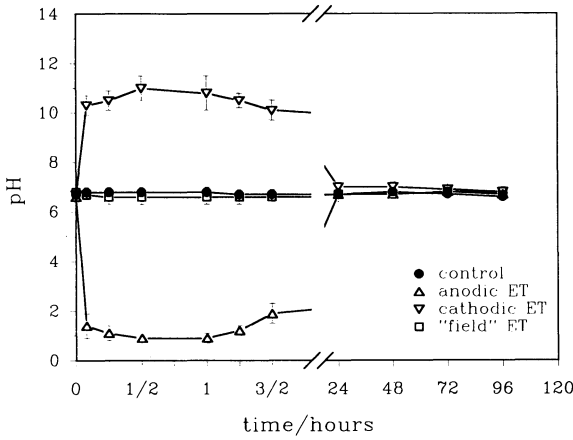


Fig. 4. Intratumoral pH measured before, during and after ET of 0.6 mA for 1 h. Each data point represents the mean of 15 or more tumors measured, with vertical bars showing the standard error.

“field” ET was applied a statistically nonsignificant drop of 0.2 pH units was observed within 15 min of the start of treatment. This persisted for 30 min after the end of ET. One day after the application of ET initial tumor pH was restored. No pH changes were observed in the control group.

The measurement of pH after 1 h anodic ET, at a site 2 mm from the center of the tumor where the anode was introduced, confirmed a large pH gradient (Fig. 5).

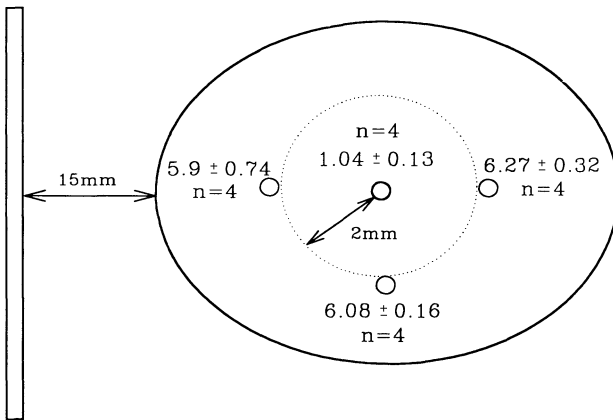


Fig. 5. Intratumoral pH measurements after ET of 0.6 mA for 1 h with the anode inserted in the center of the tumor and the cathode subcutaneously in its vicinity, at different locations. The mean and standard deviations of the four tumors measured are given indicating large pH changes only in the near vicinity of the anode.

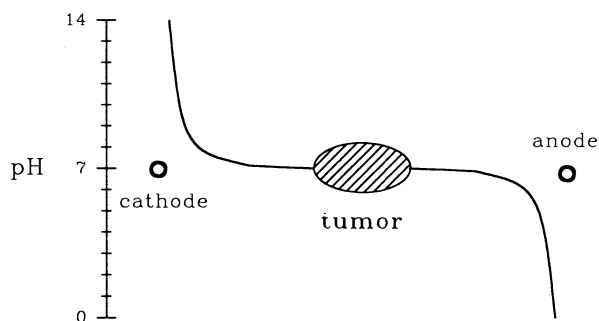


Fig. 6. Probable pH profile within the tissue after 1 h "field" ET of 0.6 mA. Both electrodes were inserted subcutaneously in the tumor vicinity indicating no change in pH within the tumor for this electrode configuration.

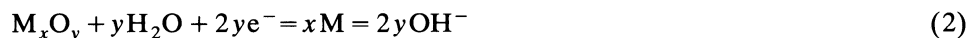
According to these results extreme pH changes are to be expected only in the close vicinity of the electrodes, as schematically presented in Fig. 6.

