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BLOOD PERFUSION IN A MURINE FIBROSARCOMA TUMOR MODEL AFTER DIRECT CURRENT ELECTROTHERAPY: A STUDY WITH ^{86}Rb EXTRACTION TECHNIQUE

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ABSTRACT

Electrotherapy with low-level direct current (DC) can induce antitumor effects in various tumor models. Applied in combination with certain anticancer drugs, it can significantly increase their effectiveness. It has been suggested that the demonstrated effects of electrotherapy arise from its modification of tumor blood flow. The effect of such treatment on blood perfusion of solid subcutaneous Sa-1 fibrosarcoma tumors in A/J mice was investigated with a ^{86}rubidium extraction technique. Following electrotherapy, the relative tissue perfusion of tumors was decreased by more than 50%. Three days after treatment, partial reperfusion of tumors occurred. The dynamics of the perfusion changes induced by electrotherapy are in agreement with tumor growth dynamics following this procedure. The effect of electrotherapy on the blood supply of tumors may be the major mechanism of antitumor action in our model. Electrotherapy could be useful as an adjuvant local procedure to other treatment modalities that require a hypoxic environment for their effectiveness.

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INTRODUCTION

It has been shown in several experimental and some clinical tumors that single-shot electrotherapy with low-level direct current (DC) can be applied locally to temporarily retard or to arrest the growth of solid tumors (1–7). When used in combination with certain drugs, the same type of electrotherapy potentiates the effectiveness of these compounds (8,9). With tumor necrosis factor-α (TNF-α), DC electrotherapy even effected the permanent removal of solid tumors in mice (10). Several possible mechanisms for the antitumor action of electrotherapy, such as its effect on pH and temperature, and deposition of electrode material in the tumor, have been investigated (11,12), but satisfactory explanations for the antitumor effectiveness of electrotherapy alone, or for its potential usefulness as an adjuvant treatment to other therapies, have not yet been provided. It has been suggested that application of electric current induces changes in tumor vasculature or blood perfusion at the site of insertion of electrodes. This seems probable, since extreme changes in pH from physiological levels have been measured in tissue surrounding the electrodes (11).

Blood perfusion of tumors has important relevance to the growth of these tumors under unperturbed conditions, and to anticancer treatment. It is well known that solid malignancies in general exhibit inadequate blood supplies (13–15); the rate of neovascularization of malignant tissue cannot match the rapid multiplication of tumor cells. Tumor neovasculature is also abnormal physiologically and functionally in comparison with the vasculature of normal tissues. As a consequence, parts of tumors are progressively deprived of sufficient supplies of oxygen and nutrients, and at the same time waste products from these regions are ineffectively eliminated. Hypoxia (both acute and chronic) is typical for tumors, but a marked intra- and intertumoral variability in perfusion and oxygenation is reported. In many cases distribution of microvessels and vessel-like structures in tumors shows surprisingly poor correlation with the distribution of necrosis. Abnormal vascularization of tumors is also an important factor in limiting the accessibility of tumor cells to various anticancer drugs. Many drugs, as well as radiotherapy, are cytotoxic only when oxygen is available at adequate levels, and this condition is not met in many tumors. On the other hand, some substances exist that work best or only in a highly hypoxic environment (13–15).

From the foregoing factors, it follows that a procedure that permits local alteration of the perfusion and oxygenation of tumors could be potentially useful as an adjuvant to other treatments that have oxygenation-dependent effectiveness. In the present work, we studied the effects of electrotherapy on perfusion of subcutaneous solid Sa-1 tumors in A/J mice by means of the rubidium (^{86}Rb) extraction technique. It has been proven that over a certain period after an intravenous injection of ^{86}Rb, the amount of this tracer extracted from tissues (except the brain) is proportional to the fraction of cardiac output reaching these tissues, and can therefore be used as a measure of relative tissue perfusion (16,17). This method has been validated for normal tissues and tumors in various animals, including mice, and was therefore suitable for our purpose.

MATERIALS AND METHODS

Animals and Tumors

The tumor model used in the study was the Sa-1 fibrosarcoma in A/J mice. Animals were obtained from Rudjer Bošković Institute (Zagreb, Croatia), and were kept

at a controlled room temperature (24°C) with a natural day/night cycle in standard animal colonies. Healthy female mice, 12 weeks of age, were used in experiments. The Sa-1 tumor cells (Jackson Laboratory, Bar Harbor, ME) were obtained from the ascitic form of the tumor. Subcutaneous solid tumors were initiated by injection of 5×10^5 viable Sa-1 cells suspended in 0.1 ml of physiological saline. Tumors were grown dorsolaterally in the right flank of mice. Approximately 8 days after transplantation, when the largest tumor diameter exceeded 7 mm as measured with a caliper gauge, animals were divided into experimental groups and subjected to either electrotherapy or control treatment.

Electrotherapy

During application of electrotherapy, animals were physically restrained. Needle electrodes of 90/10% platinum/iridium alloy (length 2 cm; diameter 1 mm; rounded tip) were inserted subcutaneously through small, superficial incisions in the skin made with a hypodermic needle. Electrodes were placed in parallel 2 cm apart into healthy subcutis surrounding an on opposite sides of the tumor, with the anode and the cathode on the caudal and cranial sides of the tumor, respectively. The tumor itself, thus situated between the electrodes, was not invaded in any way. A low-level DC of amplitude 0.6 mA, generated by a multichannel current source, was applied for 1 h. The control animals were treated in exactly the same way, except that no current was applied.

Rubidium Extraction Technique

Relative tissue perfusion (RTP) was assessed with the ^{86}RbCl extraction technique at various intervals after treatment. For each interval a separate group of animals was used, with 4 to 6 animals per group. For animals given electrotherapy, RTP was measured immediately after a 15-min treatment and at 5 intervals after completion of a 1-h treatment (10 minutes and at 4 h, 24 h, 48 h, and 72 h after treatment). In control animals, RTP was assessed at 10 min, and at 24 h, 48 h, and 72 h after control treatment.

For injection, the original 1 mCi/ml solution of ^{86}RbCl (Amersham PLC, Little Chalfont, Bucks., UK) was appropriately diluted to obtain the 50 μCi/ml solution. At predefined intervals after treatment, 0.1-ml aliquots of prepared ^{86}RbCl solution were injected into a tail vein of the animals. One minute after the injection the animals were euthanized by cervical dislocation. It has been experimentally verified that the amount of ^{86}Rb extracted by tissues at 1 min after ^{86}RbCl injection is well within the plateau region required for validity of the method (16,17). Immediately thereafter, the whole tumor, a thigh-muscle sample from the left hindleg (tumors were grown in the right flank), and the tail were removed and placed in preweighed glass vials. Muscle samples were used for assessment of effects of locally applied electrotherapy on perfusion of other tissues not directly exposed to electric current. All samples were weighed and their radioactivity was measured with a gamma counter (Institute Jožef Stefan, Ljubljana, Slovenia). In each measurement sequence, the activity of three 0.1-ml aliquots of injection solution, and the background activity, were also measured. The dose activity had to be measured regularly because the half-life of ^{86}Rb is relatively short (18.7 days).

Radioactivity measured in the sample was corrected for the background activity, and the dose radioactivity was corrected for the residual activity in the tail. All animals in which more than 10% of the injected dose was retained in the tail as a result of improper injection were excluded from further evaluation. The following equation was used to calculate the RTP of tissues expressed as a percentage of the dose extracted in the tissue sample, normalized for sample weight:

$$\mathrm{RTP}(\%/\mathrm{g}) = 100 \times \frac{(\mathrm{Activity}_{\mathrm{SAMPLE}} - \mathrm{Activity}_{\mathrm{BACKGROUND}})/\mathrm{Weight}_{\mathrm{SAMPLE}}}{\mathrm{Activity}_{\mathrm{DOSE}} - \mathrm{Activity}_{\mathrm{TAIL}}} \quad (1)$$

Statistical significance of the difference in perfusion between electrotherapy-treated and control tumors was evaluated with Student's *t* test.

RESULTS

Figure 1 shows the effect of single-shot electrotherapy on growth of subcutaneous Sa-1 tumors in A/J mice as previously reported (18). The individual RTP data for tumors and muscle tissue are shown in Figures 2a and 2b, and are summarized in Table 1 and Figures 3a and 3b.

From Figure 3a, it clearly follows that as soon as 15 min after the start of application of electrotherapy, the RTP of tumors had already reached the minimum level of about 1.5 %/g, which represented more than a 50% reduction with respect to sham-treated control tumors, in which the RTP was about 3.5 %/g. Values in the last column of Table 1 were calculated by dividing the RTP of treated tumors by the RTPs of