DISCUSSION

The idea of performing electrotherapy with low-level direct current arose from the claims of Humphrey and Seal [4] that fast-growing regions of organisms are electrically negative compared to non-growing or slower growing regions [12]. In previous work we came to the conclusion that tumors are electrically negative with respect to their surroundings [13], which is consistent with other authors [14,15]. However, attempts to change the negative bio-potential of tumors by electrotherapy with low-level direct current have failed, although growth retardation by electrotherapy was obtained [13]. Nordenström [16] has proposed a theory which is intended to explain the antitumor effects obtained by electrotherapy. This theory, however, still needs further investigation. Another possibility is Joule heating of the tumor. Although several authors have considered and rejected this, we confirmed measurements of tumor temperature prior to, during and after ET.

One very broad topic which merits careful consideration is electrochemical change arising from the current flow. Several chemical reactions occur at and in the vicinity of the electrode/tissue interface which are known to have a cytotoxic effect [17]. At the cathode likely reactions are:

oxide reduction



O₂ reduction



and H₂ production

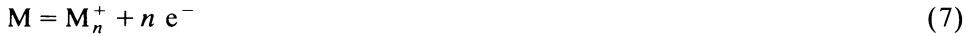


These all increase OH⁻ concentration causing a rise in the local pH. In addition, gaseous H₂ could physically damage surrounding tissue. Another possible O₂ reduction, although not very likely, is



which yields the superoxide anion, known for its toxic properties.

At the anode the most likely reactions to occur are metal corrosion



oxide formation



and O₂ production



Metal ions diffuse away from the anode and may consequently react with organic and inorganic species. It is known that the chemotherapeutic properties of cisplatin were discovered in similar way by Rosenberg [18], and that recently other metal complexes have been identified for their antitumor characteristics [19]. The three reactions mentioned above contribute directly to decreases in local pH. Additional chain reactions possibly occurring in the tissue are hard to predict and as yet none have been identified. The pH changes we recorded follow the profile suggested in Fig. 6. The characteristically large change in the immediate vicinity of the electrodes rapidly falls away to a zero net pH change at the midpoint. Since practically the same growth delay was observed in electrotherapy where electrodes were not placed into the tumor, pH is not considered to be the main cause for the observed tissue necrotization. Nevertheless, extracellular pH has been shown to affect cell survival and cell division [20], and thus it may contribute to the observed phenomena in electrotherapy where one or both electrodes are introduced directly into tumor.

Dissolved metallic ions can play a role in antitumor effects [18,19]. However, since different metals are known to dissolve at different rates under the same electrical conditions, and since the electrical parameters used in the experiments were in the order of tens of ng/g dry tumor weight and or below (this data not presented), we would like to suggest mechanisms other than (or besides) metal toxicity.

Let us consider the possible effects which can arise from current flow between the electrodes, as in case of “field” ET. Besides the possible diffusion of metal ions within the tumor, electrophoretic influences on cell morphology and ionic composition, as well as electro-osmotic influences on the water content of tissue

may occur [21]. The electric field strength within the tissue was in the order of 10^2 V/m which can result, according to Robinson [22], in hyperpolarization and depolarization of the cell membrane on cathode- and anode-facing sides of the cell, respectively. The changes in transport across the cell membrane and consequent changes in transmembrane potential may lead to conformational changes, and, together with ionic redistribution of simple ions in extracellular space, the ion flux across the cell membrane be changed. Consideration of the latter was elaborated in a previous paper, where we suggested the hypothesis of a static electric field on cell cycle regulation [23]. Poo [24] showed that receptors on the surface of the cell could be redistributed by just 10 min exposure to fields of the same order of magnitude. There are some indications that direct current can also modify the activity of relevant immune cells. Promotion of their antitumor activity has already been demonstrated [25,26] and deserves more attention in future investigations.

According to the results presented in this paper we would like to suggest that electrotherapy with low-level direct current is primarily based on the effects of the electric field and/or current imposed on the tissue. This requires a modified approach towards the understanding of phenomena reported to date and further investigations into the interaction of DC electric fields and/or currents on living tissue.

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