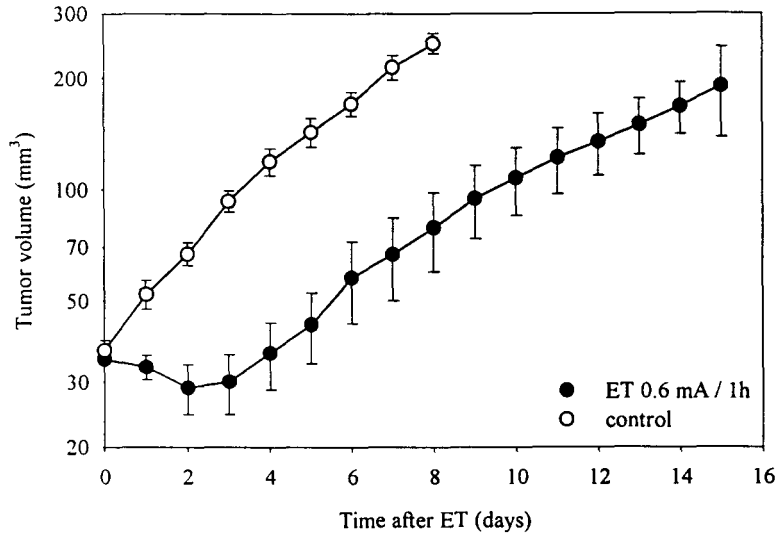
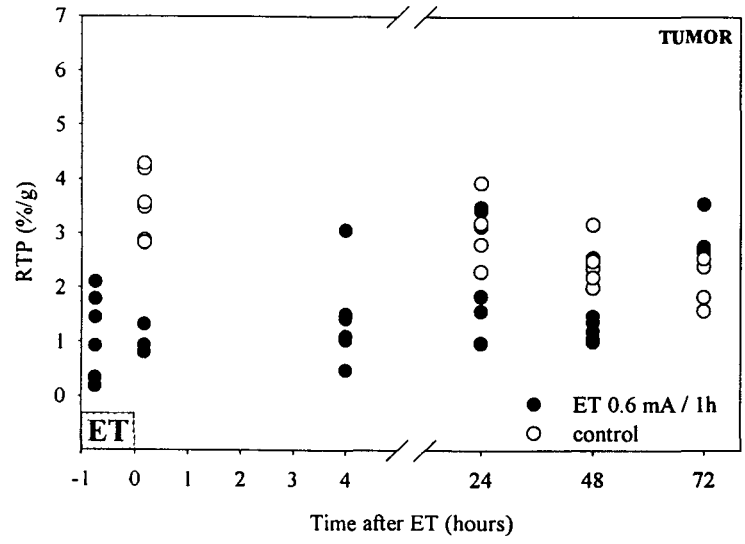
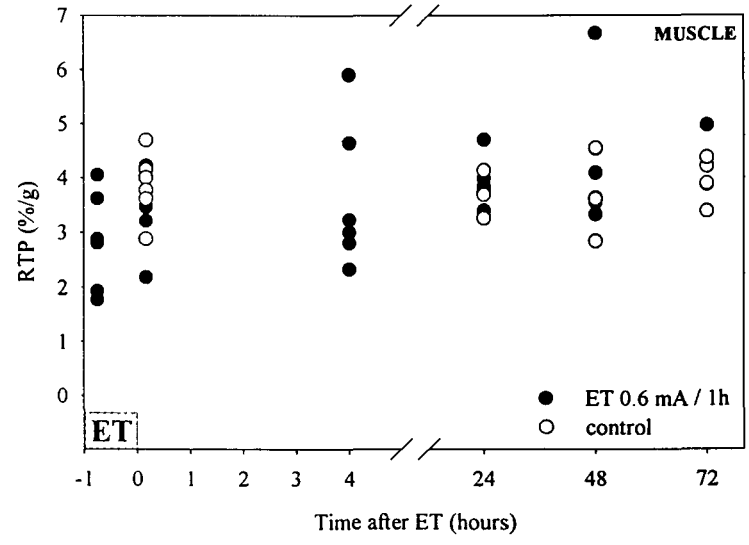


FIGURE 1. Effect of single-shot electrotherapy (ET) with 0.6 mA for 1 h on growth of solid subcutaneous Sa-1 fibrosarcoma tumors in A/J mice. Each data point is represented by the mean value and standard error (SE) bars.

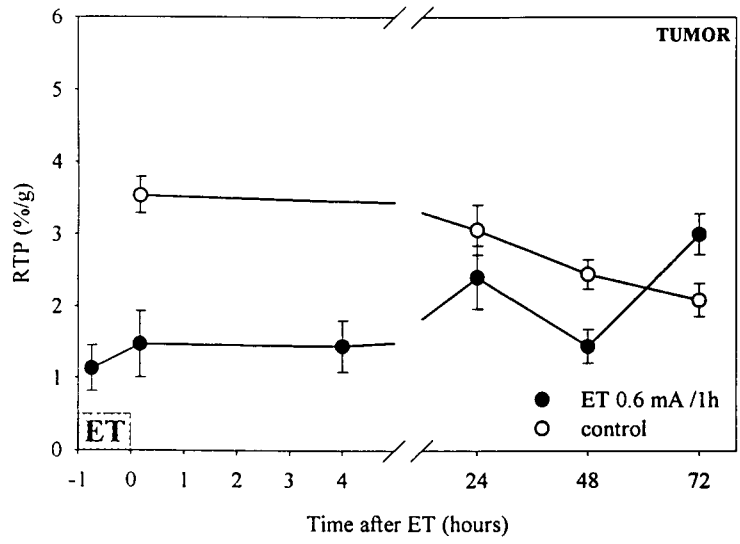


(A)

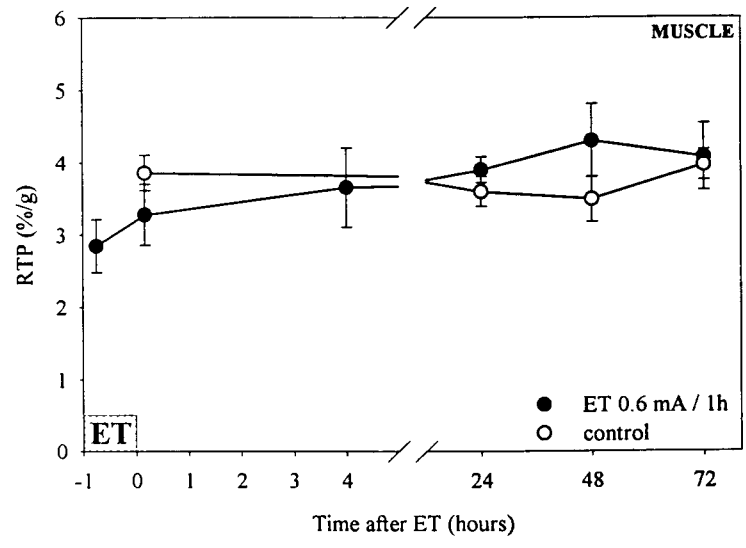


(B)

FIGURE 2. (A) Individual data for relative tissue perfusion (RTP) of control and electrotherapy-treated (ET) Sa-1 tumors in A/J mice (single-shot electrotherapy with 0.6 mA for 1 h). (B) Relative tissue perfusion (RTP) of hindleg thigh muscles of control mice and of mice whose tumors were treated with 0.6 mA electrotherapy (ET) for 1 h. Individual data are represented.



(A)



(B)

FIGURE 3. (A) Relative tissue perfusion (RTP) of control and electrotherapy-treated (ET) subcutaneous Sa-1 fibrosarcoma tumors in A/J mice (single-shot electrotherapy with 0.6 mA for 1 h) (mean ± standard error [SE]). (B) Relative tissue perfusion (RTP) of hindleg thigh muscles of control mice and of mice whose tumors were treated with 0.6 mA electrotherapy (ET) for 1 h (Fig. 2). The values represented are mean ± standard error (SE).

Table 1. Perfusion (RTP) of Tumors at Different Intervals After Treatment, by Experimental Group*

Δt[†]	RTP (%/g) Control[‡] (n)	RTP (%/g) ET[§] (n)	*p*-value[¶]	ET/control (%)[11]
–45 min		1.1 ± 0.3 (6)	<0.001	32
10 min	3.5 ± 0.3 (6)	1.5 ± 0.5 (4)	0.003	42
4 h		1.4 ± 0.4 (6)	<0.001	41
24 h	3.1 ± 0.3 (4)	2.4 ± 0.4 (6)	0.315	79
48 h	2.4 ± 0.2 (5)	1.4 ± 0.2 (6)	0.001	59
72 h	2.1 ± 0.2 (4)	3.0 ± 0.3 (3)	0.051	143

*(See Figure 3a).RTP values are given as mean ± SE (number of animals).
[†]Time interval at which ^{86}RbCl was injected following 1-h electrotherapy or control treatment ("–45 min" means that samples were taken 45 min before completion of treatment).
[‡]RTP of control tumors.
[§]RTP of tumors subjected to electrotherapy.
[¶]Statistical significance of difference between treated and control tumors (Student's *t* test).
[11]RTP of treated tumors expressed as a percentage of RTP of control tumors.

corresponding control tumors. For the first three groups, the same control group (10 min after treatment) was taken as the reference. A statistically significant decrease in RTP was maintained for 2 days, except for the group of tumors evaluated at 24 h after treatment. The mean RTP value for these tumors was surprisingly high, but this was due to three tumors that apparently were not successfully treated, the values of their RTPs being well within the range of control tumors (Fig. 2a). Volumes of these three tumors were also similar to those of tumors in control animals (data not shown). We have previously observed that not all tumors are equally responsive to DC electrotherapy and discuss the main possible reason for this, besides the biological differences between tumors, in the following section. Three days after treatment, a partial reperfusion of treated tumors was demonstrated.

Control tumors exhibited a gradual spontaneous decrease in perfusion with increasing size and age, as shown in Figure 3a at the time intervals of 24 h, 48 h, and 72 h after sham treatment. This expected tendency toward a negative correlation between RTP and tumor size is also shown in Figure 4, where the values for all control tumors in the study are included.

Samples of thigh muscles from the left hindleg were used for assessment of systemic effects of electrotherapy on tissue perfusion. No significant change caused by the application of electric current was observed in muscles (Fig. 3b). Perfusion of muscles also did not change significantly during the 3-day observation period. The mean RTP values for muscles in all treated and control animals were roughly within the range of 3%–4%.

A relatively small number of animals used per experimental group resulted in substantial variation of recorded values within individual experimental groups (Figs. 2a and 2b). In the case of tumors subjected to electrotherapy the variability was even greater, owing to variations in tumor response to electrotherapy.

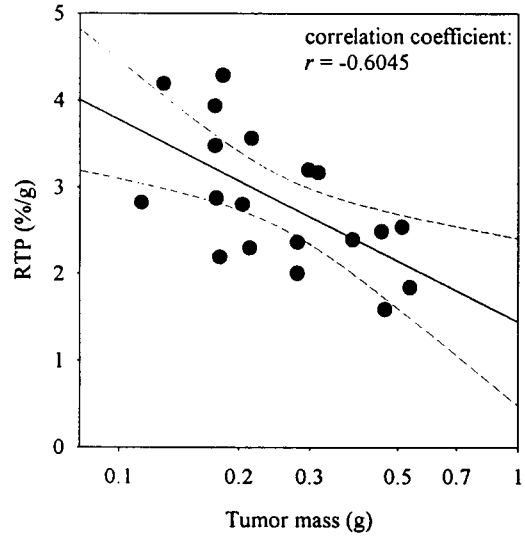


FIGURE 4. Semilogarithmic plot of relative tissue perfusion (RTP) against tumor weight for all control subcutaneous Sa-1 tumors in A/J mice. Linear regression line and 95% confidence-interval (CI) lines are shown ($r = -0.60$).

DISCUSSION AND CONCLUSIONS

The results presented here indicate that the blood supply to tumors in our study was significantly reduced by the local application of electrotherapy. The reduction in perfusion was most probably a consequence of subcutaneous vascular occlusion induced in normal tissue at the site of insertion of the electrodes. Electrolysis caused by a direct electric current results in extreme changes in pH around the electrodes, as has been demonstrated elsewhere (11). The dynamics of reperfusion of tumors shown in Figure 3a are in good agreement with tumor-growth data reported for this particular tumor model (Figure 1) (18). Three days after electrotherapy, the RTP value for treated tumors was almost the same as the RTP of tumors prior to treatment. It can also be seen in Figure 1 that 3 days after treatment, a regrowth of tumors had begun. Similar dynamics of reperfusion for this particular tumor model were also demonstrated through tissue staining with Patent Blue dye (19). It therefore seems very probable that the damage caused by electrotherapy to vessels supplying the tumor is the main determinant of the antitumor effect of such therapy for subcutaneous Sa-1 tumors in A/J mice. This would also explain the variability in response (perfusion and growth) of individual tumors to treatment. Relative proximity of the electrodes during treatment to the vessels supplying the tumor is probably crucial for induction of occlusion. In our study the electrodes were positioned in parallel, on the caudal and cranial sides of the tumor and at least 5 mm away from the tumor itself. Vessels approaching the tumor from the dorsal and from the ventral aspects would thus not be affected, since it has been shown that pH is significantly changed only in the very near vicinity of electrodes and not elsewhere (11). Therefore, tumors predominantly supplied with

blood from above and below would not be successfully treated. This probably occurred in the present study in the cases of the three tumors in the 24-h posttreatment group (Fig. 2a), whose perfusion was the same as that of control tumors, and whose size was also similar to that of tumors in the control group (data not shown).

We conclude that low-level DC electrotherapy has the potential for local modification of tumor blood flow, and that the reduction of tumor perfusion produced by the application of an electric current is probably the main cause of tumor retardation in our model. Whether the observed effect is common to other types of solid tumors, and whether it could be further exploited for enhancing the effectiveness of conventional treatment modalities, remains to be seen.

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